MedCrave
Step into the World of Research

Hematology & Transfusion International Journal

Research Article

Open Access

CrossMark

The application of next generation and whole genome sequencing in the diagnosis of hematological disorders and challenges to apply in routine diagnosis

Abstract

By assessing a patient's genetic makeup at a low cost, next-generation sequencing (NGS) technologies have begun to change the area of hematological malignancies. High-throughput, massively parallel DNA sequencing technologies are rapidly revealing new information in hematology, cancer, clinical genetics, and a variety of other diseases. It provides researchers with a new viewpoint on the onset of sickness, risk assessment, and therapeutic action. In hematology these technologies are used to diagnose hematological malignancies, inherited coagulation bleeding disorders, minimal residual diseases, hereditary hemolytic anemia, and blood typing. The use of next-generation sequencing in regular diagnostic procedures raises a number of challenges, including result interpretation, laboratory workflow, data storage, and ethical concerns.

Keywords: next generation sequencing, whole genome sequencing, hematological malignancies, hematological disorders

Volume 10 Issue 2 - 2022

Solomon Getawa, Mulugeta Melku
Department of Hematology and Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia

Correspondence: Solomon Getawa, Department of Hematology and Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Ethiopia, P.O.BOX: 196, Tel +251-914-665-736; Email solomon2525geta@gmail.com

Received: February 24, 2022 | **Published:** March 18, 2021

Abbreviations: AML, Acute myeloid leukemia; Bp, Base pair; CLL, chronic lymphocytic leukemia; DNA, deoxyribonucleic acid; FA, fanconi anemia; HCL, hairy cell leukemia, IBCD, inherited bleeding coagulation disorders; IGH, immunoglobulin heavy chain; Kb, kilo base; MRD, minimal residual diseases; NGS, next generation sequencing; PML, promyelocytic leukemia; RARA, retinoic acid receptor alpha; RBC, red blood cell; RNN, ribonucleic acid; SNV, single nucleotide variant; TCR, t cell receptor; WES, whole exome sequencing; WGS, whole genome sequencing; RUNX, runt-related transcription factor

Introduction

Both biologically and clinically, benign and malignant hematological diseases are distinct. Mutations, translocations, karyotype rearrangements, and posttranslational changes are all part of the complex and diverse genomic profile associated with these disorders.[1] This data, together with the advancement of molecular techniques, has led to a change in the current dogma of focusing on a particular gene or pathway for investigation. The development of novel molecular biology technologies has led to the identification of genetic or targeted treatment schemes with cytotoxic, anti-metabolic, or immunomodulatory capabilities.[2]

The advancement of high-throughput, massively parallel DNA sequencing technology has led to significant discoveries in hematology, oncology, clinical genetics, and a variety of other diseases.[3] The deep, high-throughput, in-parallel DNA sequencing methods known as next-generation sequencing (NGS) were created a few decades after the Sanger DNA sequencing method initially appeared in 1977 and dominated for three decades.[4] NGS technologies differ from the Sanger method in that they allow for massively parallel analysis and extremely high throughput from numerous samples at a significantly lower cost and time.[5,6]

Next-generation sequencing used to search an individual's entire genome sequence in a short period of time. NGS technology have resulted in the mapping of the human genome in a large number of people, assisting in the understanding of how genetic alterations contribute to disease.[7] It is a flexible method that can detect both new and previously reported sequence variants at the gene, exome, or whole genome level.[8] Furthermore, it can be utilized to examine the genomic component at several levels, including the transcriptome, methylome, and chromatin structure.[9,10]

Next-generation sequencing improves sequencing throughput by attaching millions of DNA fragments to a solid surface or substrate and sequencing all fragments in parallel.[8] It operates by slicing the sample into fragments and creating fragment libraries at random. Then, by ligating particular adaptor oligonucleotides to both ends of each fragment and utilizing them as sequencing templates, fragment libraries are created for sequencing. NGS platforms necessitate powerful computational tools capable of aligning reads to a reference transcriptome or genome sequence, identifying and quantifying expressed gene isoforms, and performing differential expression analysis between specimens (transcriptome profiling).[11] Through the assessment of a patient's genetic makeup, these technologies revolutionized the area of hematological malignancies and provided a unique insight in disease start, risk stratification, and therapeutic intervention.[12]

To find mutations, whole genome sequencing (WGS) entails re-sequencing the complete genome and mapping the sequence back to the human genome. Because it sequences the complete genome, including promoters and regulatory areas, it is usually used to find novel and unusual mutations. It can be used to find genes linked to cancer, diabetes, immune problems, and other diseases.[13] DNA libraries for WGS are prepared using paired-end sequencing and mate-pair sequencing methods. Approximately 100 bp are sequenced from either end of 400-bp DNA fragments in paired-end sequencing. Single nucleotide variations (SNVs), insertions and deletions, and copy-number alterations can be detected using it paired-end WGS. It requires little amounts of DNA (less than 1 mg) to generate libraries,

which is a significant benefit in the research of hematological malignancies. In mate-pair, substantially bigger DNA fragments are generated than in paired-end sequencing, with fragments ranging in length from 1 to 10 kb.[14]

Whole genome sequencing gives a single nucleotide resolution view of the whole human genome, which includes both coding and noncoding sections. It can identify somatic mutations from constitutional variants such as single nucleotide polymorphisms (SNPs) using matched normal tissue, and it can detect a wide range of mutations such as SNVs, insertion/ deletion variants, copy number modifications, and translocations.[15]

Application of whole genome and next generation sequencing in hematology

Researchers are now developing disease-specific targeted NGS panels to aid in the identification and treatment of benign and malignant hematologic disorders.[16] Hematologists are inspired by recent breakthroughs in molecular technology, particularly next generation sequencing, as a first-line technique for identifying possible mutations and determining novel causal genes in patients with blood diseases.[17] The preparation of targeted NGS panels for the diagnosis of hematologic malignancies, red cell congenital hemolytic anemia, hemoglobinopathies, genes related to RBC membrane protein disorders, RBC enzymopathies genes, congenital dyserythropoietic anemia, inherited bone marrow failure syndromes, Diamond-Blackfan anemia panel, and genes related to bone marrow failure syndromes has begun.[18]

Understanding and diagnosing hematological malignancies with next-generation sequencing

Next-generation sequencing technologies have been used to guide diagnosis, sub-classification, prognosis, minimal residual disease (MRD) diagnosis, and the discovery of new mutations in hematological disorders in a variety of scenarios.[19]

Genome analysis of chronic lymphocytic leukemia (CLL): Until a revolutionary study approach combining NGS-WES and copy number analysis was established, the molecular pathogenesis of CLL was unknown.[20] In one study, 32 genes were linked to the onset of CLL; however, a novel gene, NOTCH1, was the focus of additional investigation after it was discovered in 8.3% of CLL cases. The frequency of the NOTCH1 gene increased to 31% as the disease progressed to a more aggressive stage. Initial WGS on four CLL patients revealed 46 somatic mutations that could alter gene function, leading to the sequencing of 363 CLL tumors and the discovery of four frequently mutated genes: NOTCH1, XPO1, MYD88, and KLHL6.[21] Other new mutations include ZMYM3, FBXW7, MAPK1, and DDX3X, all of which interact with the regularly altered CLL genes NOTCH1, SF3B1, and MYD88.[22] Nine genes have been identified as driver mutations in the oncogenesis of CLL, including the previously known TP53, ATM, and MYD88 genes.[12]

Genome analysis of Hairy Cell Leukemia (HCL): A patient with HCL has five nonsynonymous coding variants discovered by WES.[23] One of these had a known activating BRAF mutation (V600E) (an amino acid alteration from a valine (V) to a glutamic acid (E) at position 600 in BRAF). Sanger sequencing of BRAF in another 47 cases of HCL indicated that the V600E mutant is present in every case, implying that it is an obligatory driver of the disease and that HCL is likely to be extremely susceptible to PLX4032.[24]

Genome analysis of Myelodysplasia: Evidence of six novel RNA splicing machinery genes carrying mutations impacting the 3'-splice site identification in pre-mRNA processing, splicing machinery abnormalities were a common discovery utilizing WES in myelodysplasia.[23] Impaired hematopoiesis and defective mRNA splicing are two major consequences, which have been linked to the PRPF40B, U2AF35, SRSF2, ZRSR2, SF3A1, and SF3B1 genes.[12]

Genome analysis of lymphomas: In a variety of lymphoid tumors, such as Hodgkin's lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, CLL, mantle-cell lymphoma, HCL, and splenic marginal zone lymphoma, NGS has also allowed for the acquisition of important molecular information.[25] The goal of NGS is to find the tumor-specific clonotype and circulating tumor-specific sequence in the blood of Hodgkin's lymphoma patients.[26] Across lymphoma entities, P53 mutation predicts poor treatment response and shorter overall survival.[27]

Cancer susceptibility gene identification in acute myeloid leukemia (AML): It's important to figure out the genetic basis for cancer susceptibility because it could influence the prevention, early identification, and treatment of related neoplasms. More than 100 genes have been linked to cancer susceptibility.[28,29] Because many of the genes that contribute to cancer susceptibility have yet to be found, genetic testing for all of these genes is prohibitively expensive, impracticable, and frequently unrevealing.[30] Whole-genome sequencing is a thorough method for detecting alterations in genes linked to cancer susceptibility.[31] The results of WGS in a female who presented with breast cancer at 37 years old, ovarian cancer at 39 years old, and rapidly fatal therapy-related AML at 42 years old were recently reported.[31,32]

Classification of AML: AML molecular classification based on karyotype and particular gene mutations appears to be superior to morphology-based categorization.[32] Whole-genome sequencing holds up the promise of improved diagnostic accuracy, as well as the potential for further identification of disease subtypes with differing outcomes and therapeutic responses.[33]

Identification of cryptic translocation

Acute myeloid and lymphocytic leukemia, myelodysplastic syndrome, and chronic myelomonocytic leukemia all include cryptic translocations and mutations in the core binding factor genes, runt-related transcription factor 1 (RUNX1) and core-binding factor subunit beta (CBFB).[34,35] The RUNX transcription factor family (Runx1, Runx2, and Runx3) encodes proteins that are involved in a variety of cell types, including blood and blood-related cell types.[36] They control normal hematopoietic development via activation or repression, and they have been shown to be useful in identifying any genetic abnormalities.[37,38]

Detection of minimal residual disease

In hematological malignancies, MRD detection and quantification are utilized to assess therapy efficacy, patient risk stratification, and long-term outcome prediction.[39] To understand the probable evolution of MRD, NGS techniques enable for searching not only for known mutations/translocations, but also for any clonal gene mutations and rearrangements present in diagnostic samples.[40]

Inherited coagulation bleeding disorders diagnosis

Inherited bleeding coagulation disorders (IBCDs) are a diverse group of coagulation factor deficiencies that includes the most

common Hemophilia A and B, as well as rare bleeding disorders involving factor II, V, VII, X, XI, and XIII deficiencies, as well as fibrinogen and fibrinolysis disorders.[41] Due to lack of accurate knowledge of the genetic, laboratory, and clinical aspects of this disorders, it can be difficult to identify using routine test methods.[42] NGS could aid in the identification of genetic problems in patients who do not fit into recognized IBCD categories, a better understanding of genotype-phenotype correlations, and identification of afflicted family members.[43]

Fanconi anemia (FA) diagnosis

Due to the clinical presentations of FA patients are so diverse, a positive chromosomal breakage test and/or pathogenic mutations in one of the FA genes are required to establish the diagnosis.[44] FA is caused by mutations in 15 different genes, and the gene products of these genes work in a pathway that deals with specific difficulties that can develop during DNA replication.[45] An Illumina GAIIx sequencer was used to generate sequence data from DNA libraries of eleven carriers of mutations in the FA genes: FANCA, FANCB, FANCC, FANCD1, FANCE, FANCG, FANCI, FANCN, and one individual having a mutation in BRCA1. Multiplexed NGS is a useful molecular diagnostics technique for FA which uses genomic DNA, minimal turnaround time, and minimal cost for detecting the disease-causing mutation.[46]

Congenital hemolytic anemia diagnosis

Hereditary hemolytic anemia are genetically diverse illnesses marked by accelerated red cell breakdown due to intrinsic or extrinsic disorders of the red cell.[47] Next-generation sequencing provides a high-throughput, very sensitive technique for the congenital hemolytic anemia. By interrogating genes encoding cytoskeletal proteins and enzymes with sequencing coverage of the coding regions, splice site junctions, deep intronic, and regulatory regions, NGS can able to detect hemolytic anemia.[48]

Blood-group typing

Pre-transfusion serological testing for ABO and RhD antigens was used to determine compatibility between blood donors and patients.[49] Research done by Lane and his colleagues WGS improved the accuracy of phenotype prediction. A controlled trial with 20 participants demonstrated that serological and single nucleotide polymorphism (SNP) array typing methods were 99.5% concordant. The approach was 99.8% and 99.9% concordant with serological and whole-genome sequencing data in 90 and 200 subjects, respectively, after controlling for slight misalignment of sequence reads.[50]

Challenges of sequencing technologies to apply in routine diagnosis

A key area of concern within sequencing technologies is that of ethical and legal concerns in reporting incidental findings.[51–53] Green et al. raised the issue of genetic variants discovered through genome sequencing that are unrelated to the condition under investigation.[54] The huge amounts of data produced by WGS and WES, most of which would not be relevant in a diagnostic scenario was the main challenge encountered in creating NGS for routine diagnostic procedure.[55] The handling of such a large amount of data poses a challenge in terms of interpreting in clinically meaningful way.[56] Other issues faced are that of the costs related to getting the equipment, software and consumables required for NGS. Besides, due to large amount of data (gigabytes) generated per sequencing in NGS platforms it requires high-performance computers to process and analyze the data quickly and effectively.[57] Validation is another key consideration when employing this technology in a hospital setting for diagnosis and treatment monitoring. As with every novel diagnostic test, various issues must be answered about its usefulness and applicability in a clinical setting by considering the workload, number of samples, and turnaround times in particular laboratory.[12]

Conclusion

High-throughput DNA sequencing technology, which has already impacted hematopoietic disease diagnosis and gene identification, has a lot of potential in the future. Genomic analysis will lead to the discovery of new disease genes and modifier alleles, new insights in diseases pathophysiology and better understanding of disease progression and stratification of risk of disease-specific complications. With better monitoring of medication by genetic biomarkers, improved therapeutic options, particularly patient-specific pharmacogenomics-based therapy, can be developed. The key hurdles of NGS technology include cost, validation, ethical, and legal issues.

Acknowledgments

None.

Competing of interest

The authors declare that there is no conflict of interest.

Funding

The authors not receive fund for this work.

Ethical approval

Not applicable.

References

1. Bowman RL, Busque L, Levine RL. Clonal hematopoiesis and evolution to hematopoietic malignancies. *Cell Stem Cell.* 2018;22(2):157–170.
2. Koutsi A, Vervesou EC. Diagnostic molecular techniques in haematology: recent advances. *Ann Transl Med*. 2018;6(12):242.
3. Merker JD, Valouev A, Gotlib J. Next–generation sequencing in hematologic malignancies: what will be the dividends? *Ther Adv Hematol*. 2012;3(6):333–339.
4. Mardis ER. Next–generation DNA sequencing methods. *Annu Rev Genomics Hum Genet*. 2008;9:387–402.
5. Mardis ER. A decade's perspective on DNA sequencing technology. *Nature.* 2011;470(7333):198–203.
6. Grada A, Weinbrecht K. Next–generation sequencing: methodology and application. *J Invest Dermatol*. 2013;133(8):e11.
7. Rebollar–Vega RG, Arriaga–Canon C, de la Rosa–Velázquez A. Clinical Applications of Next–Generation Sequencing. *Rev Invest Clín*. 2018;70(4):153–157.
8. Mori A, Deola S, Xumerle L, et al. Next generation sequencing: new tools in immunology and hematology. *Blood Res*. 2013;48(4):242–249.
9. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. *J App Gen*. 2011;52(4):413–435.
10. Metzker ML. Sequencing technologies—the next generation. *Nat Rev Genet*. 2010;11(1):31–46.
11. Garber M, Grabherr MG, Guttman M, et al. Computational methods for transcriptome annotation and quantification using RNA–seq. *Nat Methods*. 2011;8(6):469–477.

12. Black JS, Salto–Tellez M, Mills KI, et al. The impact of next generation sequencing technologies on haematological research–a review. *Pathogenesis*. 2015;2(1–2):9–16.

13. Guan Y–F, Li G–R, Wang R–J, et al. Application of next–generation sequencing in clinical oncology to advance personalized treatment of cancer. *Chin J Canc*. 2012;31(10):463–470.

14. Braggio E, Egan JB, Fonseca R, et al. Lessons from next–generation sequencing analysis in hematological malignancies. *Blood Cancer J*. 2013;3(7):e127.

15. Duncavage E, 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(s1):115–21.

16. Sankaran VG, Gallagher PG. Applications of high–throughput DNA sequencing to benign hematology. *Blood*. 2013;122(22):3575–3582.

17. Kedar P. Next–generation sequencing: emerging technology in the area of hematology and blood disorder. *Hematology Blood Disord*. 2018;1(1):1–3.

18. Johnsen JM, Nickerson DA, Reiner AP. Massively parallel sequencing: the new frontier of hematologic genomics. *Blood*. 2013;122(19):3268–3275.

19. Serratì S, De Summa S, Pilato B, et al. Next–generation sequencing: advances and applications in cancer diagnosis. *Onco Targets Ter*. 2016;9:7355–7365.

20. Fabbri G, Rasi S, Rossi D, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. *J Exp Med*. 2011;208(7):1389–1401.

21. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature*. 2011;470(7332):115–119.

22. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *New Eng J Med*. 2011;365(26):2497–506.

23. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64–69.

24. Chapman M, Warren III EH, Wu CJ. Applications of next–generation sequencing to blood and marrow transplantation. *Biol Blood Marrow Transplant*. 2012;18(1):S151–S60.

25. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines. *Blood.* 2008;111(12):5446–5456.

26. Darrah JM, Herrera AF. Updates on circulating tumor DNA assessment in lymphoma. *Curr Hematol Malig Reports.* 2018;13(5):348–355.

27. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat genet.* 2012;44(1):47–52.

28. Garber JE, Offit K. Hereditary cancer predisposition syndromes. *J Clin Oncol*. 2005;23(2):276–92.

29. Rahman N. Realizing the promise of cancer predisposition genes. *Nature*. 2014;505(7483):302–308.

30. Welch JS, Link DC. Genomics of AML: clinical applications of next–generation sequencing. *Hematology 2010, the Am Soc Hematol Educ Program*. 2011;2011(1):30–35.

31. Link DC, Schuettpelz LG, Shen D, et al. Identification of a novel TP53 cancer susceptibility mutation through whole–genome sequencing of a patient with therapy–related AML. *JAMA.* 2011;305(15):1568–1576.

32. Welch JS, Link DC. Genomics of AML: clinical applications of next–generation sequencing. *ASH Education Program Book*. 2011;2011(1):30–35.

33. Borate U, Absher D, Erba HP, et al. Potential of whole–genome sequencing for determining risk and personalizing therapy: focus on AML. *Expert Rev Anticancer Ther.* 2012;12(10):1289–1297.

34. Speck NA, Gilliland DG. Core–binding factors in haematopoiesis and leukaemia. *Nat Rev Cancer.* 2002;2(7):502–513.

35. Hart SM, Foroni L. Core binding factor genes and human leukemia. *Haematologica*. 2002;87(12):1307–1323.

36. de Bruijn M, Dzierzak E. Runx transcription factors in the development and function of the definitive hematopoietic system. *Blood.* 2017;129(15):2061–2069.

37. Sood R, Kamikubo Y, Liu P. Role of RUNX1 in hematological malignancies. *Blood*. 2017;129(15):2070–2082.

38. Fan H, Ortega V, Fanasch HM, et al. PML-RARA fusion resulting from a cryptic insertion of RARA gene into PML gene without the reciprocal RARA-PML fusion: clinical, cytogenetic, and molecular characterization and prognosis. *Eur J Haematol*. 2014;93(4):354–358.

39. Hauwel M, Matthes T. Minimal residual disease monitoring: the new standard for treatment evaluation of haematological malignancies? *Swiss Medical Weekly*. 2014;144:w13907.

40. Faham M, Zheng J, Moorhead M, et al. Deep–sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood*. 2012;120(26):5173–5180.

41. Peyvandi F, Palla R, Menegatti M, et al. Introduction: rare bleeding disorders: general aspects of clinical features, diagnosis, and management. *Semin Thromb Hemost*. 2009;35(4):349–355.

42. Bolton Maggs P, Perry D, Chalmers E, et al. The rare coagulation disorders–review with guidelines for management from the United Kingdom Haemophilia Centre Doctors' Organisation. *Haemophilia.* 2004;10(5):593–628.

43. Peyvandi F, Kunicki T, Lillicrap D. Genetic sequence analysis of inherited bleeding diseases. *Blood.* 2013;122(20):3423–3431.

44. Hays LE, Meyer S, Van De Vrugt HJ. A molecular, genetic, and diagnostic spotlight on fanconi anemia. *Hindawi;* 2012.

45. Wang W. Emergence of a DNA–damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet.* 2007;8(10):735–748.

46. Ameziane N, Sie D, Dentro S, et al. Diagnosis of fanconi anemia: mutation analysis by next–generation sequencing. *Anemia.* 2012;2012:132856.

47. Haley K. Congenital hemolytic anemia. *Med Clin North Am*. 2017;101(2):361–374.

48. Agarwal AM, Nussenzveig RH, Reading NS, et al. Clinical utility of next-generation sequencing in the diagnosis of hereditary haemolytic anaemias. *Br J Haematol.* 2016;174(5):806–814.

49. Makarovska–Bojadzieva T, Blagoevska M, Kolevski P, et al. Optimal blood grouping and antibody screening for safe transfusion. *Prilozi.* 2009;30(1):119–128.

50. Flower R, Roulis E, Hyland C. Whole–genome sequencing algorithm for blood–group typing. *The Lancet Haematol*. 2018;5(6):e233–e234.

51. Martinez–Martin N, Magnus D. Privacy and ethical challenges in next–generation sequencing. *Expert Rev Precis Med Drug Dev.* 2019;4(2):95–104.

52. Wolf SM, Annas GJ, Elias S. Patient autonomy and incidental findings in clinical genomics. *Science.* 2013;340(6136):1049–1050.

53. McGuire AL, Joffe S, Koenig BA, et al. Point–counterpoint. Ethics and genomic incidental findings. *Science (New York, NY).* 2013;340(6136):1047–1048.

54. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetic Med*. 2013;15(7):565–574.

Citation: Getawa S, Melku M. The application of next generation and whole genome sequencing in the diagnosis of hematological disorders and challenges to apply in routine diagnosis. *Hematol Transfus Int J.* 2022;10(2):24–28. DOI: 10.15406/htij.2022.10.00276

55. De Koning TJ, Jongbloed JD, Sikkema–Raddatz B, et al. Targeted next–generation sequencing panels for monogenetic disorders in clinical diagnostics: the opportunities and challenges. *Expert Rev Mol Diagn.* 2015;15(1):61–70.

56. Vestergaard LK, Oliveira DN, Høgdall CK, et al. Next generation sequencing technology in the clinic and its challenges. *Cancers*. 2021;13(8):1751.

57. Wandelt S, Rheinländer A, Bux M, et al. Data management challenges in next generation sequencing. *Datenbank–Spektrum*. 2012;12(3):161–171.

Citation: Getawa S, Melku M. The application of next generation and whole genome sequencing in the diagnosis of hematological disorders and challenges to apply in routine diagnosis. *Hematol Transfus Int J.* 2022;10(2):24–28. DOI: 10.15406/htij.2022.10.00276