Can We Use Targeted Next-Generation Sequencing an Alternative Method to the Conventional Tests in Haematological Malignancies?

Emine Ikbal Atli (emine.ikbal@gmail.com)
Trakya Universitesi Tip Fakultesi https://orcid.org/0000-0001-9003-1449
Hakan Gurkan
Trakya Universitesi Tip Fakultesi
Engin Atli
Trakya Universitesi Tip Fakultesi
Hakki Onur Kirkizlar
Trakya Universitesi Tip Fakultesi
Sinem Yalcintepe
Trakya Universitesi Tip Fakultesi
Selma Demir
Trakya Universitesi Tip Fakultesi
Ufuk Demirci
Trakya Universitesi Tip Fakultesi
Damla Eker
Trakya Universitesi Tip Fakultesi
Cisem Mail
Trakya Universitesi Tip Fakultesi
Rasime Kalkan
Near East University
Ahmet Muzaffer Demir
Trakya Universitesi Tip Fakultesi

Research

Keywords: Myeloid malignancies, Haematological Malignancies, conventional tests, next generation sequencing (NGS)

Posted Date: August 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51331/v1

Abstract

Introduction: Advanced diagnostic methods give a huge advantage for identification of the abnormalities in myeloid malignancies. Researchers tried to show the potential importance of genetic tests both before the onset of the disease and during the remission. Large testing panels prevents false negative results in myeloid malignancies. But the important question is how can be merged with conventional cytogenetic and molecular cytogenetic techniques together with NGS technologies.

Methods: In this paper, we draw an algorithm for evaluation of the malignancies. In order to evaluation of genetic abnormities we performed cytogenetics, molecular cytogenetics and NGS testing panels in hematologic malignancies. In this study, we analyzed 132 patients which are referred to Medical Genetics Laboratory within different type of hematologic malignancies. We highlighted possible algorithm for cytogenetically normal cases.

Results: We analyzed cytogenetically normal patients by using NGS 141 gene panel and we detected two or more pathogenic variations in 20 out of 132 patients.

Conclusions: Despite of long turnaround time conventional techniques is still golden standard for myeloid malignancies but sometimes cryptic gene fusions or complex abnormalities cannot easily be identified by conventional techniques that conditions advanced technologies are recommended.

Introduction

Myeloid malignancies are originating from a hematopoietic progenitor cells and characterized by defective differentiation of myeloid progenitor cells [1]. Advanced molecular diagnostic techniques have been change the diagnostic algorithm. Increased usage of next generation sequencing (NGS) can help to change the scope, timing and suitability of genetic testing in hematologic malignancies [2]. Despite the increased advances in NGS technology and rising of study findings which supports the diagnostic and prognostic usage of mutational profiling in myeloproliferative neoplasms (MPN), the clinical decision-making role is still not fully utilized [3].

Diagnostics algorithms of acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) has been changed in recent years [4, 5]. Due to the advances in NGS technology various myeloid NGS panels are commercially available and generally analyse 25–50 genes which can be classified into several functional categories including the splicing machinery, epigenetic modifiers, transcription factors, signalling molecules and chromatin modifiers [6, 7, 8, 9].

The increased knowledge of genetic abnormalities has been updated classification of the Acute Myeloid Leukaemia (AML). The World Health Organization [11] and European LeukemiaNet [12] added new subgroups of diseases and molecular genetic abnormalities have also been added in diagnostic criteria's. Increased number of mutational, epigenetic and expression studies will be help identification of the novel markers in myeloid malignancies.

National Comprehensive Cancer Network (NCCN) has been added mutations in *FLT3, NPM1, CEBPA* and *KIT* genes for risk evaluation [13] and according to the ELN guidelines *TP53, RUNX1* and *ASXL1* mutations should also be considered during the evaluation of risk. According to the studies; *SF3B1, IDH1* and *IDH2* have also be added as a marker [3, 6, 7, 14, 15]. Laboratories should consider the risks for using large panels to test for myeloid malignancies. There are significant differences in intensity and variant outputs between different laboratories. Therefore, there is an urgent need to develop some standard diagnostic kits. In addition, existing somatic variant classification rules do not include complex interactions between variants within gene pathways [3]. Using NGS based myeloid gene panels will help for identification of multiple recurrent somatic mutations in many AML patients, and additional molecular genetic mutations can be detected in most cases, even within defined AML entities [2]. In myelodysplastic syndrome (MDS), NGS allows the detection of molecular mutations in approximately 90% of patients [6, 16, 17].

As a result, the data of NGS should be interpreted in the context of other laboratory findings regarding cytomorphology, histopathology, immunophenotyping, conventional molecular genetics, cytogenetics, and clinical diagnostic parameters.

In this study, we analysed 149 patients which are referred to Medical Genetics Laboratory within different type of hematologic malignancies. We performed conventional cytogenetics, molecular cytogenetics and NGS analysis in these cases. According to the our results we highlighted possible algorithm for cytogenetically normal cases.

Methods

Patient samples

The present study included 132 patients (77 were men and 55 were women) from December 2017 to September 2018. Written informed consent was obtained from all cases. If patients are under 18 [9 children (≤ 15 years)], from a parent and/or legal guardian were obtained. Study was approved by the Ethics Committee of our university and conducted in accordance with the ethical principles established in the Declaration of Helsinki. The median age of cases was 56 years, ranging from 1 to 90 years and there were 8 children (≤ 15 years) in 132 adults. Patient characteristics were shown in Table 4. Our cohort consists of 132 patients whose diagnosed with MM (39), AML (24), CLL (14), MDS (13), Lymphoma subtypes (13), ALL (6), Pancytopenia (4), MPD (2), Castleman Disease (2), Acute leucosis (2) and 13 other hematologic malignancies. DNA was isolated from bone marrow (QIAamp DNA Blood Mini Kit (bone marrow = 132) (Qiagen, Germany) and peripheral blood (MagNA Pure system Roche Diagnostics). DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific).

Next generation sequencing

In order to evaluation of myeloid neoplasm specific 141 genes The Human Myeloid Neoplasms QIAseq Targeted DNA Panel (Qiagen, Germany) was used. This panel covers exon/intron boundaries as shown in Figure 1 and covers genes has been listed oin Table 1 [18]. MiSeq sequencing-by synthesis bench top sequencer used for sequencing of amplified targets according to the manufacturer's protocol for paired-end sequencing (Illumina, San Diego, CA, USA). Data analysis and quality assessment for calling of single-nucleotide variants and analysis of short insertions and deletions evaluated by using Ingenuity Variant Analysis (IVA) programme. Amplicons were noted as a dropout and excluded from analysis if the coverage at any analysed position in any of the two paired-end sequences (minimal coverage) was 100x, with allele frequency >5%were included for subsequent investigation. Known hotspot or clinically actionable variants detected below these thresholds were verified using orthogonal methods such as Sanger sequencing.

Cytogenetic Assessment

Karyotyping: Marrow Max and Chang media were used for cultures for bone marrow and peripheral blood specimens in a CO 2 incubator. After 24, 48, or 72 h of incubation culteres were harvested. Colcemid was used for arresting of metaphase cells and chromosome slides were stained by using for G banding protocol. International System for Human Cytogenetic Nomenclature (ISCN 2016) [19] was used for reporting and a least 20 metaphases were analysed in each culture.

Fluorescence in situ hybridization (FISH): FISH was applied according to the manufacturer's recommendations. A total of 200 interphase cells were analysed for each sample and images captured/stored by using Applied Imaging/Cytovision system. Final results were reported using the cutoff established in the laboratory for each of the probes tested [20]. Specific gene panels for FISH was applied for each malignancy. FISH panels for each of the malignancies are listed in below.

FISH Panel for MM: IGH/CCND1 t(11;14), CKS1B (1q21), IGH (14q32), p53 (17p13.1), 11q13/15q22/9q34, IGH/FGFR3 t(4;14), IGH/MAF t(14;16)

FISH Panel for AML: 5q-, -5 (5p15, 5q31, 5q33), 7q-, -7 (Cen 7, 7q22, 7q31), Trisomy 8 (Cen 8), MLL (11q23), 20q- (20q12,20qter), RUNX1/RUNX1T1 (ETO/AML1) t(8;21), PML/RARA t(15;17), CBFB inv(16), t(16;16)

FISH Panel for KLL: 6q- (6q21), MYB (6q23), ATM (11q22.3), p53 (17p13.1), Trisomy 12 (Cen 12), 13q-/-13(13q14, 13q34), CCND1/IgH t(11;14)

FISH Panel for MDS: 5q-, -5 (5p15, 5q31, 5q33), 7q-, -7(Cen 7, 7q22, 7q31), Trisomy 8 (Cen 8), MLL(11q23), 20q- (20q12, 20qter)

FISH Panel for ALL: TCF3/PBX1 (E2A/PBX1) t(1;19), Trisomy or Tetrasomy 4, 6, 10, 17 (Cen 4, Cen 6, Cen 10, Cen 17), MYC(8q24), BCR/ABL1/ASS1 t(9;22), MLL (11q23), ETV6/RUNX1 (TEL/AML1) t(12;21), IgH (14q32)

FISH Panel for Myeloproliferative Neoplasms (MPN): 5q, 7q, 8 Centromere/20q, BCR/ABL1/ASS t(9;22), FISH Panel for CML: Cen 8 (+ 8), BCR/ABL1-ASS1 t(9;22), RARA (17q21.2)/(iso17q)

Results

Characteristics of the 132 patients are summarized in Table 2. Among these patients, 77 were men and 55 were women. The median age was 56 years, ranging from 1 to 90 years and there were 124 adults and 8 children (≤ 15 years). Cytogenetic and molecular cytogenetic evaluations were normal in all of the cases. By applying NGS using the 141 gene panel to the cytogenetically normal patients we found two or more pathogenic variations in 20 out of 132 patients. Detailed mutation list has been shown in Table 3.

Next generation sequencing

Next generation sequencing of hotspot regions of 141 gene has been performed in 132 bone marrow sample which are refereed from Department of Haematology. Variables with a depth of coverage > 100x and an allele frequency of > 5% were included in this study. Known hot spots or variants identified below the threshold that may require clinically intervention were confirmed by using the Sanger sequencing. Variables of unknown significance were excluded from the clinical benefit analysis. Variants were classified as pathogenic and possible pathogenic according to the gene and clinical effects. Pathogenic variations were higher in patients with AML, MM, CLL. Two or more pathogenic variations were identified in 20 out of 132 patients. List of the variants has been shown in Table 3. *ASXL1* c.1934dupG mutation was detected in AML and Myelofibrosis patients. *NRAS* c.181C > A was detected in 2 MM patients, *IDH2* c.419G > A was identified in AML and CMML cases. *TET2* c.945delC mutation detected in AML and primary myelofibrosis cases, *SRSF2* c.284C > A mutation was identified in AML and primary myelofibrosis cases. *MPL* c.1544G > T mutation was detected in AML and primary myelofibrosis cases, *SF3B1* c.2098A > G were identified in AML, and MDS cases.

RUNX1 c.482T > C (AML, primary myelofibrosis) and U2AF1 c.101C > T (MDS) was classified as a possibly pathogenic. At the same time, 7 different variants of TP53 (TP53; c.568C > A, c.537T > A, c.596G > A, c.503A > G, c.818G > A, c.395A > G, c.638G > T) were detected in 7 different patients.

The lowest allelic frequency was observed in one MM patient in % 5, % 20 for pathogenic variation of KRAS: c.183A > C. These results show us clonally could be observed in lowest percentages. In the literature, it is recommended that to be able to determine clonally it should be evaluated up to 5% allelic fraction [21].

Discussion

Genetic and epigenetic alterations plays an important role during leukomogenesis [22]. Because of the limitations of the techniques, a variety of methods, from cytogenetic to complete genome sequencing, are needed to be able to prevent incomplete picture of genetic deviations in malignant homeopathies [23].

Advances in next generation sequencing (NGS) technology helps transformation of gene sequencing as a considerably faster and less expensive test, making it more practical in clinical practice. The validation of NGS panels are very critical and generally two-step approach is recommended for validation. The first one is related with optimization and analysis for relevant errors during the testing and the second step is related with establishment of thresholds of depth of coverage and VAF (low variant allele frequency of variations) for each type of identified variant [24].

Recent years, NGS has been used for identification of T cell clonality, recurrent cytogenetic translocations, identification of Philadelphia chromosome in Acute Lymphoblastic Leukaemia [2]. In addition of these conditions, in lymphoproliferative diseases NGS has been also used identification of clonal IGH and TCR rearrangements in MRD (Minimal Residual Disease) [21]. NGS technology can be used for identification of mutant or clonal DNA in several circulating tumor cells and with the increasing number of molecular markers, it is also essential for clinical trials based on substantially parallel next generation sequencing (NGS) [25].

Increased number of studies in this field will help to find out new mutations and gives chance to be updated WHO classification for myeloid malignancies. Moreover, those studies will be help for development of novel targeted therapeutic agents and novel therapeutic targets [26]. The discovery of new mutations in myeloid neoplasms enables us to understand the variable prognosis and pathogenesis of these diseases. The use of cytogenetic-based techniques allows the identification of "gross" chromosomal abnormalities such as translocations, amplifications, and deletions [22]. However the limitation of technique is based on size of the abnormality because genes can undergo changes in various ways (mutations, methylation, etc.) that may be critical for the onset and / or progression of malignant homeopathies. The major advance in NGS is identification of the molecular basis of leukemia because we can now classify malignant homeopathies at a molecular level that is more informative than the cytological classification [27].

Delic and colleagues analyzed 28-gene testing panel in different heamatologic malignencies (myeloproliferative neoplasms, essential thrombocythaemia, primary myelofibrosis, polycythaemia vera). Different mutations were identifed in splicing related genes (SF3B1, SRSF2 and U2AF1), chromatin modification genes (ASXL1 and EZH2) and methylation related genes (DNMT3A, IDH1, IDH2 and TET2) [28].

Here we performed sequencing of 141-gene on an Illumina MiSeq platform and highlighted its potential diagnostic importance for different haematological malignancies. We sequenced 141 genes in a cohort which consist of 132 patients whose diagnosed with MM (39), AML (24), CLL (14), MDS (13), Lymphoma subtypes (13), ALL (6), Pancytopenia (4), MPD (2), Castleman Disease (2), Acute leucosis (2) and 13 other hematologic malignancies.

We suggested to use this panel in routine clinical testing for myeloid malignancies which are cytogenetically and molecular cytogenetically reported as a normal. In this report, we identified variations in different genes which are related with epigenetic modifications, RNA modifications, transcription

factors, DNA repair and cohesin complex. This shows us in patients which are cytogenetically or FISH were reported as a normal has to check in NGS panels. Thus, this will help to prevent false negative results in those cases and can help clinicians to be able to apply true treatment strategies. Our suggested possible algorithm was shown in Fig. 2. In this work, we analyzed pathogenic / possible pathogenic variations in different types of hematologic malignancies which were reported as a normal in cytogenetic and molecular cytogenetic analysis. This shows us only cytogenetic analysis does not sufficient for evaluation of diseases. In addition, NGS gives chance to analyze the genomic copy number alteration of interest gene which triggers different questions for conventional cytogenetics during the evaluation of myeloid neoplasms. The questions are; is it essential for both NGS-based tests and cytogenetics for evaluation of newly diagnosed myeloid neoplasm patients? Which technique is the best for monitoring patients during the treatment or during relapse?

To be able to answer these questions, we examined the similarities and differences between a conventional karyotype and a targeted NGS panel.

1- Conventional cytogenetic gives information for both numerical and structural abnormalities of chromosomes but NGS is target specific and can give only numerical abnormalities. We cannot used NGS for identification of insertion or translocations.

2- Karyotype produces a genomic information in single cell resolution, but NGS provides information for a “mean” copy number changes for all cells in a sample. Also, karyotype gives chance to be able to identification of the low percentage clones which contains different aberrations. On the other side, NGS panels identifies only numerical alterations which are shared with a significant portion of the cells in the sample (typically > 30%).

3- Third, the resolution of karyotype analysis is usually > 10 Mb while NGS panels offers resolution at the gene or exon level. At the same time, cytogenetic analysis cannot be identified submicroscopic abnormalities (10 Mb) but NGS panels can do.

4- If the aneuploidy is high, the ability of karyotype analysis becomes limited. For example, in the case of a complex karyotype which contains a small“marker chromosomes”, it is difficult to identify the chromosomal origin of this abnormality. But targeted NGS panels identifies these abnormalities rapidly and reliably.

5- NGS panels measures copy numbers in relative and inaccurate proximity. The triploidy or tetraploid genome cannot be easily distinguished from the diploid genome. This can be easily determined by karyotype analysis.

These differences demonstrate that the advantages and disadvantages of targeted NGS and karyotype / targeted FISH techniques which supports the idea that complementary technologies are best used to evaluate the numerical and structural properties of neoplastic genomes.

NGS-based panel testing is widely accepted in clinical practice, and this can facilitate the construction of well-designed comprehensive NGS panels, especially during initial diagnosis. Albeit, the targeted NGS panels can evaluate the genome wide numerical imbalances, it cannot identify structural abnormalities, lack of single cell resolution and low target density, necessitate simultaneous cytogenetic analysis capable of presenting a complete picture of the genomic profile. After the diagnostic evaluation, it may be gives advantage to use NGS panel testing to perform cytogenetic analysis for patients whose NGS results show significant clonal evolution.

In this study, we provided an information about the potential uses of NGS in a routine clinical setting and emphasized the importance of the method for clinical studies and improving outcomes of haematology patients. NGS gives chance to new perspective for personalized diagnostics and targeted therapy opportunity for myeloid malignancies. The advanced sensitivity and accuracy makes NGS as a potential diagnostic test in cytogenetically normal myeloid malignancies. Identification of novel specific mutations can be help to find out new targeted therapy options which would be a cornerstone of personalized medicine in hematologic malignancies. Finally, this study provides evidence on potential uses of NGS in the routine clinical setting, and provides a framework for cytogenetically normal cases.

Declarations

Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethics committee of the Trakya University Faculty of Medicine and followed Declaration of Helsinki.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests Statement

The authors declare that they have no conflict of interest.

Funding Statement

The author(s) received no specific funding for this work.

Authors' contributions

E.I.A.- R.K. designed and performed experiments and wrote the manuscript, E.A. and D.E.-C.M. performed experiments, U.D. - A.M.D. and H.O.K. collected and characterised patients, E.I.A – H.G. and E.A. performed analysis, S.D. and S.Y. proofread and wrote parts of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

We would like to thank all of the participants in this study.

References

1. Visconte V, O Nakashima M, J Rogers H. Mutations in Splicing Factor Genes in Myeloid Malignancies: Significance and Impact on Clinical Features. Cancers (Basel). 2019 Nov 22;11(12). pii: E1844. doi: 10.3390/cancers11121844.
2. Palumbo GA et al. The Role of New Technologies in Myeloproliferative Neoplasms. Front Oncol. 2019 Apr 26;9:321. doi: 10.3389/fonc.2019.00321. eCollection 2019.
3. Bacher U et al. Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use. Blood Cancer J. 2018 Nov 12;8(11):113. doi: 10.1038/s41408-018-0148-6.
4. Shumilov, E. et al. Current status and trends in the diagnostics of AML and MDS. Review article. Blood Rev. (2018). https://doi.org/10.1016/j.blre.04.008.
5. Barbui, T. et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J. 8 (2018). https://doi.org/10.1038/s41408-018-0054-y
6. Cazzola, M., Della Porta, M. G. & Malcovati, L. The genetic basis of myelodysplasia and its clinical relevance. Blood 122, 4021–4034 (2013).
7. Papaemmanuil, E. et al. Genomic classification and prognosis in acute myeloid leukemia. N. Engl. J. Med. 374, 2209–2221 (2016).
8. Papaemmanuil, E. et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 122, 3616–3627 (2013).
9. Haley J. Abel, Eric J. Duncavage, Detection of structural DNA variation from next generation sequencing data: a review of informatic approaches, Cancer Genetics, 206,432- 440, (2013) ISSN 2210-7762, https://doi.org/10.1016/j.cancergen.2013.11.002.
10. Duncavage, E. J. & Tandon, B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. Int. J. Lab. Hematol. 37(Suppl 1), 115–121 (2015).
11. Arber, D. A. et al. The2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127, 2391–2405 (2016).

12. Dohner H. et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129:424–447(2017)
13. Patel U, Luthra R, Medeiros LJ, Patel KP. Diagnostic, Prognostic, and Predictive Utility of Recurrent Somatic Mutations in Myeloid Neoplasms. Clin Lymphoma Myeloma Leuk. 2017 Jul;17S:S62-S74. doi: 10.1016/j.clml.2017.02.015.
14. Cancer Genome Atlas Research. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N. Engl. J. Med. 368, 2059–2074 (2013).
15. Duncavage, E. J. & Tandon, B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. Int. J. Lab. Hematol. 37(Suppl 1), 115–121 (2015).
16. Yoshida K et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478: 64–69(2011)
17. 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. 28,32–42 (2015)
18. A Sample to Insight®NGS solution for myeloid neoplasms: Redefined amplicon sequencing for low variant detection and interpretation (Application Note: PROM-12533- 001)(2018)
19. Howe, B., Umrigar, A., Tsien, F. Chromosome Preparation From Cultured Cells. J. Vis. Exp. (83), e50203, doi:10.3791/50203 (2014).
20. Rack, K.A. et al. European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. Leukemia 33, 1851–1867 (2019) doi:10.1038/s41375-019-0378-z
21. Gazzola A. et al. The evolution of clonality testing in the diagnosis and monitoring of hematological malignancies. Therapeutic Adv Hematol. (2014) 5:35– 47. doi:10.1177/2040620713519729
22. Etienne De Braekeleer, Nathalie Douet-Guilbert & Marc De Braekeleer (2014) Genetic diagnosis in malignant hemopathies: from cytogenetics to next-generation sequencing, Expert Review of Molecular Diagnostics, 14:2, 127-129, DOI:10.1586/14737159.2014.872563
23. Mitelman F, Johansson B, Mertens F, editors. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. 2013. Available from: http://cgap.nci.nih.gov/Chromosomes/Mitelman
24. Kim H, Yun JW, Lee ST, Kim HJ, Kim SH, Kim JW; Korean Society for Genetic Diagnostics Clinical Guidelines Committee. Korean Society for Genetic Diagnostics Guidelines for Validation of Next-Generation Sequencing-Based Somatic Variant Detection in Hematologic Malignancies. Ann Lab Med. 2019 Nov;39(6):515-523. doi:10.3343/alm.2019.39.6.515.
25. Avila and Meric Bernstam. Next-Generation Sequencing for the General Cancer Patient. Clin Adv Hematol Oncol. 17(8):447-454(2019)
26. National Comprehensive Cancer Network. Myeloproliferative neoplasms (Version 2.2018). Available from: https://www.nccn.org/professsionals/physician_gls/pdf/mpn.pdf.Accessed September 7, 2017.

27. Kuo FC, Steensma DP, Dal Cin P. Conventional cytogenetics for myeloid neoplasms in the era of next generation sequencing. AmJHematol. 2017;92:227229. https://doi.org/10.1002/ajh.24642
28. Delic S, Rose D, Kern W, Nadarajah N, Haferlach C, Haferlach T, Meggendorfer M. Application of an NGS-based 28-gene panel in myeloproliferative neoplasms reveals distinct mutation patterns in essential thrombocythaemia, primary myelofibrosis and polycythaemia vera. Br J Haematol. 2016 Nov;175(3):419-426. doi: 10.1111/bjh.14269. Epub 2016 Jul 22.

Tables

Table 1.

The list of covered genes and related diseases in NGS panel.

Disease	Genes covered
Acute lymphoblastic leukaemia (ALL)	*ASXL2, ATM, BRAF, CALR, CDKN2A, CREBBP, CRLF2, CSF3R, CTCF, DNM2, EGFR, EP300, FBXW7, GATA2, HNRNPK, HRAS, IKZF3, IL7R, KDM6A, KDR, KMT2C, LRRC4, MAP2K1, MLH1, MSH2, MSH6, NOTCH1, NTRK3, PAX5, PDGFRA, PMS2, PRAMEF2, PTEN, RELN, SMARCB1*
Acute myeloid leukaemia (AML)	*ANKRD26, ASXL1, ATM, BCOR, BCORL1, BIRC3, BRAF, C17orf97, CALR, CARD11, CBLC, CDKN2A, CEBPA, CHEK2, CREBBP, CSF1R, CSF3R, CTCF, DAXX, DDX41, DNM2, DNMT1, ELANE, EP300, FLRT2, FLT3, GATA1, GATA2, HNRNPK, IDH1, IDH2, IKZF1, IL7R, JAK1, JAK3, KDM6A, KDR, KIT(CD117), KMT2A, KMT2C, KRAS, LRRC4, MAP2K1, MPL, MSH6, MYC, NBN, NOTCH1, NPM1, NRAS, NSD1, NTRK3, OR13H1, OR8B12, P2RY2, PCDHB1, PDGFRA, PHF6, PRAMEF2, PRPF8, PTEN, PTPN11, RAD21, RUNX1 (AML1), SF1, SF3A1, SMARCB1, SMC1A (SMC1L1), SMC3, SRP72, SRSF2, STAG2, STXBP2, U2AF1, U2AF2, WT1*
Chronic lymphocytic leukaemia (CLL)	*ADA, BIRC3, BLM, BRAF, CALR, CHEK2, CSF3R, KCNA4, KLHL6, KMT2C, MAP2K1, NBN, NPAT, NTRK3, OR13H1, OR8B12, PRAMEF2, SRP72, TAL1, TERT, TUBA3C, WAS, WRN*
Chronic myeloid leukaemia (CML)	*ABL1, CALR, CDKN2A, CEBPA, CREBBP, CSF1R, CSF3R, FBXW7, GATA2, KDM6A, MSH2, MSH6, RB1, SMC1A (SMC1L1), TP53*
Chronic myelomonocytic leukaemia (CMML)	*CALR, CEBPA, CSF1R, CSF3R, HRAS, KMT2C, LUC7L2, SRSF2*
Chronic neutrophilic leukaemia (CNL)	*CALR, CSF3R*
Multiple myeloma	*ATM, BCL6, BCR, BIRC3, BRAF, CDKN2A, CEBPA, EGFR, FBXW7, GJB3, HRAS, KDM6A, MYC, NOTCH1, PTEN, SH2D1A, SMARCB1*
Myelodysplastic syndromes (MDS)	*ATRX, CALR, CDKN2A, CEBPA, CSF1R, CSF3R, EP300, ETNK1, GNAS, HRAS, KDM6A, KMT2A, KMT2C, RAD21, RB1, SETBP1, SF1, SF3A1, SMC3, SRSF2, STAG2, U2AF1, U2AF2, XPO1, ZRSR2*
Myeloid malignancies	*CBL, CBLB, DNMT3A, EED, ETV6, EZH2, PRPF40B, SUZ12, TET2, TP53*
Myeloproliferative neoplasm (MPN)	*ABL1, ASXL1, CALR, CSF1R, JAK2, JAK3, KAT6A (MYST3), KRAS, MPL, NF1, NRAS, RB1, SETBP1, SF3B1, SH2B3, SRSF2, STAG2.*
Myelofibrosis (MF)	*CALR, CHEK2, IDH1, IDH2, CSF1R, SRSF2*
Other myeloid neoplasms	*BRAF, CDKN2A, CEBPA, FBXW7, HRAS, IKZF3, KLHDC8B, KMT2C, MSH6, NTRK3, PTEN, SRP72, TPMT*
Other myeloid neoplasm genes	*BRCA1, BRCA2, BRINP3, CUX1, FAM47A, FAS, KCNK13, MYD88, PML, PRF1, SAXO2, STAT3, TERC, TNFRSF13B*

Table 2.
WHO classification of our cohort and results of genetic analysis.

WHO classification	n	Detected pathogenic variant	Detected likely pathogenic variant	Detected VOUS	FISH	Karyotype
Multiple Myeloma	39	15	2	12	N	N
Acute myeloid leukemia (AML)	24	18	11	15	N	N
Chronic lymphocytic leukemia (KLL)	14	16	-	16	N	N
Myelodysplastic syndrome (MDS)	13	4	-	4	N	N
Acute lymphocytic leukemia (ALL)	6	3	6	2	N	N
Lymphoma and subtypes	13	3	1	7	N	N
Pancytopenia	4	-	-	3	N	N
Chronic myeloproliferative diseases (MPD)	2	3	-	-	N	N
Castleman disease	2	-	-	1	N	N
Acute leukosis	2	2	-	1	N	N
Primary myelofibrosis	1	4	1		N	N
Chronic myelomonocytic leukemia (CMML)	1	1	-	-	N	N
Plasma cell leukemia	1	-	-	-	N	N
Isolated neutropenia	1	1	-	-	N	N
Bicytopenia	1	-	-	-	N	N
Essential thrombocytosis	1	-	-	-	N	N
Hemolytic anemia	1	-	-	-	N	N
Others	6	1	1	1	N	N

Table 3. The list of identified mutations and their distributions of diseases.

Mutation List	Disorders
TP53 c.568C>A	LYMPHOMA
TP53 c.537T>A	AML
TP53 c.596G>A	AML
TP53 c.503A>G	AML
TP53 c.818G>A	MM
TP53 c.395A>G	CLL
TP53 c.638G>T	CLL
NRAS c.35G>A	LYMPHOMA
NRAS c.181C>A	MM
	MM
NRAS c.183A>T	MM
JAK2 c.1748G>A	LYMPHOMA
JAK2 c.3G>A	CASTLEMAN D.
IDH2 c.419G>A	AML
	CMML
PTPN11 c.227A>G	AML
PTPN11 c.184T>G	ACUTE LEUKOSIS
PTPN11 c.205G>A	CLL
TET2 c.2746C>T	AML
TET2 c.945delC	AML
	PRIMARY MYELOFIBROSIS
TET2 c.3543_3544delCT	AML
TET2 c.4182+1G>A	MPD
TET2 c.822delC	ALL
FLT3 c.1784_1804dupGAGAATATGAATATGATCTCA	AML
FLT3c.1770_1793dupCTACGTTGATTTCAGAGAATATGA	AML
FLT3 c.2678C>T	MM
FLT3 c.1748G>A	CLL
FLT3c.1810_1811insGGGAATATGAATATGATCTCAAATGGG	ALL
FLT3c.1755_1778dupAGATAATGAGTACTTCTACGTTGA	AML
SF3B1 c.1866G>T	MM
SF3B1 c.1866G>T	CLL
SF3B1 c.2225G>A	CLL
NOTCH1 c.4721T>C	ALL
NOTCH1 c.7541_7542delCT	CLL
NOTCH1 c.7330C>T	CLL
BIRC3 c.1639delC	ISOLATED NEUTROPENIA
WT1 c.1153_1157dupCGGTC	AML
ASXL1 c.1141G>T	MM
ASXL1 c.1900_1922delAGAGAGGCGGCCACCACTGCCAT	MM
ASXL1 c.2129delG	MPD
ASXL1 c.1934dupG	AML
	AML
	PRIMARY MYELOFIBROSIS
ATM c.7328G>A	AML
ATM c.2250+2T>A	MM
ATM c.6848_6855delCAGTTAGC	CLL
CHEK2 c.1312G>T	ALL
BCORL1 c.2916T>A	AML
NF1 c.4537C>T	AML
SRSF2 c.284C>A	AML
	PRIMARY MYELOFIBROSIS

DNMT3A c.2645G>A	MM
MPL c.1544G>T	AML
	PRIMARY MYELOFIBROSIS
NPM c.860_863dupTCTG	ACUTE LEUKOSIS
TNFRSF13B c.310T>C	MM
KRAS c.182A>G	MM
KRAS c.183A>C	MM
TERT c.2035T>G	MM
MYD88 c.684G>A	MM
BRCA2 c.4446_4451dupAACAGA	MDS
BRCA2 c.9097dupA	CLL
NBN c.871C>T	CLL
RB1 c.2431delA	CLL
XPO1 c.1711G>C	CLL
GATA2 c.1076T>C	MPD
PHF6 c.1024C>T	ALL
KDM6A c.1578delG	BONE MARROW INVOLVEMENT
EP300 c.6627_6638delCCAGTTCCAGCA	AML
SF3B1 c.2098A>G	AML
	MDS
SF3B1 c.1866G>T	AML
ASXL1 c.2056A>T	AML
ATM c.7466C>T	CLL
ATM c.3032delC	ALL
CHEK2 c.444+1G>A	HODGKIN LENFOMA
CHEK2 c.480A>G	SYSTEMIC MASTOCYTOSIS
ETV6 c.163+1G>T	AML
SRSF2 c.284C>T	ALL
RUNX1 c.482T>C	AML
	PRIMARY MYELOFIBROSIS
DNMT3A c.2645G>A	AML
DNMT3A c.976delC	NHL
CREBBP c.5213_5216dupATGC	AML
STAG2 c.1414G>T	AML
CEBPA c.779_783delACCCCinsG	AML
GNAS c.1376C>G	MM
BCOR c.2428C>T	MDS
BCORL1 c.2916T>A	AML
IKZF3 c.485T>G	CLL
CBLB c.2008C>T	CLL
U2AF1 c.101C>T	MDS
	MDS
FBXW7 c.1393C>T	ALL
ASXL1 c.143G>T	MM
SF3B1 c.422A>G	MM
	MM
NOTCH1 c.7357delG	CLL
ATM c.7466C>T	AML
ATM c.3605G>C	MDS
BCORL1 c.2446C>T	CLL
NF1 c.1921A>G	MANTLE CELL LENFOMA
ETV6 c.909dupC	DBBHL
MPL c.121T>C	AML
	CLL
MPL c.1481T>G	MM
DNMT3A c.1555-8_15551delCTGTCTAG	AML
MYD88 c.815G>A	MM

BCORL1 c.2446C>T	CLL
NBN c.163_171+3delACCAACCTGGTA	CLL
IKZF3 c.998A>G	CLL
CBLB c.2434G>A	MM
CBLB c.1927A>G	MM
CBLB c.1472A>G	MM
CBLB c.815G>A	MDS
JAK1 c.2948A>G	BONE MARROW INVOLVEMENT
JAK1 c.1951G>A	AML
JAK1 c.2221G>A	CLL
DNM2 c.-4C>T	FOLLICULAR LYMPHOMA
EP300 c.7238T>A	PANCYTOPENIA
EP300 c.6967C>T	CLL
BLM c.3416G>C	MANTLE CELL LENFOMA
BLM c.274A>G	LYMPHOMA
	ALL
BLM c.2237C>T	MM
BLM c.1693G>A	CLL
EZH2 c.943A>G	DBBHL
AKAP13 c.7265G>A	AML
DNAH9 c.10555C>A	AML
SMC1A c.2152G>A	AML
OR8B12 c.353C>T	AML
CALR c.-2C>T	CLL
CALR c.682C>T	AML
	MDS
	CLL
KAT6A c.4108G>A	AML
ASXL2 c.833T>A	AML
NPM1 c.733G>C	AML
PRPF40B c.1103C>T	AML
PMS2 c.2321A>T	AML
ADA c.179A>G	AML
SETD2 c.6685G>A	AML
	CLL
PAX5 c.1096C>T	ACUTE LEUKOSIS
AKAP13 c.7265G>A	MM
CSF3R c.355G>A	MM
SF3A1 c.458T>C	MM
IKZF1 c.949_951delAAC	PANCYTOPENIA
CNOT3 c.1847C>T	PANCYTOPENIA
NTRK3 c.121A>G	MDS
NTRK3 c.2293-3C>T	CLL
ZRSR2 c.1098_1099delGA	CLL
ETNK1 c.749C>T	CLL
IL7R c.602A>G	CLL
TUBA3C c.544G>A	CLL

VOUS **: Likely Pathogenic** **:Pathogenic :**

Figures

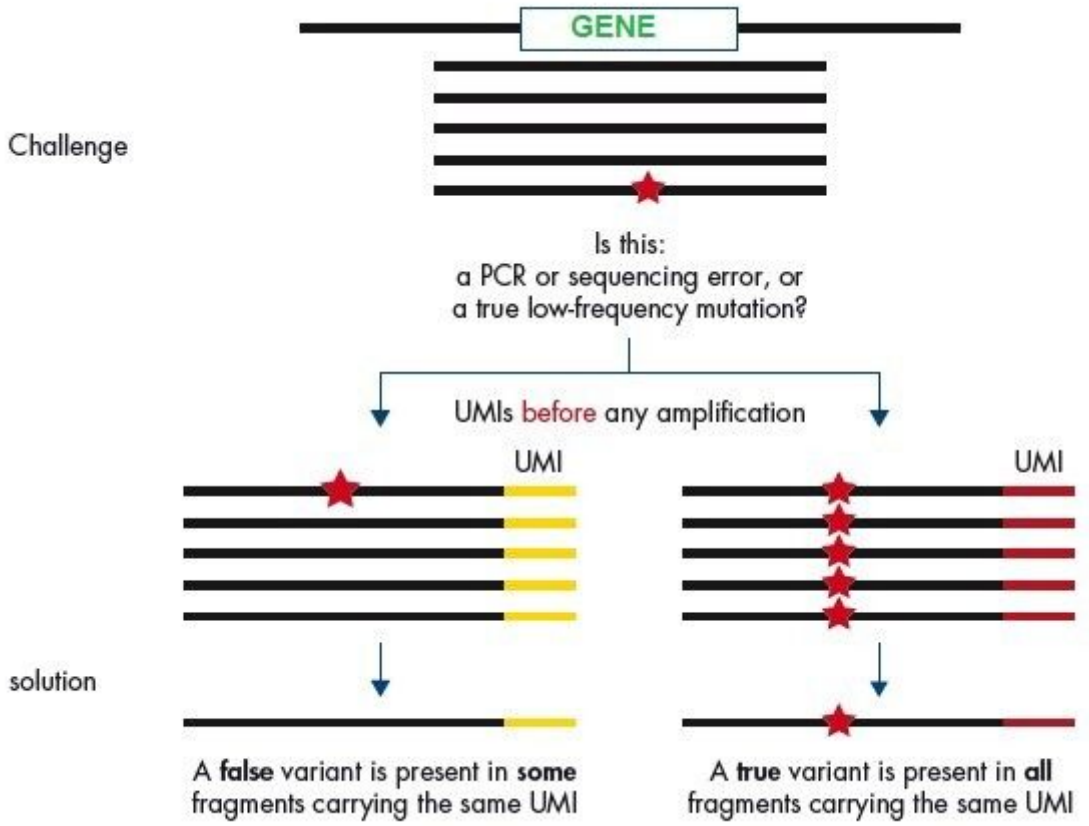

Figure 1

Mechanism of unique molecular indices (UMIs).

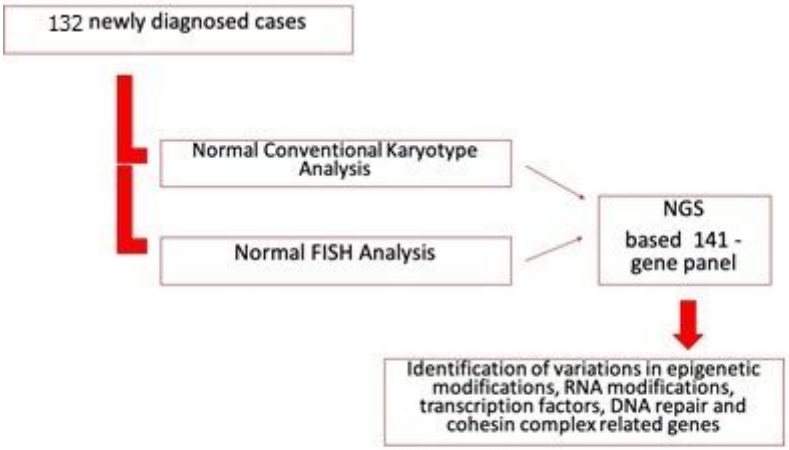

Figure 2

Suggested algorithm for cytogenetically normal cases.