Technological Challenges for Management of Genetic Complexities of Myelodysplastic Syndromes

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Abstract

Background: Chromosomal abnormalities (CA), including del(3q, 5q, 7q, 11q, 12p, 17p, 20q); loss of 5, 7, and Y; trisomy (8,19); i(17q); and balanced and unbalanced translocations have been demonstrated as prognostic markers in 5-tier risk-grouping and WHO-2016 classification of myelodysplastic syndromes (MDS). However, monosomal karyotype (MK) in the presence or absence of a complex karyotype (CK) has not been considered in the WHO classification. Additionally, a plethora of somatic mutations of MDS-specific and elderly populations collected through a-CGH, SNP-array, next-generation, and targeted sequencing has led to understanding of their impact on MDS-phenotype, initiation and progression of the disease, and treatment outcome in single or cooperating effects of comutations of several pathway-mechanisms.

Methods: The present review on technological challenges has been raised on the information available through Google-search using MDS-genetics, mutations of MDS, diagnosis and prognosis of MDS, etc. with a view to understanding the possibilities in low-resource settings.

Results: Mutual exclusivity and cross-talk of such mutations help in self-renewal of leukemic stem cells. However, molecular screening is not only time-consuming but also expensive in poor-economic settings. Nevertheless, the significance of unspecified and uncalled mutations is yet to be understood. In contrast, conventional cytogenetic assays have specific aberrations of prognostic and therapeutic values, which cover the whole genome in a cost-effective manner. However, since somatic mutations of clonal hematopoiesis of indeterminate potential (CHIP) in asymptomatic and/or patients with idiopathic cytopenia of undetermined significance (ICUS) have the potential for favoring the leukemic onset to progression, molecular screening has inherent importance within the disease-mechanism.

Conclusion: The WHO-2016 risk-classification has considered mutations of SF3B1, TP53, and MLL for management of MDS, and also powered conventional cytogenetics for diagnosis and risk-stratification of MDS.

Keywords: Chromosomal rearrangements, Mutational complexities, Myelodysplastic syndrome, Somatic mutations, Technological challenges.

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Introduction

Myelodysplastic syndromes (MDS) are characterized as heterogeneous diseases having premalignant clonal changes of the hematopoietic system. The disease is typically presented with blood cytopenia, ineffective hematopoiesis, and a higher propensity of transformation to acute myeloid leukemia (AML). The conventional cytogenetic study, which is chosen as the first line of diagnosis, has frequently demonstrated chromosomal abnormalities (CA), including deletions (del) of 3q, 5q, 7q, 11q, 12p, and 20q, monosomy 5/7, trisomy 8/19, i(17q), and loss of Y of “very good” to “very poor” prognostic implications in IPSS-R.1-6 Independent impact of monosomal karyotypes (MK) has not gained importance in IPSS-R stratification because the missing chromosome can be rearranged on normal chromosomes and appear as a marker; however, MK alone or in association with structural aberrations result in genomic instability.5,6 Normal karyotypes of over 30% de novo and 50% therapy-related MDS leads to silent progression and transformation of the disease. Therefore, utilization of advanced techniques for understanding the mutational spectrum in hematopoietic stem cells (HSC) and its clonal and sub-clonal developments facilitated risk-estimation of MDS and its evolution.3,7,8

Molecular techniques such as comparative genomic hybridization (CGH), single nucleotide polymorphism (SNP), and sequencing of the whole genome or exome have identified a plethora of non-random ‘founder’ mutations lead to the initiation of the disease mechanism.9,10 Somatic mutations of signal transduction kinases: FLT3-ITD/MPL/GNAS/JAK2/KIT (15%), transcription factors: RUNX1/TP53/ETV6/GATA2 (15%), tumor suppressors: TP53/WT1, epigenetic modifiers: TET2/ASXL1/IDH1/IDH2/EZH2/DNMT3A (45%), RNA splicing: SF3B1/U2AF1/SRSF2/ZRS2 (64%), RAS-pathway: KRAS/NRAS/CBL/NF1/PTPN11 (12%), cohesin complex: STAG2/RAD21/SMC3/SMC1A (13%), and many other mutations such as SETBP1/miRNA/ABCB7 (10%) have led to re-defining MDS-pathogenesis.9,10,13-17 Direct association of MDS-status has been demonstrated with some of the mutations such as mutations of PRPF8 and SF3B1 and ring sideroblasts; reduced TET2 activity and increased DNA-methylation; mutations of DNMT3A and reduced overall survival (OS) with faster AML-transformation; TET2-mutations and response to hypomethylating drugs; del5q and response to lenalidomide; and so on.18-20

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TET2-like epigenetic mutations in asymptomatic older individuals guided to understand that the MDS is an age-related disease, also contributes to clonal hematopoiesis of indeterminate potential (CHIP) for disease-initiation. Mutagenic events of RNA-splicing and epigenetic machineries are frequently reported in MDS. DNMT3A was frequent over ASXL1 and TET2 mutations in apparently healthy elderly population. CHIP and MDS-specific mutations have postulated that a single and dormant mutant clone could progress through a pre-leukemia to an overt leukemia with accumulation and cooperation of driver mutations.

In view of the above facts, it is understood that age-related pre-leukemic MDS is an acquired the disease of HSC, which could be characterized by a conventional cytogenetic study, and a wide range of molecular techniques for recognition of clonal and sub-clonal aberrations of chromosomes and path-way genes, which are important for risk-classification and outcome-analysis of MDS. However, availability and affordability for the screening of mutations of MDS patients of low-resource settings is a matter of concerns. Therefore, the present review has addressed the disease-specific CA, somatic mutations, and cross-talk among them for initiation to transformation of the disease and technologies available for risk-assessment and clinical management of MDS patients.

Mutations of MDS and Complexities

Chromosomal alterations, including loss/gains of chromosomes/segments, balanced/unbalanced translocations (t(1;3)/t(2;11)/t(3;21)/t(6;9)/t(11;16)/etc.), CK with ≥3 aberrations, MK with at least one structural rearrangement along with monosomy of one autosome, etc. have been reported in 7–8% de novo MDS with an unfavorable outcome. MK indicates a poor prognosis and that worsens when combined with CK and advancing age; however, it has lost its distinction as an independent prognostic factor when associated with ≥5 aberrations. Deletions indicate haploinsufficiency wherein del(7q)/−7 is associated with a poor prognosis. Del(17p)/i(17q) results in loss of tumor suppressor TP53 and is associated with an unfavorable clinical outcome, whereas inv(3)[q21−q26] leads to increased blasts and a rapid transformation to AML. There are several abnormalities such as trisomy 8, del(20q), −Y, del(9q), −13/del(13q), del(11q)/t(11q), del(12p)/t(12p), ±19/19(t), idic(Xq13), etc. which are not MDS-specific but generally present in myeloid neoplasia. Del(5q) has been classified as a distinct 5q-syndrome with refractory anemia (RA). RPS14-haploinsufficiency in del5q-patients perturbs P53-signaling and ribosomal biogenesis, block erythroid differentiation and that results in severe macrocytic anemia in MDS. Del(5q) is a good prognosticator; however, in association with TP53 mutations, this disease progresses to AML and develops resistance to the immune-modulating agent lenalidomide.

Besides landscapes of mutations detected in MDS patients, at least one mutation has been reported by sequencing with a median of 10 mutations/patient in >90% patients, which includes non-pathogenic ‘passengers’ as well as non-random ‘founder’ and ‘driver’ mutations, wherein mutations of RNA-splicing and DNA-methylation systems are reported as ‘founders’. Mutations in −40 genes have been demonstrated as ‘drivers’, which are involved in clonal evolution in ~80% of MDS, whereas mutations of chromatin modification and signaling systems occurred later as ‘sub-clonal’. Targeted deep-sequencing has reported mutations in TP53, EZH2, ETV6, RUNX1, and ASXL1, and that conferred a poor prognosis collectively in 439 patients; while mutations in SF3B1, TET2, SRSF2, and ASXL1, and TET2, SF3B1, ASXL1, SRSF2, DNMT3A and RUNX1 were reported in ~10% MDS in each of the two separate studies.

Mutual exclusivity has been reported in a number of mutations such as TET2 and IDH1/2 was mutually exclusive within the same functional pathway; SF3B1 was mutually exclusive when co-occurred with ASXL1 and IDH1; while co-occurrence of splicing-mutations was rare. Interestingly, SF3B1 confers a good prognosis; in contrast, ASXL1 and IDH1 render poor prognosis. Co-operating effect of TET2 and SRSF2 caused monocytosis, resulting in progression to a chronic myelomonocytic leukemia (CMML). Collectively, biological interactions of concomitant mutations and their mutual exclusivity has led to understanding that a higher quantum of mutations contributes to a higher degree of clonal expansion in high-risk subtypes (RAEB1 and RAEB2), and also, shorten the disease-free OS. It is further hypothesized that multiple RNA-splicing mutations drive MDS to AML-transformation through the acquisition of multiple clonal cytogenetic and molecular alterations of chromatin modulation and signaling, and dictates the phenotype of MDS-disease. Occurrence of recurrent CA has been implicated as secondary events and outcome of genomic instability, where CK has demonstrated a very poor outcome. Therefore, the independent expression of single mutation and accumulation and concomitant occurrence with others have negative effects on the clinical significance for risk-classification and targeted therapeutic outcome.

It is noteworthy that healthy individuals, especially the elderly population, harbor some of the mutations and chromosomal aberrations of leukemic reference such as BCL2 and BCR-ABL rearrangements; CNVs at 5q/11q/17p/20q in individuals of >70 years age; altered expression of >40 point-mutations; non-pathogenic ‘passenger’ mutations without clonal expansion; and mutations of DNA-methylation system, particularly DNMT3A and TET2, which are detected in both patients with MDS/AML as well as in the healthy individuals in an average of >2% and ~6% of >70 years age. Intensely treated AML patients had demonstrated the presence of DNMT3A mutation in complete remission state. Somatic mutations of DNMT3A, TET2, and ASXL1 were also reported in diabetes mellitus, coronary heart disease, and ischemic stroke. Furthermore, MDS-specific clonal mutations increased from 9.6% (70–79 years) to 18.4% (≥90 years) in line of the aging process. Thus, the question was raised on the age-related clonal CHIP-mutations in apparently healthy population and risk of malignancy owing to acquisition and cooperation of further mutations, and contribution of co-morbidities leading to disarrayed erythropoiesis. There was evidence of hematologic neoplasia-specific CHIP mutations in the elderly population with an allele frequency of ≥2% and 0.5–1% annual risk of hematologic neoplasia; however, these mutations do not meet the diagnostic criteria of MGUS/PNH/MBL though indicate cytopenia or normal blood count and leads to disease-progression. Spontaneous CHIP-mutations may occur many years before initiation of clonal expansion, remain dormant in the sub-clinical state, and develop frank/overt malignancies in association with other accumulated mutations coupled with CK. Thus, CHIP-mutations have been designated as ‘seeds’ of leukemia, which also facilitates self-renewal of leukemic HSCs and deregulation of cellular differentiation and maturation.

The reported studies have indicated the need for further studies on a different population of the different morbid condition with a view to preventing the onset and malignant transformation of MDS, and also to designing meaningful targeted drugs.
the employment of technologies and the endpoints are matters of serious concerns as it depends on the economic condition of the country and its patients. The low- to middle-income countries are truly not equipped, mainly because of financial constraints, for supporting screening of disease-specific mutations and management of diseases. Otherwise, opportunistic screening of CHIP mutations could have prevented neoplastic development, especially age-related pre-leukemic conditions. Therefore, the present review has discussed technological sensitivities and specificities for risk-classification and therapeutic management of MDS.

**Technologies for Understanding MDS Disease-state**

**Conventional Cytogenetics**

Conventional bone marrow cell culture and chromosomal analysis in a karyotypic form following the traditional ‘G-banding’ is useful for investigation of all chromosomes on one screen. It facilitates recognition of balanced/unbalanced rearrangements, including translocation, inversion, deletion, ring, dicentric and marker chromosomes, and also numerical alterations, including hypodiploid/hyperdiploid conditions and aneuploidies. In addition to disease-specific aberrations, conventional cytogenetics allows detection of additional chromosomal abnormalities, including the expansion of clonal and sub-clonal developments. In MDS, del(3q)/5q/7q/11q/12p/20q), monosomy 5/7, trisomy 8/19, i(17q), −Y, etc. have been frequently reported as single exclusive or complex aberrations (CK), wherein monosomal karyotypes (MK) with loss of whole/partial chromosome occurred owing to complete monosomy and/or deletion of chromosomal segments. Thus, the efficiency of a comprehensive chromosomal investigation by conventional the cytogenetic technique has bagged the certificate as the “gold standard” technique for understanding and management of hematologic malignancies.¹

Therefore, the conventional cytogenetic study is important for every new MDS-diagnosis and also for patients having differential diagnosis with persistent unexplained cytopenias. Such patients may represent the incipient stage of MDS even in the absence of morphologically identifiable dysplasia. Thus, a baseline cytogenetic profile is of immense importance for all suspected MDS. Chromosomal rearrangements are not static in MDS; however, accumulation of abnormalities results in genetic instability, indicating an adverse prognosis and disease progression. Hence, conventional cytogenetic monitoring is recommended in the revised WHO 2016 classification.²

**Fluorescence In Situ Hybridization (FISH)**

The use of sequence-specific probes in FISH facilitates detection of gains/losses and cryptic rearrangements on interphase nuclei. However, several studies have experienced that FISH can detect 70% of the abnormalities detected by conventional cytogenetics.³ Twenty-five percent of MDS samples may represent low-grade mosaicism, compound lesions, etc. Fluorescent in situ hybridization is superior to conventional cytogenetics in the detection of rearrangements in 15–20% more cases compared to conventional cytogenetics. However, low-degree mosaicism, inversions and balanced translocations, and thus, conventional karyotyping tools demonstrated no additional advantages compared to conventional cytogenetics.⁵ Therefore, refinement of CGH technology based on the microarray using bacterial artificial chromosome (BAC), oligonucleotide, and SNPs has significantly improved the resolution (BAC: 75–200 kb, oligonucleotide: 25–85 mers; smaller insert clones, cosmids: 30–40 kb; fosmids: 40–50 kb).⁶ The genomic resolution of different aCGH platforms is defined by spacing and length of the DNA probes. Array-CGH is a combination of conventional CGH and FISH; however, it does not require metaphase chromosomes. Thus, it is a useful tool in cases with a low or poor metaphase yield and also powerful in the normal cytogenetic scenario.

In MDS, aCGH reported 39% more cryptic alterations compared to conventional cytogenetics.⁷ Application of aCGH facilitates a genome-wide simultaneous detection of CNVs at multiple loci and analysis of hundreds to thousands of genes considered on the microarray in one single experiment. Commercially available aCGH platforms reproduce a 50-fold higher resolution with chromosomal rearrangements in 15–20% more cases compared to conventional cytogenetics. However, low-degree mosaicism, inversion, balanced translocations, polyploidy, etc. cannot be detected by this technique.⁸ In general, DNA from patient and control reference DNA are labeled with fluorochrome dyes and co-hybridized to the chip containing arrayed genomic clones or oligonucleotide probes and the arrays are scanned using a fluorescent imaging scanner. The fluorescent signal ratio (excess or gain in red, under-representation of tumor DNA; excess of green, over-representation of tumor DNA) of patient and control DNA indicates CNVs along the genome and the results are analyzed using bio-statistical algorithms.⁹ Targeted aCGH focuses only on specific regions of a genome or specific genes.

**Comparative Genomic Hybridization (CGH) and aCGH**

Conventional CGH uses FISH technique on metaphase chromosomes and detects CNVs across the whole genome. However, it has a low resolution (3–10 MB). Also, CGH cannot detect mosaicism, inversions and balanced translocations, and thus, demonstrated no additional advantages compared to conventional cytogenetics.⁵⁵ Therefore, refinement of CGH technology based on the microarray using bacterial artificial chromosome (BAC), oligonucleotide, and SNPs has significantly improved the resolution (BAC: 75–200 kb, oligonucleotide: 25–85 mers; smaller insert clones, cosmids: 30–40 kb; fosmids: 40–50 kb).⁶ The genomic resolution of different aCGH platforms is defined by spacing and length of the DNA probes. Array-CGH is a combination of conventional CGH and FISH; however, it does not require metaphase chromosomes. Thus, it is a useful tool in cases with a low or poor metaphase yield and also powerful in the normal cytogenetic scenario.

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**Genome-wide BAC-aCGH**

However, Genome-wide BAC-aCGH has detected novel CNVs in 47% of MDS patients, including deletion of RUNX1 (344 kb) gene in three MDS patients at the time of AML transformation, and also EVI1 (3q26.2), APC (5q22), TCERG1 (5q32), EMP1 (12p13.1), KITLG (12q21.3),
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In another study on 183 $10^9$ stem cells from a large group of patients, deregulation of multiple ribosomal genes, and genes involved in the initiation of translation in 5q-syndrome. The discovery of MDS-specific homozygous mutations through SNP-array has tremendously contributed to the development of DNA-methyltransferase inhibitors such as azacytidine and decitabine.62,63 Employment of SNP-array studies of del(5q) and abnormalities of 17(p) dictated the severity of disease phenotype in hematologic malignancies.15,48,49,64

Microarray-based gene expression profiling on bone marrow CD34+ cells of 55 MDS patients revealed deregulation of mitochondrial genes in RARS-MDS, and down-regulation of multiple ribosomal genes, and genes involved in the initiation of translation in 5q-syndrome.65 In another study on 183 patients, deregulation of immunodeficiency, apoptosis, and chemokine signaling pathways were described on CD34+ cells at the early MDS stage, whereas deregulation of DNA-damage response and checkpoint pathways were evidenced in advanced MDS.66 A significant association of gene expression profiles and deregulated pathways were also demonstrated in patients with del(5q), trisomy 8, or -7/del(7q). Clinical accuracy of gene-expression profiling was found to be 93% accurate in sub-typing of leukemias; however, only 50% of MDS was correctly classified owing mainly to marked heterogeneity of MDS.10 A signature-expression of 20 genes on CD34+ cells from a large group of MDS patients help segregated a good from poor prognosis. Investigation of the effect of mutations and gene expression demonstrated the power of transcriptome on prognostic scoring and prediction of treatment outcome.67

The advantages of high-resolution, genome-wide coverage and minimal DNA requirement in SNP-array and yield of quality-result have widened its application. In MDS, SNP-array investigation is expected to detect novel clonal changes with a promising direction towards understanding its pathogenesis, transformation, and response to treatment. However, challenging potential of SNP-array needs validation on a large database of genomic CNVs, association with disease onset and progression, and outcome of treatment. Also, variation in genomic CNVs largely rests on different platforms and the methodology used, though the use of the bone marrow and peripheral blood presented no difference.

**Sequencing**

The incredible advantage of next generation sequencing (NGS) technology for detection of mutations in MDS through global sequencing has implicated several specific and unspecific mutant genes, which will certainly be of clinical significance. NGS is able to detect fusion rearrangements in balanced translocations, deletions, duplications, CNVs and UPD, and also numerical changes of cytogenetic failure.8,10,13,15,21,22,68 Therefore, NGS stands as a judicial combination of conventional cytogenetics, aCGH and SNP-array, and a comprehensive platform for a genome-wide investigation of as precise as the low frequency of mutations.

Analysis of sequencing data with low-frequency or non-coding mutations, and cost and time required for NGS are the limiting factors for a routine clinical workup. Moreover, cytogenetic anomalies detectable by conventional karyotyping could be underestimated by NGS, and thus, NGS cannot replace conventional karyotyping in the clinical setting. Nevertheless, detection of key mutations could be accomplished through targeted sequencing of small groups of genes of diagnostic and therapeutic interest. NGS is an important tool for diagnosis of otherwise diagnostically challenged cases. In MDS, rapid screening of identified genes of prognostic and diagnostic importance in large cohorts could be undertaken for understanding actual implications of mutations on treatment outcome following homogeneous treatment with disease-modifying agents. Sequencing of clinically relevant mutations in a standardized manner within affordable time-frame could guide clinical validation and acceptance of the technique.

Mutation analysis has increasingly become important for MDS-management and thus, molecular assay-platforms are being incorporated into diagnostic algorithms for patients suspected with hematologic neoplasia. Academic hemato-pathology groups, commercial pathology laboratories, and bioinformatics groups are in the process of refining data-filtration for calling disease-causing mutations and drawing an interpretation of findings. Targeted deep-sequencing appears important for extending translational opportunities for a further dissection of clonal myelodysplasia, non-clonal cytopenia, and clonal hematopoiesis arising upon aging or in the context of acquired aplastic anemia.10,66 Therefore, large-scale genetic and molecular profiling of multiple targeted genes by combining molecular karyotyping and sequencing would be invaluable for sub-classification and prognostication of MDS and further guide to design an individualized therapeutic program. Further focus on developing simple methods for detection of mutations would be important, since establishing sequencing facilities and use of the technology for identification of driver mutations at bedside is difficult in the far future, especially for low- and middle-income countries.

**NEW WHO RECOMMENDATION ON TECHNOLOGICAL APPLICATION**

It has been recommended in the WHO 2016 classification that chromosome abnormalities (CA) will continue to be classified as MDS-specific and carry its clinical significance. WHO-2016 has exclusively recommended conventional cytogenetics for detection of CA.32 in the absence of dysplasia and other diagnostic morphology, CAs, including trisomy 8, del(20q), and loss of Y, are not considered MDS-specific. Del(5q) as single or in combination with one additional abnormality of the low-risk group has been re-classified to indicate a similar prognosis.32,68 MK has not been considered in the new risk-classification to avoid disputes on its risk
when present with CK.\textsuperscript{5,8–10} Thus, conventional G-banding study has been powered by WHO-2016 classification amidst technical advancements for CA-based risk-grouping of MDS,\textsuperscript{12,48} though a concomitant use of SNP-array and conventional cytogenetics has a significant outcome of clinical prediction of OS, EFS, and PFS.\textsuperscript{32}

Although landscapes of point mutations have been identified in MDS, mutations of SF3B1, MLL, and TP53 have been recommended by WHO for prognostic stratification of MDS with ring sideroblast, del(5q), and other categories. However, CHIP mutations, though developed bona-fide MDS in some individuals, have not been considered for risk-stratification in the new classification owing mainly to a lack of clear understanding of their expression and interaction with other co-operating mutations. Therefore, molecular screening of somatic mutations, CNVs, UPD, CHIPS, ICUS (idiopathic cytopenia of undetermined significance), etc. have not attracted much interest in the new classification system of MDS. However, further studies might lead to the incorporation of more mutations of clinical significance in MDS-classification and therapeutic development, especially that of epigenetic mechanism.\textsuperscript{10,48,49,51} Clonal cytogenetics with ASXL1, RUNX1, and TP53 mutations did not present features of MDS or specific CA; however, clonal CA might increase the risk of MDS in patients with persistent ICUS.\textsuperscript{26} Thus, mutation analysis would be important to refine the MDS-cases from ICUS for clinical management, though molecular screening of mutations is still far to reach in low-economic settings. Nevertheless, molecular aCGH, SNP array, and sequencing techniques extract genome-wide information on mutations at a better resolution, irrespective of the karyotype status, and most importantly in interphase cells in the absence of, or inadequate chromosome morphology. Thus, dynamic opportunistic screening of CHIP-mutations in ICUS or asymptomatic healthy elderly individuals could prevent hematopoietic malignancies. However, conventional cytogenetics facilitates a genome-wide screening of balanced and complex rearrangements, which are not recognized by molecular techniques.\textsuperscript{32,48} Therefore, it is clear that WHO-2016 has recommended a conventional cytogenetic study mandatory for MDS-diagnosis and risk-grouping.

**Conclusion**

MDS has been characterized by CA and categorized in different risk-groups. The conventional cytogenetic study relies on metaphase chromosomes of a good morphology and covers the whole genome for detection of inter- and intra-chromosomal rearrangements. Advancement of technological innovation and development of bioinformatics tools have facilitated the collection of a wide spectrum of somatic point mutations in MDS and AML patients; however, many of these are uncalled for clinical understanding. However, mutations of SF3B1 of RNA-splicing and epigenetic factors are demonstrated as founders and drivers of MDS-pathogenesis, and also targeted for therapeutic development. Some of the MDS/AML-specific mutations have been detected as CHIP in apparently healthy elderly individuals, which might favor leukemogenesis and disease progression. Screening of such mutational spectrum has an immense value of prediction of disease-development and management; however, clinical or laboratory facilities of low-resource setting have yet to establish such facilities for molecular screening of mutations. On the basis of the knowledge gained on the clinical impact of somatic mutations, CA, and hematopoietic phenotypes, WHO has revised risk-stratification of MDS where conventional cytogenetics has been powered for MDS-management, while mutations of SF3B1, MLL, and TP53 have been recommended for understanding MDS-phenotype and therapeutic outcome. Therefore, conventional cytogenetic characterization of bone marrow cells could enable disease management in the absence of information on point-mutations.

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