Assessing Copy Number Aberrations and Copy Neutral Loss of Heterozygosity Across the Genome as Best Practice: An Evidence Based Review of Clinical Utility from the Cancer Genomics Consortium (CGC) Working Group for Myelodysplastic Syndrome, Myelodysplastic/Myeloproliferative and Myeloproliferative Neoplasms

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Highlights

- Assessment of clinically significant copy number alterations (CNAs) and copy-neutral loss-of-heterozygosity (CN-LOH) in myeloid malignancies by chromosomal microarray (CMA) can improve diagnostic yield, refine risk-stratification and provide genomic information to guide therapy. The Cancer Genomics Consortium (CGC) Working Group for Myeloid Neoplasms performed an extensive systematic examination of the peer-reviewed literature to evaluate the clinical value of CMA testing in the workup of myelodysplastic syndrome (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and myeloproliferative neoplasms (MPN). Based on the evidence, this review describes the specific clinical scenarios where CMA can complement the information obtained by current standard-of-care testing modalities. An example of a testing algorithm illustrating how CMA can be incorporated in selected settings within the backbone of the current testing guidelines is provided. In addition, the current review provides an exhaustive list of recurrent CNAs and CN-LOH observed in these myeloid neoplasms and their clinical significance.

List of Acronyms

aCGH, array-based comparative genomic hybridization

CN-LOH, copy neutral loss of heterozygosity

MDS, myelodysplastic syndrome

MPN, myeloproliferative neoplasm

CMA, Chromosomal Microarray

CGAT, chromosome genomic array testing

RARS-T (Refractory anemia with ring sideroblasts and thrombocytosis

SNP-A, single-nucleotide polymorphism array

NCCN, National Comprehensive Cancer Network

WHO, World Health Organization

NGS, Next Generation Sequencing

FISH, Fluorescence In Situ Hybridization

Abstract

Multiple studies have demonstrated the utility of chromosomal microarray (CMA) testing to identify clinically significant copy number alterations (CNAs) and copy-neutral loss-of-heterozygosity (CN-LOH) in myeloid malignancies. However, guidelines for integrating CMA as a standard practice for diagnostic evaluation, assessment of prognosis and predicting treatment response are still lacking. CMA has not been recommended for clinical work-up of myeloid malignancies by the WHO 2016 or the NCCN 2017 guidelines but is a suggested test by the European LeukaemiaNet 2013 for the diagnosis of primary myelodysplastic syndrome (MDS). The Cancer Genomics Consortium (CGC) Working Group for Myeloid Neoplasms systematically reviewed peer-reviewed literature to determine the power of CMA in 1) improving diagnostic yield, 2) refining risk stratification, and 3) providing additional genomic information to guide therapy. In this manuscript, we summarize the evidence base for the clinical utility of array testing in the workup of MDS, myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and myeloproliferative neoplasms (MPN). This review provides a list of recurrent CNAs and CN-LOH noted in this disease spectrum and describes the clinical significance of the aberrations and how they complement gene mutation findings by sequencing. Furthermore, for new or suspected diagnosis of MDS or MPN, we present suggestions for integrating genomic testing methods (CMA and mutation testing by next generation sequencing) into the current standard-of-care clinical laboratory testing (karyotype, FISH, morphology, and flow).

Keywords: Copy Number Aberrations, Copy Neutral Loss of Heterozygosity, Microarray, Myelodysplastic Syndrome, Myeloproliferative Neoplasm, Next-generation Sequencing

Introduction

The integration of genetic data into the clinical and pathological assessment of myeloid neoplasms underscores the expanding role of genomic changes in the diagnosis, prognosis, classification and therapeutic implications of precision medicine. Myeloid neoplasms include myelodysplastic syndrome (MDS), myelodysplastic/ myeloproliferative neoplasm (MDS/MPN), myeloproliferative neoplasm (MPN) and acute myeloid leukemia (AML). The myelodysplastic syndromes (MDS) comprise a very heterogeneous group of clonal myeloid disorders characterized by peripheral blood cytopenias, a bone marrow aspirate/biopsy showing dysplasia in one or more hematopoietic lineages and/or the presence of characteristic chromosome abnormalities [1, 2]. In addition, karyotype is a critical component of the International Prognostic Scoring System (IPSS), the gold standard used to predict overall survival and risk of AML transformation in primary MDS patients [3]. The recently revised IPSS or IPSS-R refined the cytogenetics categories listed in the original IPSS and provided "greater weight" to the cytogenetic categories, underscoring the importance of genetic-based testing in the myeloid malignancies [4]. However, karyotype analysis only detects chromosome abnormalities in ~ 50% of primary MDS patients. Thus, to further improve the genetic diagnostic and prognostic precision in MDS and identify therapeutic targets, molecular genetic assays such as CMAs and NGS are needed. MPNs are clonal hematopoietic disorders characterized by proliferation of one or more of the myeloid lineages, while MDS/MPNs have features of both MDS and MPN at the time of initial presentation [1]. Per current NCCN guidelines for MPN, the diagnosis of MPN is based on the 2016 WHO criteria and requires a combination of clinical, laboratory, cytogenetics, and molecular testing [1, 5, 6]. For chronic myeloid leukemia (CML), defined by the presence of *BCR/ABL1* rearrangement, RT-PCR or FISH with or without conventional karyotype are recommended for diagnosis. For *BCR/ABL1*-negative MPNs, in the absence of mutations of *JAK2*, *MPL* and *CALR*, chromosomal abnormalities can represent markers of clonality. Similarly, diagnosis of certain subcategories of MDS/MPN such as chronic myelomonocytic leukemia is facilitated by detection of chromosomal abnormalities, especially in the absence of diagnostic morphologic features [1].

Numerous studies described below have demonstrated the utility of chromosomal microarray (CMA) testing to identify copy number alterations (CNAs) and copy neutral loss of heterozygosity (CN-LOH) in myeloid malignancies for diagnostic evaluation and assessment of prognosis; certain CN-LOH have significant therapeutic implications due to underlying mutations that could be potential therapeutic targets or predict treatment response. However, CMA, also known as array comparative genome hybridization (aCGH), single nucleotide polymorphism array (SNP-A), chromosome genomic array testing (CGAT), DNA microarray testing, genomic array or simply referred to as array, is still not a standard of practice across all cancer care institutions. Assessment of genomic aberrations by CMA testing has not been addressed by the WHO 2016 or the NCCN guidelines for clinical work-up of hematological malignancies [1, 7, 8]; European LeukaemiaNet 2013 has suggested the use of CMA testing for the diagnosis of primary MDS [9]. However, at this time, there are no specific guidelines available for clinical utilization (*i.e.*, when and how to perform CMA analysis).

To evaluate the clinical utility of CMA in hematological malignancies, the Cancer Genomics Consortium (CGC) Working Group for Myeloid Neoplasms was formed comprising cytogenetics, molecular genetics, and pathology experts under the auspices of the CGC. An extensive systematic examination of the peer-reviewed literature was performed to evaluate the clinical value of CMA and to identify the recurrent CNAs and CN-LOH in various myeloid malignancies. According to the 2016 WHO classification, diseases reviewed in this manuscript include MDS, MDS/MPN, and MPN including CML. For each recurrent CNA (gain or loss) or CN-LOH, the clinical significance of the affected gene(s) in various myeloid disorders and their corresponding impact on clinical management were assessed.

Here, we present the evidence base for the clinical utility of array testing in myeloid neoplasms (MDS, MDS/MPN and MPN), and provide suggestions for clinical utilization and methodology considerations.

Materials and Methods

Literature search and review

A literature search was performed for articles on PubMed using a combination of the following terminologies: "MDS; MPN; MDS/MPN; chronic myelomonocytic leukemia (CMML) and myeloid neoplasms" with "microarray; SNP array; array CGH; loss of heterozygosity/LOH; uniparental disomy/UPD; copy number. A total of 66 peer-reviewed articles were reviewed in-depth up to 2017. These studies utilized one of the three common microarray platforms, namely, Agilent copy number (CN) or CN+SNP arrays, Affymetrix CN+SNP arrays, or Illumina-SNP arrays. The following data from each of the articles were collected: type of study, array platform, total number of cases in the study, disease type and WHO sub-classification whenever available; time point of testing during the disease course, criteria for making the calls (gains/ losses/ CN-LOH), diagnostic yield, recurrent CNA and CN-LOH findings and their clinical significance (diagnostic/ prognostic/ therapeutic targets) and their role in clonal evolution and disease transformation from MDS or MPN to AML. The primary literature was also evaluated to identify the spectrum of recurrently affected genomic regions and genes, regardless of known clinical significance, in MDS, MDS/MPN or MPN as ascertained through chromosomal microarray analysis. Review articles and articles related to primary or secondary AML cases were excluded.

Recurrent CNA and CN-LOH detected across the myeloid neoplasms were retrieved. Clinical significance was based on the utility for a) diagnosis; b) prognostication; c) predictive marker for therapeutics (targeted agents or precision medicine); and/or d) correlation of other clinical-pathological findings of interest, e.g., morphologic subtypes, flow cytometry immunophenotype, association with somatic mutations, microRNAs, etc. Because variable criteria for aberrant CNA and CN-LOH calls were used in the literature, we applied the following consistent inclusion criteria for the purpose of this review to obtain comparable data across all articles: included CNAs generally ≥100 Kb in size and CN-LOH regions of ≥ 10 Mb and telomeric for CN-LOH regions that occurred in ≥ 2 patients in a single study unless of known clinical significance or proven somatic by paired germline tissue array analysis. For each recurrent CNA and CN-LOH, gene content (if known), disease type and clinical significance were recorded.

The level of evidence for clinical significance of CNAs was assigned as follows: Level 1, well established: present in current WHO classification (adapted from IPSS [3]/IPSS-R [4]for MDS) [1, 2, 4] and/or professional practice guidelines (NCCN for MDS [7] and MPN [5] and International MDS/MPN Working Group's recommendations for MDS/MPN [10]); Level 2, emerging: defined here as recurrent (≥5 cases) in well-powered studies with expert consensus; and Level 3, other recurrent abnormalities present in either ≥5 cases that do not meet levels 1 or 2 or in ≥2 cases of deletion that overlaps a myeloid-associated gene with previously described loss-of-function mutations. The level of evidence for clinical significance of CN-LOH was assigned as follows: Level 1, well established, present in current WHO classification and/or professional practice guidelines; Level 2, emerging, defined here as present in ≥2 cases, including a known myeloid gene from NCCN guidelines (22 genes), ≥10 MB, and at least one study proved the affected region is not germline; and Level 3, other recurrent abnormalities present in ≥2 cases that do not meet levels 1 or 2 and includes at least one known myeloid gene from a non-NCCN guidelines source (99 genes) and ≥10 MB [11].

Results

The results of this analysis are organized into different sub-headings for the sake of clarity:

I. Evidence of improved diagnostic yield by CMA in myeloid neoplasms
   a. Detection of CNAs
   b. Detection of CN-LOH
   c. Utility in cases with non-informative karyotype

II. Summary of the disease-based prognostic and therapeutic implications of CMA findings:
   a. Myeloid disorders classified per 2016 WHO classification: MDS, MDS/MPN and MPN(CML and BCR/ABL1 negative MPNs)
   b. Myeloid disorders with specific genetic abnormalities: del(5q), *TET2* alterations, *TP53* mutations, Trisomy 8 and del(20q)
   c. Bone marrow failure syndrome (BMFS)
   d. Precursor myeloid entities: idiopathic cytopenias of undetermined significance (ICUS), idiopathic dysplasia of undetermined significance (IDUS), clonal

cytopenias of undetermined significance (CCUS) and clonal hematopoiesis of indeterminate potential (CHIP)

III. Important Pre-analytical and Post-analytical considerations for CMA and limitations of CMA testing
   a. Peripheral Blood vs. Bone Marrow
   b. Formalin fixed paraffin embedded (FFPE) material

IV. Limitations of CMA

I. CMA facilitates improved diagnostic yield in myeloid neoplasms

a. Detection of CNAs

The overall detection rate by CMA in all myeloid neoplasms ranged between 19 and 83%. In patients with normal karyotype, the detection rate ranged between 33% and 62% [12]. In patients with both normal karyotype and normal FISH, CMA detection rate was 25% whereas in patients with normal FISH, karyotype and NGS studies, the detection rate was 10% (6 of 59) of patients in a large study [13]. The higher detection rate of CMA is due to its ability to detect sub-microscopic CNAs beyond the resolution of karyotype and FISH. In addition, CNAs detected by CMA are potentially targetable by on-label and off-label FDA approved therapies in 46% of patients with myeloid malignancies [13]. The results are summarized in **Table 1**. Recurrent CNAs include gain of chromosomes 1p, 8, 9p, 13 and deletions of 4q, 5q, 7q, 11q, 12p, 17p, 20q, 21q, among others (see **Table 2-4** for the complete list).

Overall, CMA identified 54% cryptic/submicroscopic CNAs in myeloid malignancies with normal/ non-informative karyotype [14, 15]. Of those with normal karyotype (study sample size ranged between 33 patients to over 200), detection rate was 15%-40.1% [16-21]. The median sizes of CNAs were 0.3 Mb and 0.625 Mb for deletions and duplications, respectively [17]. Of particular importance, *TET2* deletion, noted in 5.6% of myeloid malignancies, is cytogenetically cryptic in 50% of cases. CMA is helpful to identify *TET2* deletions since FISH is not routinely performed in clinical labs [22, 23]. The concordance between FISH and CMA for *TET2* deletions was 100% [24].

Focusing only on MDS, we selected studies with unbiased patient cohorts of at least 30 WHO-defined MDS patients regardless of karyotype and IPSS/IPSS-R risk scores to enable a more accurate estimation of CMA abnormality rate. The review revealed 1) an overall detection rate ranging between 28% and 83% [12, 14, 16, 21, 25-31]; 2) detection rate ranging between 10%-80% in patients with normal karyotype [12, 14, 16, 18, 19, 21, 25, 26, 29-32]; 3) additional aberrations identified in MDS patients with del(5q) or del(7q) [33, 34]; and 4) a detection rate of up to 50% in MDS cases with unsuccessful cytogenetics [31, 35].

b. Detection of CN-LOH

One of the most important advantages of CMA is the identification of CN-LOH that cannot be detected using any other standard laboratory techniques. CN-LOH is a frequent chromosomal lesion in MDS, CMML, and MDS/MPN [36] and could involve almost any chromosome (**Table 2**). The overall frequency of CN-LOH in myeloid neoplasms ranged between 6% and 41% (**Table 1**) although the frequency in MDS was much lower than in MDS/MPN [31, 36]. Akagi et al reported that 32% of AML/MDS patients with normal karyotype had CN-LOH with a median size of 30.91 Mb [17]; Heinrichs et al reported CN-LOH in 15% of MDS patients, with all CN-LOH validated as somatic by comparison to buccal cells. The latter study concluded that the presence of acquired CN-LOH helped in making the diagnosis of MDS based on identification of a clonal genetic abnormality [16]. The presence of 4q24 CN-LOH correlated with myeloproliferative features and was mostly noted in MDS/MPN whereas 4q24 microdeletions were more common in MDS (enriched in MDS with ring sideroblasts and multilineage dysplasia sub-category) and secondary AML (sAML) [23, 37]. CN-LOH of 17p was noted in 18% of 72 newly diagnosed MDS patients with complex chromosomal alterations, all of which had a *TP53* mutation [38].

Identification of CN-LOH is a marker of clonality and pinpoints a possible underlying homozygous gene mutation; for example, CN-LOH of 1p, 11q, 9p, 13q and 17p are associated with mutations in *KIT/NRAS, CBL, JAK2, FLT3* and *TP53* genes, respectively; homozygous mutations in the latter four genes have been associated with disease progression [23, 36, 39-41]. The pathogenic significance can be inferred by the identification of characteristic clinicopathological findings associated with specific CN-LOH: advanced MDS/AML in the

presence of 17p CN-LOH; mixed MDS/MPN, monocytosis and a high propensity for AML transformation in the presence of 11q CN-LOH [36]. Furthermore, homozygous mutations due to CN-LOH, such as *JAK2* mutations with 9p CN-LOH, *FLT3* ITD mutations due to 13q CN-LOH, *TP53* mutations due to 17p CN-LOH, and *CBL* mutations due to 11q CN-LOH have been associated with disease progression [36, 39-41]. Aside from the mutations, CN-LOH by itself can confer poor prognosis, as shown by poor outcome in MDS patients with CN-LOH of 7q, similar to MDS with del(7q) [12, 16, 38, 41].

c. Utility in cases with non-informative karyotype (failed or less than 20 metaphases)

Gondek et al. reported CNAs in up to 44% of myeloid neoplasms with non-informative karyotype [12]. Arenillas et al. identified abnormalities in 50% of patients including CN-LOH of 3q in addition to common abnormalities of 5q, 7, and 8 [35]. The authors identified significant differences in overall survival (OS) between IPSS and IPSS-R cytogenetic risk groups that were calculated based on the CNA data obtained from SNP arrays [35]. CMA can also help refine the nature of ambiguous cytogenetic findings,, such as additional material (add), marker and ring chromosomes (mar, ring), double minutes (dmin) which can represent amplification (e.g. *MLL/KMT2A*, *MYC*) and delineate the breakpoints in chromosomal rearrangements [42].

Summary: Taken together, these data emphasize the considerable diagnostic yield of CMA in detecting submicroscopic CNAs and CN-LOH in myeloid neoplasms. Specifically, because CMA recapitulates most of the findings of karyotype studies in normal and non-informative (failed or limited growth) cases, it adds diagnostic value. In addition, CN-LOH pinpoints regions harboring possible homozygous mutations.

II. Summary of the disease-based prognostic and therapeutic implications of CMA findings

Because of the heterogeneous nature of the disease subtypes included in this review, we detail the relevant prognostic and therapeutic implications of CMA findings within various disease entities based on WHO classification, including MDS, MDS/MPN (CMML and MDS/MPN-U), CML, and *BCR/ABL1*-negative (Ph-negative) MPNs, as shown below. Bone marrow failure

syndrome (BMFS) and precursor MDS are also discussed separately. **Table 1** highlights the literature review results, and **Table 2-4** lists all recurrent CNAs or CN-LOH reported to date. In both tables, we classify the types of recurrent CMA findings based on the level of evidence as defined in Methods.

a. Summary of the disease-based prognostic and therapeutic implications of CMA findings in myeloid disorders classified per 2016 WHO classification

Myelodysplastic syndrome (MDS)

Multiple studies have shown that detection of additional aberrations by CMA in patients diagnosed with MDS has prognostic value [14, 26, 42, 43]. Tiu et al. showed that the outcome of patients with chromosomal defects detected by either karyotype or array was worse than that of patients in whom no lesions were detected for OS (16 vs 43 months; $P \leq 0.0001$), event-free survival (EFS) (12 vs 20 months; $P = 0.0006$), and progression-free survival (PFS) (11 vs 17 months; $P = 0.002$) [14]. Regardless of prior karyotype, survival of patients with new defects uncovered by array testing was significantly inferior compared to patients with a negative result [14]. Multivariable analysis showed that the presence of new array-detected lesions and an increased number of such lesions (2 vs 1 or none) were independent predictors of inferior OS and EFS in patients with MDS and related myeloid malignancies [14]. Due to higher yield of chromosomal abnormalities, Tiu and colleagues suggested that CMA testing facilitates better prognostic stratification of MDS using the IPSS scoring system leading to significant impact on treatment selection. Within MDS patients with IPSS intermediate-1 risk group, the survival curves for patients with and without additional abnormalities by CMA diverged (median survival 28 versus 9 months, $P=0.03$) [12]. Within the low-risk IPSS groups, patients with additional CMA–detected defects had worse OS although EFS or PFS did not differ; this finding did not extend to the high-risk group [14]. Further, total genomic aberrations (TGA) measured by CMA can further stratify MDS patients with both low and high IPSS/IPSS-R scores [26, 44].

The prognostic impact of CNAs in MDS with normal karyotype was also confirmed by Thiel et al. among 107 patients from the German (Duesseldorf) registry [18]. A total of 43 (40.1%) MDS cases revealed both common recurrent (4q, 5q, 7q, 21q) and other individual CNAs. The median

survival among the patient group without CNAs was 56 months in comparison to 20 months in the group with CNAs (P=0.002) [18]. A few other papers that focused on MDS with normal karyotype did not show significant prognostic impact by multivariable analysis, presumably due to small sample size [19, 20]. Nevertheless, 20% of low-risk (good or very good) MDS cases had a major cryptic CNA [21]; therefore the risk category was modified for more accurate stratification of these patients.

A common concern is whether CMA-detected abnormalities convey the same prognostic effect of well-defined karyotypic abnormalities. Gondek et al. compared the survival outcome among patient groups with 1) normal CMA testing results, 2) previously known deletion 7/7q by karyotype, and 3) those with normal karyotype but new cryptic lesions of chromosome 7 detected by CMA (including 7q deletions and CN-LOH). The patients with new cryptic lesions by CMA showed similar outcomes as the patients with previously known deletion 7/7q; as expected, their outcome was significantly worse than patients with normal karyotype by karyotype and CMA (median survival 6 vs 8 vs 39 months, respectively, P=0.002) [12].

In terms of predictive markers in MDS, the best known is del(5q). Patients with this abnormality respond well to lenalidomide. As an example, CMA helped to identify cryptic del(5)(q31.3q33.2) (12 Mb) in a patient [19] whose WHO diagnostic classification was subsequently changed from MDS-RA to 5q- syndrome (included in the commonly deleted region); neither karyotype analysis nor MDS FISH probes using the most common 5q- probe targeting *EGR1* at 5q31 could identify del(5)(q31.3q33.2). However, a FISH probe targeting the more distal region of 5q33 was able to confirm the CMA finding [19].

Low-risk vs high-risk MDS (based on IPSS or IPSS-R)

Identification of cryptic aberrations using CMA analysis can facilitate prognostic stratification in lower-risk IPSS patients [45]. 20% of MDS patients with low-risk (good or very good) had a major cryptic CNA [21]. Within low-risk MDS (IPSS $\leq$ 1), Starczynowski et al. showed that the presence of aberrations of more than 3Mb was associated with a lower OS and more frequent transformation to AML [26]. In a large series of 119 low-risk MDS patients, there was a correlation between a higher IPSS score and presence of CNAs. Specifically, deletions were

associated with higher IPSS scores compared with amplifications (p=0.007) [45]. Although univariate analysis showed that deletions and IPSS scores correlated with OS, only IPSS scores retained prognostic significance by multivariate analysis [45]. In low-risk MDS patients with normal karyotype, a significantly shorter OS was observed for patients with additional aberrations compared to patients without additional aberrations (p=0.017) [18]. Similar findings were observed independently in another study where MDS patients with low-risk IPSS with additional CMA abnormalities had worse OS (but not EFS or PFS) [14].

In addition, CMA improved patient stratification even in high-risk MDS patients. The detection rate of CMA abnormalities was much higher (up to 80% for new aberrations not identified by karyotype) in MDS patients with abnormal karyotype [46, 47]. In a study on high-risk MDS patients treated with azacitidine, identification of CMA abnormalities greater than 100 Mb correlated with worse OS [44]. Within high-risk MDS/AML patients with del(5q) or highly complex karyotypes, the amount of genetic rearrangements and fragmentation status had an effect on outcome and response to treatment [48]. Specifically, total genomic aberration size (<200 Mb) was predictive of improved OS. Within these patients, *TP53* mutation was associated with therapy refractoriness only if accompanied by heavily rearranged chromosomes [48]. In newly diagnosed MDS patients with complex chromosomal aberrations, CN-LOH of 17p (~18% of patients) correlated with aggressive clinical course [38]. Thus, CMA analysis has a significant prognostication value in both low-risk and high-risk MDS.

Summary: CMA adds prognostic value in MDS patients with normal karyotype and in MDS patients with low or intermediate IPSS-R risk, especially when on the interface of an IPSS-R range, by providing genomic-based evidence (CNAs or CN-LOH) to either upgrade or downgrade risk to optimize patient management.

Myelodysplastic/ Myeloproliferative neoplasms (MDS/MPN)

Within MDS/MPN, chromosomal aberrations were detected in 75% of patients by CMA as opposed to 37% by conventional cytogenetic studies [12]. Recurrent CNAs included gains of chromosomes 8 and 21q and losses of 4q, 5q, 7q, 12p, 13q, 17p, and 20q (**Table 2**). The overall survival of patients with MDS/MPN and sAML with additional lesions by arrays was lower than

patients with normal karyotype and array results [12]. When patients with MDS/MPD-U who progressed to AML were compared to those with a stable course of the disease, CMA showed, as expected, a greater number of lesions detected in the first group; however, no survival difference was noted between patients with or without previously cryptic defects, likely due to the small sample size [43].

In addition to cryptic CNAs, CN-LOH is frequently observed in MDS/MPN and often as a solitary lesion and may represent clonality [37] (see**Table 3** for details). CN-LOH was more frequent in patients without a *JAK2* mutation (frequently involved chromosome 11) compared to MDS/MPN patients with a *JAK2* mutation (frequently involved chromosome 9) [43]. Dunbar et al. reported frequent CN-LOH in both CMML (48%) and MDS/MPN-unclassifiable (38%) and also in secondary AML arising from MDS/MPN [36]. The authors discovered novel mutations in the *CBL* gene at 11q23.3 in 58% of patients [36] thereby establishing CN-LOH of chromosome 11q as an important clue to homozygous *CBL* mutation [36]. Similarly, Jankowska et al. found that CN-LOH of chromosome 4q was also frequent in MDS/MPN and in secondary AML arising from MDS/MPN including CMML; however, it was absent in RARS-T (Refractory anemia with ring sideroblasts and thrombocytosis) or atypical CML. In contrast, microdeletions of 4q24 were noted in MDS [23]. CN-LOH of 4q was associated with *TET2* mutations in all cases, but *TET2* mutations were less frequent in cases with microdeletions. Morphologically, myeloproliferative features were apparent in cases with CN-LOH of 4q and not in deletion of 4q (*TET2*), suggesting that either CN-LOH of 4q or *TET2* mutation conferred these features [23].

Summary: MDS/MPN patients could benefit from CMA because of high CN-LOH frequency in this disease group, which cannot be otherwise detected, and the additional CMA lesions have significant survival impact and are associated with disease progression.

Myeloproliferative Neoplasms (MPN)

Chronic myelogenous leukemia (CML)

CNAs are not infrequent in CML even in chronic phase. Four studies of unique CML patients have been reported with CMA analysis with a total of 259 patients, 214 of which also had a

karyotype [15, 49-52]. Overall, CMA identified 121 CNAs in 84 patients and the one study that assessed LOH identified 65 LOH regions (>3Mb) in 19 patients [49].

In one large study, CMA detected abnormalities in 21% with the size ranging between 0.1 and 52 Mb [49]. Submicroscopic deletions at 9q34 and 22q11.2 were seen in 12%, with half occurring right at the *BCR* or *ABL1* breakpoint. 1p CN-LOH and 9p CN-LOH (*JAK2* mutation positive) were seen in one patient each, but are known to be recurrent [49]. Another study also showed a detection rate of 24% in chronic phase CML patients; recurrent losses of 9q34 and 22q11.2 were noted at t(9;22) breakpoints [15].

Nowak et al. explored the genomic alterations in tyrosine kinase inhibitor (TKI) resistant CML patients. In addition to t(9;22), 26 of 45 (57.8%) patients had an abnormal CMA result. On average, there were 1.68 CNAs per TKI-resistant patient. These included a total of 36 deletions, 29 duplications, and 9 types of CN-LOH. Recurrent lesions in this cohort included 1p and 19q. The common secondary findings at time of TKI resistance were extra *BCR/ABL1*, trisomy 8 and deletion of *TP53* [50].

Boultwood et al. explored a gamut of chromosomal alterations during disease progression in 41 CML patients using array testing. Twelve of the 41 patients in this cohort had paired samples in chronic and blast phases. Overall, 75.6% patients showed abnormalities by array, including unique findings of 41 CN-LOH and 9 CN gain in 27 patients with available karyotype for comparison. However, most CN-LOH were not convincing because a low-resolution 50K array was used and the cut-off was set below 5Mb, unless lesions were noted only during the blast phase of the paired-sample analysis. Recurrent deletions >1 Mb involved chromosomes 12p and 17p (*TP53*). Mutation in *ASXL1* exon 12 was detected in 15% patients in both chronic and blast crisis phase. Of note, all patients in this cohort were of pre-imatinib era [51].

Summary: Although CMA could identify many additional clonal findings in CML patients, especially those at the time of TKI resistance and disease progression, no clear prognostic and predictive value of CMA findings has been established to date.

BCR/ABL1-negative MPN

CMA is able to detect all clonal abnormalities seen in *BCR/ABL1*-negative MPN by karyotype, such as +1q (14%), gain or loss of 6p (7%), +8, and deletions of 12p, 13q and 20q in primary myelofibrosis [53, 54]. In addition, frequent additional alterations uniquely detected by CMA included 6p CN-LOH (12.5%), 9p gain/CN-LOH (18.8%), and 22q deletion (12.5%) [54]. In MPN and MDS/MPN, 9p CN-LOH was the most common, accounting for 41% overall and 100% in polycythemia vera (PV) [55]. *BCR/ABL1*-negative MPN with homozygous *JAK2* mutations had frequent 9p CN-LOH while those with heterozygous *JAK2* mutations had no detectable 9p CN-LOH [43]. Recurrent CN-LOH of 1p associated with *MPL* mutations in essential thrombocythemia (ET) and 11q CN-LOH associated with *CBL* mutations have been reported in myelofibrosis.

The main concern for MPN patients is disease progression, either to myelofibrosis or to acute leukemia. Several studies compared the genomic profiles of stable disease vs. progression among MPN patients. In MPNs, the average number of aberrations increased over the course of disease progression (3 vs. 0.6 in patients with and without progression, respectively). When excluding 9p CN-LOH, the incidence of genomic changes (both CNA and CN-LOH) was significantly higher in patients with disease progression than in patients without disease progression (63% and 0%, respectively, $p=0.01$) [55]. Similarly, Thoennissen et al. reported up to 3-fold more genomic changes in MPN at the time of leukemic progression compared to chronic phase ($p<0.001$) [56]. Rumi et al. also demonstrated that disease progression of PV or ET to either secondary myelofibrosis or AML was associated with a significant increase in the number of chromosomal aberrations, and no change in the mutant allele burden of *JAK2* mutation [39]. This was also true in patient without CN-LOH of 9p [39]. In a series of 408 samples, Klampfl et al. reported that changes involving 1q and 9p were strongly associated with secondary myelofibrosis or progression to accelerated phase whereas, changes involving chromosomes 1q, 3q, 5q, 6p, 7p, 7q, 19q, and 22q were associated with post-MPN AML when compared to chronic phase MPN [57]. Thoennissen et al. reported trisomy 8 or 8q24 amplification was almost exclusively detected in *JAK2*V617F negative patients with MPN blast phase [56]. Also, CN-LOH of either 7q or 9p including homozygous *JAK2*V617F was related to decreased survival after leukemic transformation ($P=0.01$ and $P=0.016$, respectively) [56].

Among patients with progression, 80% showed a CMA abnormality at baseline. All patients with 9p CN-LOH as a sole abnormality did not progress, suggesting this was a favorable marker [55] even though a higher *JAK2* mutant burden (>50%) in PV has been reported to associate with a higher risk of developing myelofibrosis [58]. Nevertheless, AML transformation arose in either the clone with 9p CN-LOH and homozygous *JAK2* mutation or a new *JAK2*-negative clone with normal chromosome 9 [43]. In PV patients, 9p aberrations (either as CN-LOH and/or gain) were associated with progression to post-PV MF, and this may result in a higher *JAK2* mutant allele burden [39]. More importantly, there was a significant association between the acquisition of aberrations of chromosome 5, 7, or 17p and progression to blast phase [39]. The presence of one or more of these aberrations was independently associated with reduced overall survival from the time of diagnosis of MPN (HR 18, 95% CI 1.9–164, $P = 0.011$) and progression to AML (OR 5.9, 95% CI 1.2–27.7, $P = 0.006$) [39].

Puda et al. compared the CNAs between secondary AML or blast transformation of MPN and chronic phase of MPN or MDS. Within secondary AML or blast transformation of MPN, the detection rate was 83.1%; recurrent CN-LOH, according to descending frequency, included 9p, 11q, 17p, 1p or 22q, 4q or 19q, and 6p. Deletions of polycomb repressive complex 2 (PRC2) members were significantly enriched in secondary AML compared with chronic phase MPN or MDS: *JARID2* on 6p, *AEBP2* on 12p, *SUZ12* on 17q, and *EZH2* on 7q; in contrast, *PRC2* sequence mutations were rare, thereby suggesting that deletions were the main type of defect of *PRC2* loci in myeloid malignancies [59].

Summary: CMA testing in the workup of *BCR/ABL1*-negative MPN has clinical value. The detection of increasing number of genomic lesions was associated with disease progression, and CN-LOH was common. Specific changes were associated with the type of progression (1q/9p with myelofibrosis and 3q, 5q, 6p, 7p, 7q, 19q, and 22q with post-MPN AML), and acquisition of certain abnormalities (5, 7, or 17p) was independently associated with survival. It is helpful that 80% of patients with progression showed CMA abnormalities at baseline. Therefore, CMA can be helpful to identify patients who are more likely to progress.

b. Summary of the disease-based prognostic and therapeutic implications of CMA findings in myeloid disorders with specific genetic abnormalities

Del(5q)

Monosomy 5 or deletion 5q abnormalities are frequent in myeloid malignancies. Chromosome 5 abnormalities were identified in approximately 440 cases in this review series by karyotype /FISH (n=390) or CMA (n=440) with a concordance of ~90% between these techniques [12, 14, 15, 19, 26, 28, 29, 33-35, 39, 43, 46, 48, 60-64]. A total of 14 cases (3.2%) were missed by either CMA (n=4) or karyotype/FISH (n=10) and array was able to identify chromosome 5 abnormalities in 43 (11%) cases in which karyotype analysis failed.

Deletion of 5q as the sole abnormality in primary MDS is associated with a good cytogenetic risk in IPSS-R. From our review, of the 392 abnormal karyotypes, 209 had a 5q abnormality as the sole abnormality by karyotype analysis; but not all studies reported on the analysis of regions outside of 5q by CMA. For the studies that addressed additional aberrations identified by CMA compared to karyotype, 100 cases showed sole 5q abnormalities by karyotype compared to only 53 cases by CMA. This finding could potentially upgrade the IPSS-R determined using conventional karyotype. One study that assessed these patients for response to lenalidomide showed no significant difference in the response of patients with 5q deletions as the sole abnormality and those with additional abnormalities detected by CMA [60].

Despite the lack of correlation with treatment response, one study identified significant differences in OS between patients with del(5q) as the sole abnormality by karyotype (median OS = 34 months) compared to 5q abnormalities (loss and CN-LOH) identified by CMA only (OS = 15 months) [14]. Furthermore, using CMA to refine 5q deletion breakpoints, Stengel et al. have shown in MDS, MPN and MDS/MPN cases that the size of 5q deletion correlated with the number of additional CNAs detected by array, and *TP53* mutations were correlated with a larger del(5q) size and with disease progression and worse prognosis [62].

According to the WHO 2016 recommendation, in patients with isolated del(5q), which may include one additional abnormality with the exception of del(7q) or monosomy 7, testing for

TP53 mutation is recommended to identify an adverse subset of del(5q) syndrome [1]. *TP53* mutations/deletions are markers of clonal progression and predictors associated with a poor response to lenalidomide and an increased risk of AML transformation in del(5q) patients [65-67].

Summary: Almost 50% of cases with del(5q) as the sole abnormality had additional chromosome aberrations identified by CMA. As only one study has addressed the response to treatment, further studies are necessary to potentially identify particular chromosomal regions that could predict response to therapy.

TET2 alterations

TET2 alterations (deletion, CN-LOH, and mutations) are evident in every type of myeloid malignancies (**Table 2**). *TET2* deletions are cytogenetically cryptic in 50% of cases, and need either FISH or CMA for identification [24]. In a study on 893 adult patients with myeloid malignancies, using FISH, *TET2* deletion was found in 5.2% AML, 4.8% MDS, 6.9% CMML, and 6.3% MPN [24]. By using CMA, the size of the *TET2* deletions was variable, ranging between 0.6 and 17.2 Mb. While concordance between FISH and CMA is high, CMA has the advantage of detecting CN-LOH. In *TET2*-deleted patients, *TET2* mutations were detected in 19/37 (51.4%) by NGS, including 10/14 (71.4%) CMML, 6/16 (37.5%) AML, 2/4 (50%) MDS and 1/3 (33%) MPN. *JAK2* V617F was detected in 6/18 *TET2* deleted patients (33%). *CBL* mutation was also found in 2/36 (5.5%) patients. In *de novo* AML (n=301), *TET2* deletion was associated with intermediate- and poor-risk cytogenetics; among patients with intermediate-risk cytogenetics, *TET2* alteration had worse OS and EFS [24].

Importantly, alterations of *TET2* could be a potential marker associated with response to demethylation agents. Specifically, clonal *TET2* mutations (>10% variant allele frequency) were associated with improved response to treatment with hypomethylating agents, although there was no improvement in overall survival [68-70]. The response was more robust in the absence of *ASXL1* mutation [68]. Similarly, mutations in *TET2* and/or *DNMT3A* independently predicted better response to DNA methyltransferase inhibitors [71].

Summary: CMA can help uncover *TET2* deletion as it is cytogenetically cryptic in 50% of cases and FISH is not routinely performed. *TET2* deletion is associated with *TET2* mutation, which could be a marker of improved response to hypomethylating agents.

TP53 mutations

A number of studies have reported deletions, mutations and CN-LOH of the short arm of chromosome 17p encompassing the *TP53* gene as a recurrent abnormality in MDS (Table 1 and 2). *TP53* mutations were frequently associated with MDS with del(5q) and complex cytogenetic abnormalities [72]. In a study of 106 patients with MDS, MDS/MPN, and MPN associated with deletion 5q, using CMA, the size of the deletion ranged between 16 and 119 Mb with a median of 70 Mb. In that study, the highest mutation frequency was reported in *TP53* (overall frequency 31%, frequency in MDS was 36%) followed by *JAK2* (23%) and *DNMT3A* (18%). While there was no significant differences in size of the deletions between the various WHO defined entities, cases with larger deletions (defined as ≥70 Mb) had a significantly higher frequency of *TP53* mutations [62]. In a separate study of 72 newly diagnosed MDS patients with complex chromosomal abnormalities, 17p CN-LOH was detected by CMA in 18% of the patients, distributed as follows: 38.4% RAEB-2, 46.1% MDS-AML, in 7.6% of RCMD and 7.6% of MDS-unclassified. CMA characterized the average size of the CN-LOH region to 8.2–20.8 Mb encompassing the *TP53* gene. All of the 17p CN-LOH patients in this study also had mutations of *TP53* [38]. Within high-risk MDS/AML patients with del(5q) with and without additional cytogenetic abnormalities treated with a sequential combination of azacitidine and lenalidomide, *TP53* mutations were associated with increased genomic instability, and the total number of genomic alterations <200 Mb was predictive of improved OS (p=0.046), while *TP53* mutations by itself did not predict response to therapy. Further, *TP53* mutated patients showed therapy refractoriness only when accompanied by heavily rearranged chromosomes, while *TP53* mutated patients without heavily rearranged chromosomes responded to treatment [48].

Summary: CMA study has shown that cases with 17p CN-LOH were often accompanied by homozygous mutations of the *TP53* gene. LOH (deletion, mutation, or CN-LOH) at 17p was strongly associated with a complex karyotype and deletions of 5q and 7q. In a newly diagnosed MDS patient, this could trigger a rapid clonal evolution with high risk [38].

Trisomy 8 and del(20q)

Trisomy 8 and del(20q) are most common among sole cytogenetic abnormalities but are not diagnostic of MDS in the absence of morphological dysplasia. In one study, CMA analysis on trisomy 8 MDS/AML patients revealed additional submicroscopic CNAs in 40% of cases, including a recurrent 12p deletion encompassing the *ETV6* locus [73]. A possible association was reported between *IDH* mutations and trisomy 8 [74]. Two or more additional CNAs/CN-LOH identified by CMA in these patients would reclassify a patient from intermediate-risk to high-risk because of complex karyotype, defined as 3 or more clonal abnormalities [75]. In a study of 306 MDS patients, the commonly deleted region (CDR) for del(20q) was defined as 4.6 Mb in size encompassing 96 genes, flanked by *PTPRT* at 20q13.11 and *EYA2* at 20q13.12 [76]. CMA analysis on 30 of these patients showed no significant difference in deletion size in early or advanced MDS cases. Additional aberrations equal to or greater than 3 by karyotype were seen in 10.2% of patients with significantly worse 2-year OS (0% vs. 87.7%, HR 27.5, p=0.003) by multivariate analysis. Sequence analysis identified mutations in *U2AF1* (20%), *SRSF2* (19.5%), *ASXL1* (16.3%), *RUNX1* (8.9%), and *SF3B1* (5%); only *ASXL1* mutation status had a significant impact on prognosis (2-year OS of 45.5% vs. 87.9% with and without *ASXL1* mutation, respectively, p=0.002). *ASXL1* mutations typically occurred outside of CDR and were associated with advanced MDS [76].

Summary: CMA may identify abnormalities in addition to trisomy 8 or del(20q) in patients with MDS or suspected MDS. Aside from complex karyotype, the prognosis of these additional findings is unclear. Larger studies are necessary to determine if additional abnormalities, identified by CMA but not by karyotype, impact prognosis.

c. Summary of the disease-based prognostic and therapeutic implications of CMA findings in bone marrow failure syndrome (BMFS)

Revised 4th edition of WHO classification has recognized myeloid neoplasms with germline predisposition [1]. CMA testing is especially useful in these settings as cells from patients with bone marrow failure syndromes such as aplastic anemia (AA) are typically hard to grow *in vitro*

[77]. CMA can detect additional aberrations in AA patients and hypocellular MDS beyond karyotype. Serial longitudinal CMA analyses were able to identify monosomy 7 in four AA patients, consistent with progression, earlier than karyotype analysis [77]. CN-LOH of 6p involving the HLA locus was a frequent abnormality seen in 11% of AA patients [78, 79]. CN-LOH of 6p is a mechanism for cells evading the immune system, rather than a malignant process and is often associated with multiple clones. None of the AA patients with 6p CN-LOH developed MDS or additional MDS-related cytogenetic abnormalities [79]. However, none achieved long-term remission with immune suppressive therapy either [79]. An important consequence to note is that HLA typing of peripheral blood could give inaccurate or ambiguous results if CN-LOH 6p is present.

A comprehensive CMA analysis was performed on 91 patients with various BMFS including AA with and without PNH, Fanconi Anemia (FA), Dyskeratosis Congenita, Diamond Blackfan Anemia, Shwachman Diamond Syndrome, severe congenital neutropenia and BMFS that could not be classified. CMA facilitated identification of a number of pathogenic abnormalities (low-level acquired CNAs, CN-LOH, and inherited regions of homozygosity) that were not identified by conventional karyotype. Using CMA, delineation of the breakpoint of ring chromosome 21 was possible in a case of *RUNX1* haploinsufficiency. Further, inherited regions of homozygosity (ROH) were frequent in BMFS, and were located in the regions containing genes with autosomal recessive mutations, such as *FANCA* mutation in FA, *DOCK8* mutations on chromosome 9 in primary combined immunodeficiency syndrome. Sequential CMA analysis in 25 patients at different time points including diagnosis, routine follow-up and relapse showed that 2 of 4 relapsed patients had acquired CN-LOH. Hence, the authors suggested that repeating CMA at the time of suspected disease relapse had the highest yield. Interestingly, acquired CN-LOH was significantly more frequent in patients with acquired AA (aAA) than in the combined category of non-aAA BMFS patients ($p<0.01$). The most frequent acquired CN-LOH in aAA involved 6p. Other less common aberrations included 5q15qter, 6q12qter and 15q12qter, and these were mostly small clones [78].

Some patients with familial platelet disorders with germline *RUNX1* mutations have deletions encompassing multiple exons that cannot be detected by targeted NGS-based testing. In such

situations, CMA testing is one of the easiest methods of identification of such alterations, as illustrated in a patient in one study [80]. Further, CMA testing can help in detection of acquired secondary somatic alterations in these patients that often accompanies transformation to MDS/AML. In a study by Antony-Debre et al., 2 of 9 patients with familial platelet disorder with germline *RUNX1* mutations who transformed to AML had CN-LOH of chromosome 21 that was only detectable by CMA [81].

Summary: A key question in the evaluation of BMFS patients is differential diagnosis of MDS or transformation to MDS/AML. Literature evidence suggests that CMA is a valuable tool to address this question. Another differential diagnosis is AA or hypoplastic MDS. The presence of CN-LOH of 6p would suggest AA.

d. Summary of the disease-based prognostic and therapeutic implications of CMA findings in precursor myeloid entities of ICUS, IDUS, CCUS, and CHIP

CMA and targeted mutation profiling have identified MDS-associated alterations in the hematopoietic cells of normal individuals signifying that acquired somatic alterations were not restricted to patients with myeloid neoplasms [82-84]. Additionally, some patients with suspected MDS present with unexplained cytopenias and others present with dysplasia without cytopenias and fail to meet the standardized morphologic parameters of MDS. Because these individuals do not meet the WHO diagnostic criteria for MDS or other hematological disease, provisional descriptive entities have been introduced into clinical practice to classify these diagnostically challenging patients. These entities include: a) patients with persistent peripheral blood unexplained cytopenias, normal bone marrow morphology, but no clonal karyotypic and MDS-associated mutations are classified as having idiopathic cytopenias of undetermined significance (ICUS); b) patients with a dysplastic bone marrow without cause, no or mild cytopenias and no clonal aberrations are classified as having idiopathic dysplasia of undetermined significance (IDUS); c) Clonal cytopenias of undetermined significance (CCUS) describes individuals with idiopathic cytopenias and clonal hematopoiesis (MDS-associated mutations and/or CMA-defined or non-MDS defining cytogenetic lesions), and d) clonal hematopoiesis of indeterminate potential (CHIP) has been proposed for patients with a clonal

alteration/mutation associated with hematologic neoplasia without cytopenias or dysplasia [82, 85-88].

Currently, little is known about the natural history of these preclinical entities but some degree of risk for either MDS or other hematologic malignancy is inferred. Molecular characterization of ICUS/IDUS cases using CMA and mutation profiling report a subset of these diagnostically ambiguous patients have acquired MDS-associated alterations (reported range, 35%-62%) [21, 89, 90]. Allen et al. reported 5 of 12 IDUS/ICUS patients with normal cytogenetics showed clonal aberrations by array, indicating reclassification to CCUS. With a median of 28 months of follow-up, 3 of 3 CCUS patients reevaluated by subsequent bone marrow evaluation met the criteria for MDS or MDS/MPN within 6 months. Among the seven patients who did show clonal hematopoiesis, three ICUS patients subsequently met the criteria for MDS within 9 months. In a prospective study, Kwok et al. [89] studied 144 patients with unexplained cytopenias in which 35% of ICUS patients carried a somatic mutation or chromosome aberration indicative of clonal hematopoiesis. In a different study designed to distinguish preclinical MDS from healthy individuals, Cargo and colleagues [90] reported 63 of 69 (91%) ICUS/CCUS patients showed either an array-based abnormality (23%) or a MDS-associated mutation in their non-diagnostic marrow. The number of mutations and the variant allele fraction (VAF)/clonal size were notably greater in the ICUS patients vs. healthy individuals. For the 59 ICUS patient with a follow-up marrow sample, 39 patients eventually progressed to MDS or AML.

The risk of progression for CCUS patients is unknown but suggested to be between CHIP and MDS. Because CHIP involves a mutated hematopoietic stem cell or immature progenitor cell, CHIP is currently viewed a precursor state for a broad range of hematopoietic neoplasms with a rate of progression to a hematologic neoplasm in the 0.5% to 1% per year, similar to the transition of monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma [88].

Summary: The high frequency of CNAs/CN-LOH by CMA and somatic MDS-associated mutations recently reported in precursor myeloid entities provide potential objective markers of

disease. As these pre-malignant clinical entities evolve, molecular genetic testing is warranted for ambiguous morphology/diagnostically challenging patients.

III. Important Pre-analytical and Post-analytical considerations for CMA and limitations of CMA testing

a. Peripheral Blood vs. Bone Marrow

For myeloid malignancies, studies have demonstrated a high level of concordance (95%) between CMA aberrations detected in peripheral blood (PB) and bone marrow (BM) [29]. Some authors have suggested that PB granulocytes may be a viable option for patients in which bone marrow/ karyotype analysis cannot be performed [61]. This is particularly helpful in elderly patients or in patients with fibrotic marrow and dry taps. Two studies have demonstrated that CNAs can be more readily identified in CD34+ cells compared to granulocytes or whole mononuclear cells [16, 61]. There is a significant correlation between gene aberrations detected in CD34+ cells of MDS patients by CMA with gene expression data [91]. However, isolating CD34+ cells is usually not feasible for routine clinical testing. Furthermore, in order to distinguish somatic from germline aberrations, it would be ideal to test BM samples with buccal DNA as matched germline control [16, 92, 93]. Again, this is not always feasible in a clinical laboratory. Therefore, some laboratories may consider testing for remission samples instead.

b. Formalin fixed paraffin embedded (FFPE) material

FFPE tissue in the haematological setting offers an alternative tissue source when other more easily extracted tissue such as bone marrow aspirate samples are not available. FFPE tissue is an excellent source of DNA, despite the detrimental effect that the fixatives used can have on the quality of the nucleic acids retrieved [94-97]. Many studies have demonstrated comparable performance between DNA obtained from FFPE samples and fresh frozen tissue, especially with modified protocols, and have shown concordance of CNVs obtained from FFPE and fresh frozen specimens [94, 98, 99]. CMA testing is reliable from FFPE specimens if optimised protocols are used [100, 101], ensuring minimum DNA quality, adequate input quantity and sufficient tumor burden. Decalcified FFPE specimens are especially challenging. Nevertheless, Stevens-Kroef et al. demonstrated a high concordance between CMA on decalcified BM biopsy samples and

karyotyping on corresponding BM aspirates with a much higher resolution [102]. This is particularly valuable in obtaining clinically important genomic information in MDS cases with fibrosis and dry tap lacking cytogenetic data [102].

IV. Limitations of CMA

A variable number of aberrations were missed by CMA compared to karyotype or FISH, mostly due to low level clones, depending on the resolution of CMA testing and coverage [12, 26, 35, 47, 61]. There are many commercial CMA platforms available and each should be independently validated in the laboratory to establish the limit of detection. The detection limit varies considerably in the studies reviewed as a consequence of biases and variability including disease selection, metaphase chromosome abnormality selection, differing CMA platforms, differing calling criteria and whether CMA analysis was performed blind to the metaphase chromosome analysis. In one study 142 cases of deletion 5q identified by metaphase chromosome analysis were identified by CMA analysis [33]. This contrasts to another study where 4/30 (13%) cases with CNAs of del(5q), del(7q), del(20q) and del(17p) by metaphase chromosome analysis with clonal levels of between ~10 to 15% remained undetected by CMA [28]. The detection limits of CMA and metaphase analysis are difficult to compare since karyotype by chromosome banding analysis evaluates only dividing cells while CMA analysis evaluates the entire cell pool.

CMA analysis can only detect copy number changes and CN-LOH. Rearrangements that are genuinely balanced such as translocations and inversions, having no loss or gain of genetic material at the molecular level, cannot be detected using CMA technology, although one study estimated that 7% of abnormal CMA cases had an “unbalanced translocation” [42]. This implies that aberrations that appear balanced by metaphase chromosome analysis may be unbalanced by CMA technology due to sub-microscopic CNAs at one or more of the rearrangement breakpoints. Indeed, balanced rearrangements by karyotype have been shown to be unbalanced at the sub-microscopic level in 37% cases by CMA [103]. These imbalances may highlight the presence of a recurrent rearrangement, such as a CNV near the *MECOM* gene may represent a rearrangement of the *MECOM* gene. In this literature review, with the exception of CML, most studies reported either none or one case where a balanced rearrangement was missed; this

included one case of an inv(3)(q21q26) [104]. In MDS, other than inv(3) present in 1%, the common, recurrent chromosome abnormalities and prognostically significant abnormalities named in IPSS-R are copy number changes [4]. Therefore, CMA is ideal for MDS, MDS/MPN, and Ph-negative MPN. In general, if balanced rearrangements are also expected in the cancer type being studied, CMA should be supplemented by karyotype analysis and/or FISH [103]. Another limitation of CMA includes the inability to identify independent clones and the complexity of the sub-clones, i.e., clonal architecture that is obtainable by karyotype analysis. While clonal evolution can be deduced, this complexity may not be captured by CMA analysis alone [47, 105].

Discussion

The recent implementation of CMA into clinical laboratories has been an exciting and practical advancement in cytogenetic testing since the introduction of FISH technology as a new molecular cytogenetic tool in the 1990s. Despite the heterogeneity in study design, patient cohort, disease stage, CMA platform used, and criteria for aberration calls, the overall clinical diagnostic impact and advantages of CMA in MDS, MPN and MDS/MPN is evident. The CMA technology has a considerably higher resolution for whole genome coverage; for example, a CMA platform with a functional resolution of 10-20 Kb has at least a 1000-fold increase in resolution when compared to karyotype analysis (5-10 Mb). It also allows for more precise identification of genes involved in genomic abnormalities. There is no requirement for dividing cells, and both fresh and formalin-fixed paraffin-embedded tissues could be used. It showed equivalent diagnostic yield for MDS between blood and marrow, which is especially helpful. CMA is generally considered technically less laborious and more cost-effective than karyotype with great potential for multiplexing and automation. However, evidence for clinical utility is badly needed for routine utilization of this test by hemato-oncology providers and for better insurance coverage to benefit our patients.

Key questions from providers typically include: (1) Are deletions and gains detected by CMA the same in terms of diagnosis and prognosis as those detected by karyotype and FISH? (2) What does a finding of CN-LOH mean to my patient? (3) How is CMA different from NGS testing? Are they redundant? (4) Is CMA testing recommended by NCCN-guidelines? (5) Is

CMA paid by insurance companies? (6) How do I use these new results? We will highlight the answers to these questions as we summarize the evidence base below.

Here, we present literature-based evidence on the application of CMA as a clinical diagnostic test in the work-up of MDS, MPN and MDS/MPN in specific clinical settings. We provide an example of a diagnostic testing algorithm for genomic testing in these neoplasms based on the complementary nature of CMA testing with current diagnostic modalities (**Figure 1**).

Summary of Evidence Base:

Table 1 summarizes the most important or unique CMA findings and their clinical utility in each disease entity while **Tables 2-4** provides a comprehensive list of all the recurrent CMA findings reported in the literature based on our review. Diagnostic yield is generally high across the myeloid neoplasms (up to 80%) and is around 50% among those with normal or failed karyotype. Even for patients with an abnormal karyotype, CMA can detect additional aberrations in approximately half of the cases. The number of CMA abnormalities is usually low, with a median between 1 and 2. Higher numbers are typically associated with advanced disease, disease progression, or high-risk disease. The concordance between FISH and CMA is excellent for CNAs, and the diagnostic and prognostic impact of the deletions and gains identified by CMA is equivalent to those by karyotype and FISH even in the setting of a clinical trial [12, 18, 21, 24, 106].

In specific clinical scenarios, CMA testing offers advantages over routine karyotype as detailed below and summarized in **Figure 1A**. CMA is particularly useful in myeloid neoplasms with insufficient (<20 metaphases) or failed karyotype either due to the poor quality of the specimen or due to factors inherent to the disease condition such as bone marrow failure. In such situations, CMA testing of DNA extracted from residual cell pellets, aspirate smears or touch preps or FFPE sections is an alternative to karyotype and/or FISH.

CN-LOH is prevalent in MDS/MPN and *BCR-ABL1*-negative MPN, with a reported frequency between 6%-41%. Currently, CMA is the only feasible technique available for identification of

CN-LOH. In addition to being a clonal marker, identification of CN-LOH can direct appropriate mutation analysis of specific genes of clinical significance.

In the setting of normal karyotype, CMA provides additional information of clinical value. Additional aberrations detected by CMA over conventional karyotype are associated with worse survival in MDS patients [14, 26, 42, 43], MDS/MPN [12], and MPN [39] and also with disease progression to secondary leukemia or myelofibrosis [39, 55, 57, 59, 63, 107]. Additional CMA-detected genomic aberrations and total genomic aberration measured by CMA can be used for further risk-stratification in both low and high IPSS/IPSS-R risk MDS patient groups [12, 14, 26, 44]. Therefore, CMA can be helpful in low or intermediate-risk MDS patients, especially when straddling the prognostic range to more accurately assess the prognostic risk based on objective genetic data.

CMA can detect potential markers of clonality in diagnostically challenging settings. These include cases with ambiguous morphology not diagnostic of a myeloid neoplasm, recently recognized as precursor myeloid entities, to differentiate BMFS from hypoplastic MDS, and assess progression of BMFS to MDS.

Predictive markers remain scant even with the help of CMA. Specifically, response to lenalidomide among MDS patients with del(5q) and TKI resistance among CML patients did not correlate with CMA findings even though additional CNAs found by array and *TP53* mutations/17p deletions are associated with disease progression and worse prognosis [60, 62]. However, CMA is useful in detecting cryptic *TET2* deletions and 4q CN-LOH. Recent evidence suggests that *TET2* alterations could be a potential marker for response to demethylation agents, especially in the absence of *ASXL1* mutation [68-70].

Current Practice of CMA in Myeloid Neoplasms (expert opinion)

Overall, CMA is extremely useful for work-up of patients with MDS, MDS/MPN, and Ph-negative MPN along with karyotype, FISH and gene mutation analysis, where CNAs and CN-LOH are the most common abnormalities in these diseases and balanced chromosomal abnormalities do not have a major role. CMA provides a whole genome view at 1000x

resolution, from a gene to chromosome level in a single assay that does not require cell cultures. CMA facilitates detection of submicroscopic CNAs below the resolution of conventional karyotyping and adds precision with regards to breakpoint locations and the gene content. Accurate definition of breakpoints can suggest haploinsufficiency or disruption of a key gene and provide direction for appropriate mutation testing if clinically relevant. Most importantly, CMA is the only technique that allows detection of large CN-LOH, some of which are of prognostic value. This finding can also trigger mutational analysis of target genes of potentially predictive significance. In cases where karyotyping fails or cannot be performed, CMA allows detection of diagnostic and prognostic CNAs. For MDS, there is a growing body of literature challenging the value of FISH following an adequate, normal karyotype analysis (He et al. 2016, Coleman et al. 2012, Jiang et al. 2011), prompting the recent Center for Medicare Services (CMS) proposal to eliminate coverage for MDS FISH when karyotype is successful. This further emphasizes the clinical utility of CMA in MDS. Limitations of CMA include inability to detect balanced rearrangements (noted in about 5% of the MDS patients), higher limit of detection than FISH, and reduced ability to fully resolve individual clones.

Many clinical laboratories have implemented CMA for hematological malignancies. The robustness of CMA offers great confidence in reporting new clonal findings which aid in diagnosis and prognosis. With a resolution of 10-20 Kb, CMA cannot detect point mutations and indels except those at the SNP loci covered by the array design. NGS is ideal to detect single nucleotide point mutations and small indels up to 1 Kb. NGS may also infer copy numbers but are not yet as accurately as CMA. Therefore, NGS and CMA can be utilized as complementary genomic testing tools. Mutation analysis, karyotype and FISH are recommended in practice guidelines available for myeloid neoplasms. However, CMA has not been explicitly mentioned, which raises concerns among providers regarding the value of CMA.

We present an example of an evidence-based testing algorithm to help promote the best clinical practice for diagnostic workup of MDS, MPN or MDS/MPN neoplasms by integrating CMA into current standard-of-care clinical laboratory testing tools (karyotype, FISH, morphology and flow) **(Figure 1B**). Many variations of this testing strategy can be applied based on local institutional standard of care. Initial evaluation typically includes karyotype analysis along with

morphology, and whenever possible, multiparameter flow cytometry immunophenotypic analysis. NGS panel testing consisting of genes recommended by the NCCN guidelines is usually performed. We suggest that CMA can be performed in specific scenarios of clinical significance: Anytime karyotype is unsuccessful, CMA is recommended. In MDS, if the karyotype is successful with an abnormal karyotype with a poor or very poor cytogenetics group classified per IPSS-R, no CMA testing is needed; patients are likely to receive aggressive or clinical trial regimens regardless of CMA findings. If the karyotype is successful with a very good, good or intermediate cytogenetics risk group, CMA is most helpful in patients with normal karyotype or in patients with cytogenetic risk-scores with uncertain IPSS/IPSS-R to accurately risk-stratify the patient. Baseline FISH for the key stem clone abnormality based on the karyotype or CMA findings could be considered to identify an informative probe for future monitoring of minimal residual disease after therapy.

Establishing a diagnosis of MDS may be challenging in the absence of diagnostic morphologic findings or a MDS-defining cytogenetic abnormality. In such patients, identification of CNAs, or CN-LOH in the regions of myeloid-related genes would trigger a more rigorous follow-up schedule in comparison to patients lacking these adverse prognostic markers. Our example MDS testing algorithm includes testing for ambiguous morphology/diagnostically challenging patients. Future large-scale prospective studies should assist in further refining the spectrum and number of specific mutations/chromosome aberrations involved in preclinical risk progression.

For Ph-negative MPN, CMA may be helpful as a reflex to normal or failed karyotype while mutation testing appropriate for the specific subtype diseases should be carried out; for instance, *JAK2* for PV and ET, *CALR* and *MPL* for myelofibrosis, and *CSF3R* for chronic neutrophilic leukemia.

In CML, however, although higher numbers of chromosome abnormalities detected by CMA in addition to t(9;22) are clearly associated with blast crisis (76% vs. 58% in chronic phase), they have no predictive value for TKI resistance. Therefore, CMA is not currently of high value in this scenario. Quantitative real-time PCR is the preferred monitoring method; hence a baseline

Q-PCR for *BCR/ABL1* is essential. Occasionally, Q-PCR may be uninformative due to rare rearrangement breakpoints and FISH would be preferred for monitoring instead.

The proposed algorithm may prove to be cost effective when CMA replaces extensive FISH panels routinely performed at diagnosis and that mutation testing could be by single- or multiplex-PCR or NGS depending on the availability at various clinical settings.

Summary of cost/reimbursement information and published social economic consideration

Payers, insurance companies and Medicare have their own policies based on research to determine reimbursement approvals and rates. For example, Aetna considers CMA testing medically necessary for evaluating histologically equivocal Spitzoid melanocytic neoplasms (http://www.aetna.com/cpb/medical/data/700_799/0787.html). Furthermore, NCCN guideline on "Melanoma" (Version 3.2015) states to "Consider use of comparative genomic hybridization (CGH) or fluorescence in situ hybridization (FISH) for histologically equivocal lesions". The majority of such policy decisions is driven by consensus statements from professional societies or published clinical utility literature. For example, an NGS cancer panel was approved by Palmetto (a contractor for the Centers for Medicare and Medicaid Services) for patients with advanced non-small cell lung cancer based on ASCO guidelines. However, there have been no clear guidelines about when to use CMA testing in the context of hematological malignancies. Therefore, practicing laboratories have received widely varied rates of reimbursement for CMA.

The Cancer Genomics Consortium (CGC) Working Group for Myeloid Neoplasms, in an international effort, has evaluated through a systematical peer-reviewed literature review, the clinical utility of CNA and CN-LOH evaluation by CMA in the non-AML myeloid neoplasms, and developed a working algorithm for optimal genome-wide testing by CMA. The data indicate such testing is appropriate in some scenarios for clinical management with equitable reimbursement similar to other molecular based testing is warranted in the light of this clinical utility information.

Future directions

It is an exciting time when new technologies like CMA, NGS, exome sequencing, and whole genome sequencing are providing increasingly higher-resolution and cost-effective clinical test options for precision medicine. CMA is likely a cost-effective alternative to FISH panels (when karyotype is inadequate) for studies of copy number changes and allelic imbalances that provides more information. It is a whole-genome analysis approach unlike most of the current NGS panels that would only detect changes in the targeted regions. NGS is invaluable in mutation characterization, but the limitations of NGS panel based testing to detect CNVs in somatic/tumor samples and the advantages of using CMA for this purpose has been widely studied by various groups. Furthermore, CN-LOH analysis can be cumbersome with NGS panel testing [96, 97]. Exome and whole-genome sequencing can help close the current gap of identifying genomic aberrations in the range between 1 Kb and 100 Kb in clinical use. Eventually whole-genome sequencing or whole-exome sequencing will allow accurate assessment of CNAs and CN-LOH. Currently, CMA offers the best high-resolution tool for this application.

This review clearly identified areas where we should further demonstrate the clinical utility of CMA. Precursor myeloid entities were only recently recognized. Diagnosis based on genomic findings in the absence of morphological evidence remains to be established and require additional larger studies. Many questions remain to be answered in myeloid disorders. Should CN-LOH be considered provisional evidence of a myeloid neoplasm as advocated by Gronseth et al [108]? What is the significance of CN-LOH in various chromosome arms? How are they correlated with mutations or other structural aberrations important for diagnosis and prognosis? Last but not least, which of the recurrent findings may serve as predictive markers? Clinical laboratories should join forces to address these questions by multicenter studies and clinical trials that include karyotype, FISH, NGS and CMA in order to establish the best genetic testing algorithm. One of the goals of CGC is to facilitate efforts in this direction by bringing multiple institutions and professional organizations under one umbrella.

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Conflict of interest

None

Table 1. Evidence for the clinical utility of chromosomal microarray testing (CMA) in myeloid disorders excluding acute myeloid leukemia

Disease	Overall CMA detection rate	Key and unique CMA aberrations	Altered gene(s)	Impact	References
MDS	28-83% (Normal karyotype only: 11-39%)	Total genomic alteration		Prognostic poor survival	[26, 31, 35, 44, 48]
		1p CN-LOH		Prognostic for progression to AML	[14, 25, 36, 60, 104]
		1q gain		Recurrent	[14, 21, 30, 104]
		4q loss	*TET2*	Prognostic for poor survival	[14, 21, 23, 24, 46]
		4q CN-LOH	*TET2*	Prognostic for poor survival	[12, 14, 16, 21, 30, 35-37, 45, 63, 109]
		5q loss		5q loss "size" prognostic for progression to AML	[14, 15, 21, 26, 33, 62, 104, 110]
		7q loss	*CUX1, EZH2*	Prognostic for poor survival	[14, 15, 18, 30, 32, 38, 45, 60, 63, 78, 102, 104, 107, 110]
		7q CN-LOH		Recurrent	[12, 14, 21, 25, 30, 36, 48, 91, 109]

		11q CN-LOH	*CBL*	Prognostic/ recurrent	[12, 14, 15, 25, 35, 36, 63, 104]
		12p loss	*ETV6*	Recurrent	[14, 16, 30, 32, 46]
		13q loss	*?RB1*	Recurrent	[14, 21, 32, 35, 104]
		17p loss	*TP53*	Recurrent	[14, 30, 34, 46, 102]
		17p CN-LOH	*TP53*	Diagnostic for advanced MDS/sAML	[21, 30, 35, 36, 38]
		20q loss		Recurrent	[14, 60, 61, 64, 102, 107, 110]
		21q CN-LOH or deletion	*RUNX1*	Prognostic for progression to AML	[14, 18, 32, 46, 60, 91]
MDS/MPN	73%/NA	4q CN-LOH	*TET2*	Recurrent	[12, 20, 23, 63, 64]
		7q CN-LOH	*Likely CUX1*	Recurrent	[12, 20, 36, 60, 63]
		11q CN-LOH	*CBL*	Recurrent	[12, 20, 23, 36, 43]
MPN	>56%/NA	1q gain		Recurrent	[39, 54, 55]
		4q loss	*TET2*	Prognostic for progression to AML	[24, 57]
		9p CN-LOH	*JAK2*	Predictive for JAK2 inhibitors; Prognostic for PV progression to MF	[39, 43, 54, 55, 111]
		14q CN-LOH		Presence of CNAs/CN-LOH prognostic for progression to AML	[39, 54, 55, 60]
		20q loss		Recurrent	[39, 54, 112]
CML	21-24%/NA	17p loss	*TP53*	Recurrent, progression, associated with TKI resistance	[50, 51]

		2q CN-LOH		Diagnostic (only seen in BC)	[51]
		8p CN-LOH		Diagnostic (only seen in BC)	[51]
BMFS	19% (AA)	6p CN-LOH	*?HLA genes*	Recurrent	[77-79]

*Recurrent indicates recurrent aberration with no established prognostic significance

AA, Aplastic anemia

BMFS, Bone Marrow Failure Syndrome

MDS, Myelodysplastic Syndrome

MDS/MPN, Myelodysplastic/ myeloproliferative Neoplasm

MPN, Myeloproliferative Neoplasm

CML, Chronic Myelogeneous Leukemia

sAML, secondary AML

TGA, Total genomic aberration

TKI, tyrosine kinase inhibitors

Table 2. A comprehensive list of CNAs and CN-LOH of known or likely clinical significance in MDS detected by CMA testing

Chromosome	Disease	Abnormality Type (Gain, Loss, CN-LOH)	Region	Relevant Genes (if known)	Clinical Significance*	Level of Evidence	References
1	MDS	Gain	1p36.33-p33	*MPL*	Recurrent	3	[15] [14, 61]
1	MDS	CN-LOH	1p	*MPL*	Recurrent	2	[14, 25, 36, 60]
1	MDS	Gain	1q		Recurrent	2	[14, 21, 30, 104]
2	MDS	CN-LOH	2pter-2p13.3	*DNMT3A*	Recurrent	2	[14, 62, 63, 113]
3	MDS	CN-LOH	3q21.3-qter	*MECOM, GATA2*	Recurrent	3	[14, 16, 19, 35, 60]
4	MDS	Loss	4q24	*TET2*	T***	2	[14, 18, 21, 23, 24, 32, 46]
4	MDS	CN-LOH	4q12-qter	*TET2*	Recurrent	2	[12, 14, 16, 21, 29, 30, 36, 37, 45, 63, 109]
5	MDS	Gain	5p	Suggestive of i(5p) with 5q del	Recurrent	3	[14]
5	MDS	Loss	5q	*RPS14*	D, P (Good when isolated)	1	[14-16, 18, 19, 21, 26, 30, 33-35, 38, 42, 45, 48, 61-64, 102, 104, 110, 114]

7	MDS	Loss	7q	*EZH2, CUX1*	D, P (Intermediate)	1	[14, 15, 18, 19, 26, 30, 32, 34, 38, 45, 48, 60, 78, 102, 104, 110]
7	MDS	CN-LOH	7q21.11-qter	*EZH2, CUX1*	Recurrent	2	[12, 14, 16, 19, 21, 25, 30, 36, 48, 91, 109]
7	MDS	Loss (Monosomy)	7	Whole Chromosome	D, P (Poor)	1	[34, 38, 45, 63, 78, 102, 104, 107, 110]
8	MDS	Gain (Trisomy)	8	Whole Chromosome	P (Intermediate)**	1	[14, 21, 30, 34, 46, 48, 60, 61, 73, 78, 102, 110]
9	MDS	Gain	9p	*JAK2*	Recurrent	3	[14, 30, 46]
9	MDS	CN-LOH	9pter-p24.2	*JAK2*	Recurrent	2	[14, 31, 35]
11	MDS	Loss	11q14.1-q24.3	*CBL*	D, P (Very Good)	1	[14, 61]
11	MDS	CN-LOH	11q13.3-qter	*CBL*	Recurrent	2	[12, 14, 15, 25, 35, 36, 63, 104, 107]
11	MDS	Gain (Trisomy and q-arm)	11 / 11q	*CBL*	Recurrent	3	[14, 16, 26, 30, 63]
12	MDS	Loss	12p	*ETV6*	D, P (Good)	1	[14, 16, 30, 32, 46]
12	MDS	CN-LOH	12pter-p11.23	*ETV6*	Recurrent	2	[35, 63]
13	MDS	Loss	13q	*RB1*	D, P (Intermediate)	2	[14, 21, 35, 104]
13	MDS	CN-LOH	13q12.3-qter	*FLT3, RB1*	Recurrent	3	[14, 36, 63]
13	MDS	Gain (Trisomy)	13	Whole Chromosome	Recurrent	3	[14]

14	MDS	CN-LOH	14q24.2-qter	*CHGA*	Recurrent	3	[14, 15, 25, 29, 36]
16	MDS	Loss (Monosomy and q-arm)	16 / 16q	*CDH1*	Recurrent	3	[14, 46, 104]
16	MDS	CN-LOH	16q22.1-qter	*CDH1*	Recurrent	3	[14, 91]
17	MDS	Loss	17p	*TP53*	P (Poor)	1	[14, 30, 34, 42, 46, 48, 102]
17	MDS	CN-LOH	17pter-p11.2	*TP53*	Recurrent	2	[16, 21, 30, 33, 35, 36, 38, 48]
17	MDS	Loss	17q11.2	*NF1*	Recurrent	3	[32, 46]
17	MDS	CN-LOH	17q11.2-qter	*SRSF2, NF1*	Recurrent	2	[14, 19, 25]
19	MDS	CN-LOH	19pter-p13.11	*DNMT1, PRDX2*	Recurrent	3	[60, 63]
19	MDS	Loss	19p13.13	*PRDX2*	Recurrent	3	[26]
19	MDS	Gain (Trisomy)	19	Whole Chromosome	Recurrent	2	[14, 60]
20	MDS	Gain	20p	Suggestive of ider(20p) with 20q del	Recurrent	3	[14]
20	MDS	Loss	20q	*ASXL1*	P (Good)**	1	[14, 21, 26, 42, 48, 60, 61, 64, 91, 102, 107, 110] [15, 30, 45, 112, 115]
20	MDS	CN-LOH	20q11.21-qter	*ASXL1*	Recurrent	2	[48, 91]
21	MDS	Loss	21q22.12	*RUNX1*	D, P (Poor)	2	[14, 16, 18, 21, 32, 34, 46]
21	MDS	CN-LOH	21q21.1-qter	*RUNX1*, *U2AF1*	Recurrent	2	[14, 25, 48, 109, 114]
21	MDS	Gain (Trisomy)	21	Whole Chromosome	Recurrent	2	[14, 30, 102]
22	MDS	CN-LOH	22q11.23-qter	*MN1, SF3A1, EP300*	Recurrent	3	[14, 104]

Legend: D- diagnostic significance; P-prognostic significance; T- therapeutic significance.
Recurrent indicates recurrent aberration with no established prognostic significance.
* Clinical significance based on WHO classification using IPSS-R (Greenberg et al., Blood 2012; Schanz et al., J Clin Oncol 2011).
** Isolated trisomy 8 or del(20q) are not diagnostic of MDS in the absence of morphologic findings of disease.
***Potential marker for responsiveness to hypomethylating agents or DNA methyltransferase inhibitors (Bejar et al., Blood 124:2705-12, 2014;Traina et al., Leukemia 28:78-87, 2014).

Table 3. A comprehensive list of CNAs and CN-LOH of known or likely clinical significance in MDS/MPN detected by CMA testing

Chromosome	Disease	Abnormality Type (Gain, Loss, CN-LOH)	Region	Relevant Genes (if known)	Clinical Significance*	Level of Evidence	References
1	MDS/MPN	CN-LOH	1p21.3	*MPL*	Recurrent	2	[36]
4	MDS/MPN	Loss	4q24	*TET2*	Recurrent**	2	[23]
4	MDS/MPN	CN-LOH	4q12.4-qter	*TET2*	Recurrent	2	[12, 20, 23, 36, 63, 64]
5	MDS/MPN	Loss (Monosomy and q-arm)	5 / 5q	*RPS14*	P (Intermediate)	1	[23, 28, 33, 43, 55, 62]
7	MDS/MPN	Loss	7q	*EZH2, CUX1*	P (Poor)	1	[12, 43]
7	MDS/MPN	CN-LOH	7q21.11-qter	*EZH2, CUX1*	Recurrent	2	[12, 20, 36, 60, 63]
8	MDS/MPN	Gain (Trisomy)	8	Whole chromosome	P (Poor)	1	[55, 63]
9	MDS/MPN	CN-LOH	9pter-p13.3	*JAK2*	Recurrent	2	[36]
11	MDS/MPN	CN-LOH	11q13.2-qter	*CBL*	Recurrent	2	[12, 20, 23, 36]
12	MDS/MPN	Loss	12p	*ETV6*	P (Intermediate)	1	[20, 28]
13	MDS/MPN	Loss	13q	*RB1*	P (Intermediate)	1	[43, 55]
14	MDS/MPN	CN-LOH	14q	*CHGA*	Recurrent	3	[36]
17	MDS/MPN	Loss	17p	*TP53*	P (Poor)***	1	[55]
20	MDS/MPN	Loss	20q	*ASXL1*	P (Intermediate)	2	[43]
21	MDS/MPN	Gain	21q22.12	*RUNX1*	P (Intermediate)	2	[20, 23]
21	MDS/MPN	CN-LOH	21q22-qter	*RUNX1*	Recurrent	2	[20, 36]

Legend: D- diagnostic significance; P-prognostic significance; T- therapeutic significance.

Recurrent indicates recurrent aberration with no established significance.

* Clinical significance based on International MDS/MPN Working Group recommendations [10]; No NCCN guidelines available. Low risk (normal, isolated –Y), Intermediate (others), High risk (+8, abnormal 7, complex).

**Potential marker for responsiveness to hypomethylating agents or DNA methyltransferase inhibitors [68, 71]

***Haploinsufficiency of 17p as part of an isolated isochromosome may be a distinct disease entity with further increased risk of AML progression relative to 17p loss in a complex karyotype.

Table 4. A comprehensive list of CNAs and CN-LOH of known or likely clinical significance in MPN detected by CMA testing

Chromosome	Disease	Abnormality Type (Gain, Loss, CN-LOH)	Region	Relevant Genes (if known)	Clinical Significance*	Level of Evidence	Reference (PMID)
1	MPN	CN-LOH	1p21.3	*MPL*	Recurrent	2	[39]
1	MPN	Gain	1q21.2-q32.1		Recurrent	2	[39, 54, 55]
4	MPN	Loss	4q24	*TET2*	Recurrent	2	[24, 57]
5	MPN	Loss	5q	*RPS14*	P (Poor)	1	[62]
6	MPN	Loss	6p23-22.3	*JARID2*	Recurrent	3	[55, 59]
7	MPN	Loss	7q	*EZH2, CUX1*	P (Poor)	1	[39]
7	MPN	CN-LOH	7q22.1-qter	*EZH2, CUX1*	Recurrent	2	[51]
8	MPN	Gain (Trisomy)	8	Whole chromosome	P (Poor)	1	[50]
9	MPN	Gain	9p	*JAK2*	Recurrent	2	[39, 54, 55]
9	MPN	CN-LOH	9pter-p13.3	*JAK2*	Recurrent	2	[39, 43] [54] [55, 111]
9	CML	Loss	9q34		Recurrent	3	[49, 50]
9	CML	Gain	9q34 (+Ph)	*ABL1*	Recurrent	1	[50]
11	MPN	CN-LOH	11q13.4-q25	*CBL*	Recurrent	2	[39, 54]
12	MPN	Loss	12p13.3-p12.2	*ETV6*	P (Poor)	1	[51]
13	MPN	Loss	13q	*RB1*	Recurrent	1	[54]
14	MPN	CN-LOH	14q	*CHGA*	Recurrent	3	[39, 54, 55, 60]
17	MPN	Loss	17p	*TP53*	P (Poor)	1	[50, 51, 54]
20	MPN	Loss	20q	*ASXL1*	Recurrent	1	[39, 54, 112]
22	CML	Loss	22q11.2		Recurrent	3	[49, 50]
22	CML	Gain	22q11.2 (+Ph)	*BCR*	Recurrent	1	[50]

Legend: D- diagnostic significance; P-prognostic significance; T- therapeutic significance. Recurrent indicates recurrent aberration with no established significance.

* Clinical significance based on NCCN guidelines [5]; For myelofibrosis, unfavorable [complex karyotype or sole or two abnormalities that include inv(3), 5/5q-, 7/7q-, +8, 11q23 rearrangement, 12p-, and (17q)]

Figure Legends

1A

Clinical Scenarios where detection of CNA and CN-LOH is of additional value to current standard of care testing modalities	
Disease entity	**Clinical Scenario**
Any disease below	• Unsuccessful karyotype
Myelodysplastic Syndromes	• Successful karyotype with very good, good or intermediate cytogenetic risk groups: a. Normal karyotype b. Uncertain IPSS/IPSS-R
Myelodysplastic/ Myeloproliferative Neoplasms	• Normal karyotype
BCR/ABL1-negative Myeloproliferative Neoplasms	• CMA at baseline can predict progression
Precursor myeloid entities	• Normal karyotype without diagnostic morphologic features of a myeloid neoplasm
BM failure syndromes	• Differential diagnosis between aplastic anemia (AA) and hypoplastic MDS (CN-LOH 6p favors AA) • Assess transformation to AML/MDS

1B

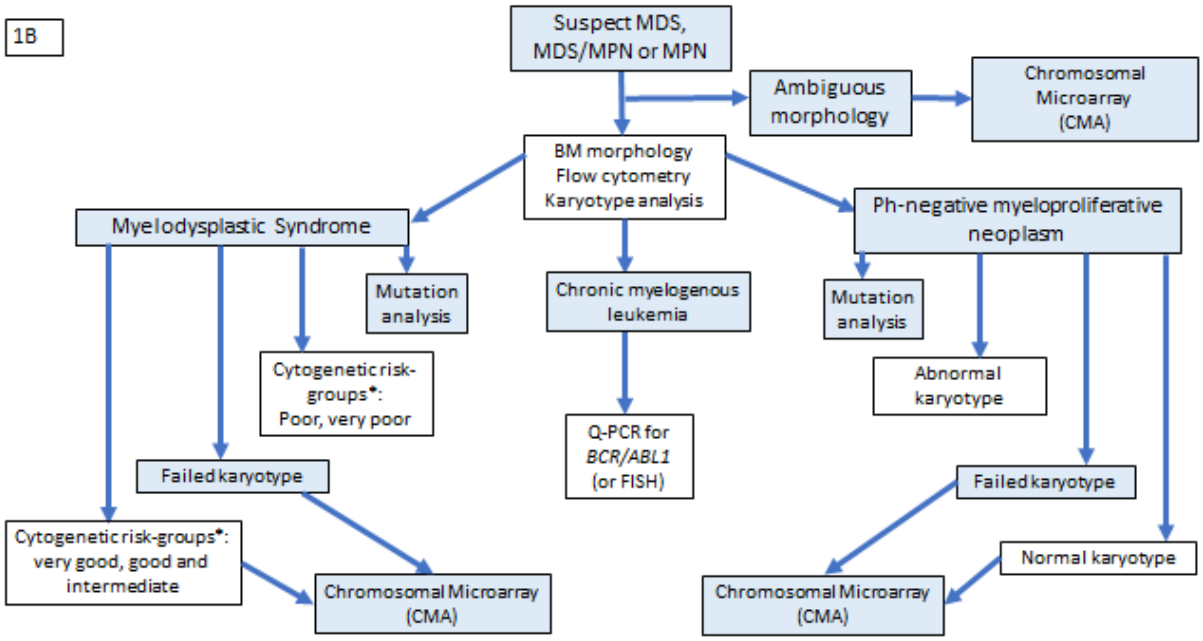

Figure 1. (A) Clinical Scenarios where detection of CNA and CN-LOH is of additional value to current standard of care testing modalities. **(B)** An example testing algorithm for MDS, MDS/MPN and MPN in a clinical laboratory. Every patient with a suspected diagnosis of MDS or MPN should have karyotype analysis performed at diagnosis, along with morphology

evaluation, flow cytometry analysis and mutation analysis (if considered appropriate and feasible). In the case of CML with t(9;22) by karyotype, quantitative real-time PCR is the preferred monitoring test. For patients with suspected MDS, MDS/MPN or Ph-negative MPN diagnoses, chromosomal microarray testing (CMA) is recommended if the karyotype is unsuccessful; for MDS patients with successful karyotype showing very good, good or intermediate cytogenetic risk-groups per IPSS-R stratification, CMA is especially helpful in patients with normal karyotype and in patients with uncertain IPSS/IPSS-R risk score to further assist with diagnostic uncertainty and risk stratification**. In MDS patients when del(5q) is detected by karyotype as a sole abnormality or along with one additional abnormality other than del(7q)/-7, 2016 WHO classification system recommends performing *TP53* mutation studies for identifying those patients with poor response to lenalidomide. The genes in the mutation analysis panel should be based on the NCCN guideline and may be tailored to the needs and technology available at each institution. *SF3B1, ASXL1, RUNX1, EZH2, ETV6* and *TP53* for MDS and *JAK2, CALR, MPL, TET2* for MPN are typical examples. If feasible, when clonal copy number alterations (CNAs) are identified by either CMA or karyotype, a baseline FISH for the key stem clone could be considered to establish an informative FISH marker for future monitoring of minimal residual disease post-treatment. In patients with ambiguous morphology, CMA is helpful for identification of clonal markers.

* Cytogenetic risk-groups classified per IPSS-R [2, 4]

** At this time, IPSS-R is based on karyotype assessed by chromosome banding analysis only. Further multi-center studies to comprehensively evaluate the prognostic impact of additional findings by genomic arrays compared to IPSS-R need to be undertaken.

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