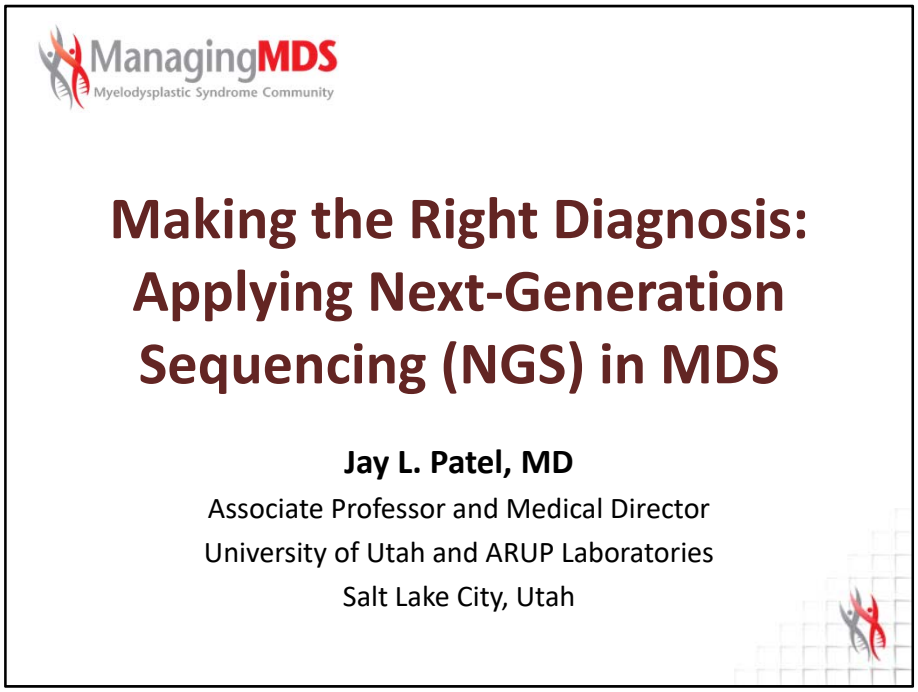
Making the Right Diagnosis: Applying Next-Generation Sequencing (NGS) in MDS



Welcome to *Managing MDS*, I am Dr. Jay Patel. Today I will be presenting Making the Right Diagnosis: Applying Next-Generation Sequencing in MDS. In this presentation, I will outline the current status of NGS in the clinical setting, as well as describe the advances in genomics and the role of next-generation sequencing in the diagnosis and prognosis of MDS, and finally, we will apply next-generation sequencing in the diagnosis and management of patients with MDS.

Disclosure

- Dr. Jay Patel has no relevant financial disclosures

I have no relevant financial relationships to disclose.

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WHO 2008	WHO 2016
Refractory cytopenia	**MDS with single lineage dysplasia**
Refractory cytopenia with multilineage dysplasia	**MDS with multilineage dysplasia**
Refractory anemia with ring sideroblasts	**MDS with ring sideroblasts** **- Single lineage dysplasia** **- Multilineage dysplasia**
MDS with isolated del(5q)	**MDS with isolated del(5q)**
Refractory anemia with excess blasts	**MDS with excess blasts**
MDS, unclassifiable	**MDS, unclassifiable**

Arber D, et al. *Blood*. 2016;127(20):2391-2405.

I would like to begin by reviewing the recently updated WHO criteria and classification for MDS diagnosis. As we all know, the classification scheme for myeloid malignancies has evolved significantly over the years; and as of the 2016 publication of the WHO classification, the MDS classification has been updated. However, I would note that the substantive changes relative to the 2008 WHO classification are really cosmetic in nature for the most part. Instead of refractory anemia as part of the 2008 classification, we have MDS with single-lineage dysplasia, for example. This is really to serve a purpose for clarifying the diagnosis and the extent of dysplasia observed. Now this stands in contrast to the extensive genomic understanding of myeloid malignancies and myelodysplasia in general that has been accumulated over the last several years. Despite the significant advances in genomics, the WHO classification changes are really marginal.

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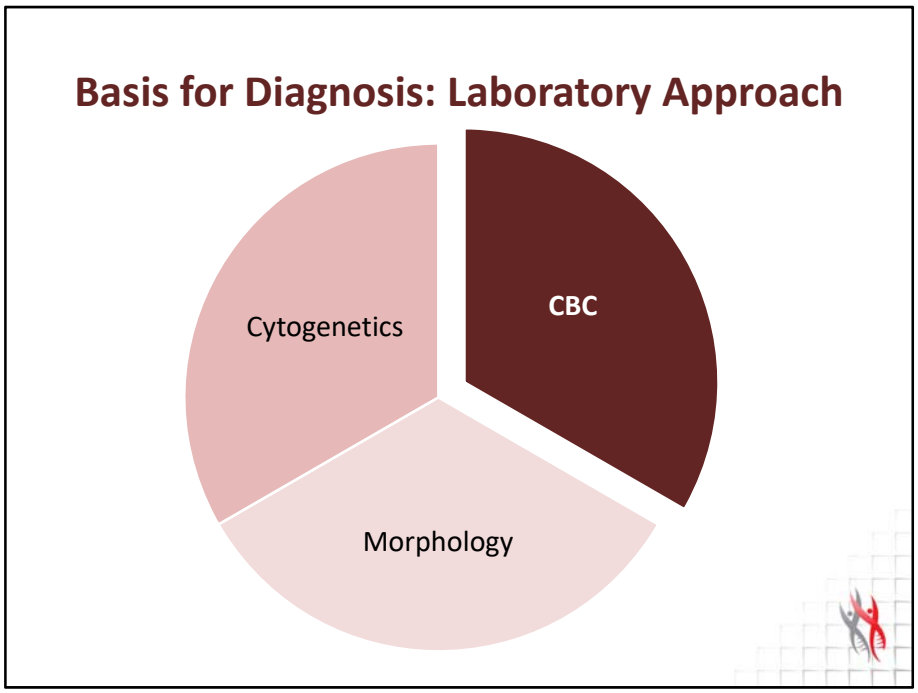

As we take a step back and look at the basis for a diagnosis of myelodysplastic syndrome, I want to remind us that in terms of the laboratory approach, there are three main parameters which have been used for the last many years. These, combined with clinical history and clinical findings, have been the basis for MDS diagnosis. Now we will start with the CBC.

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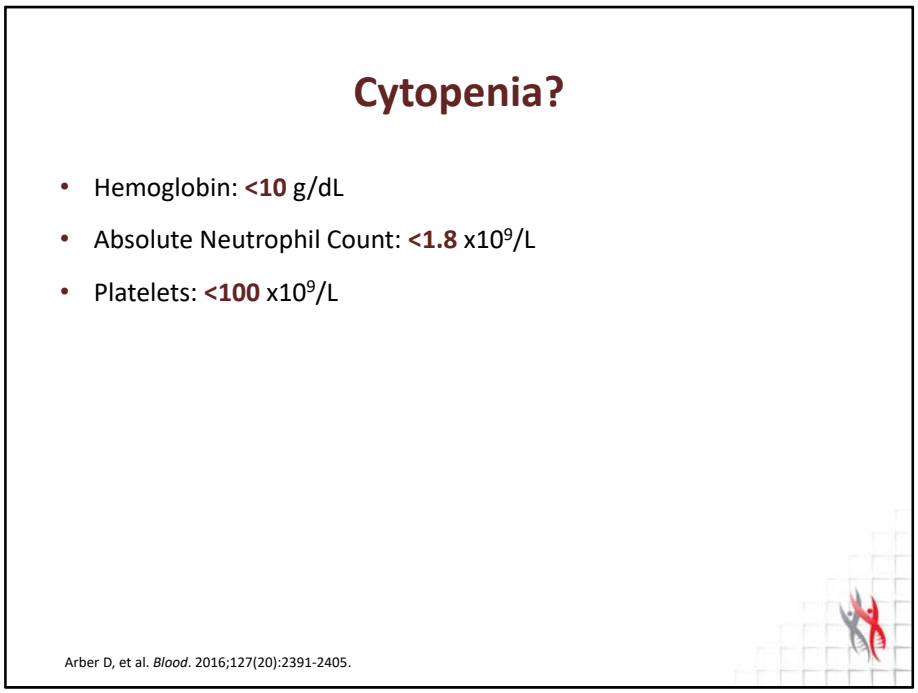

Cytopenias are the hallmark of MDS and are thus required for a diagnosis; so one or more cytopenias is necessary. Patients with bona fide MDS typically have significant cytopenias. By this I mean a hemoglobin value less than 10 g/dL, absolute neutrophil count less than 1.8 or 1800 per microliter, or a platelet count less than 100,000 per microliter. This is to say that borderline cytopenia should not necessarily lead us to think about myelodysplasia first. In fact, borderline cytopenias are very rare in true cases of myelodysplasia.

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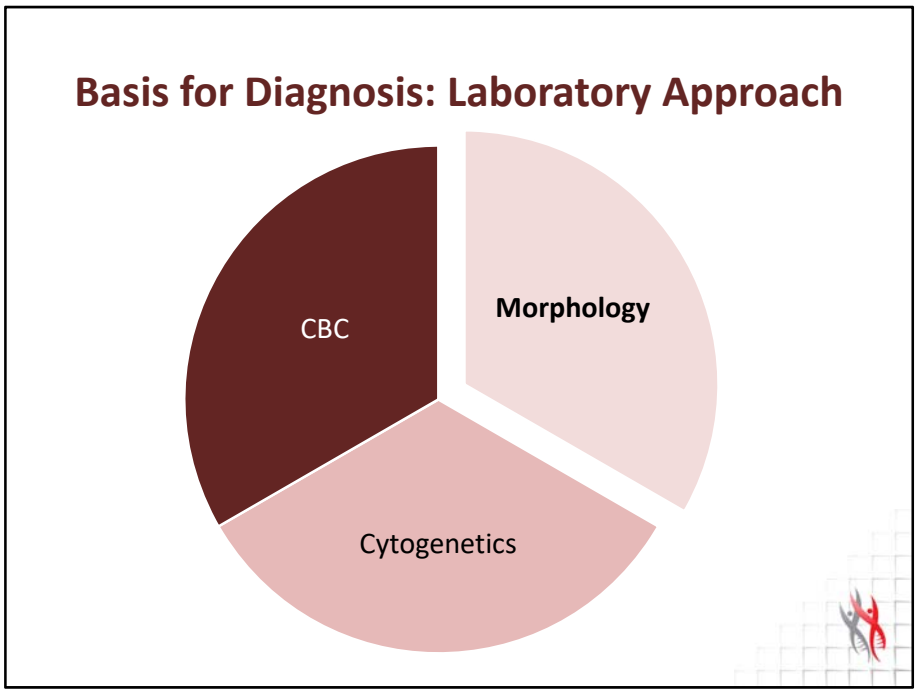

Morphology is the second mainstay for MDS diagnosis.

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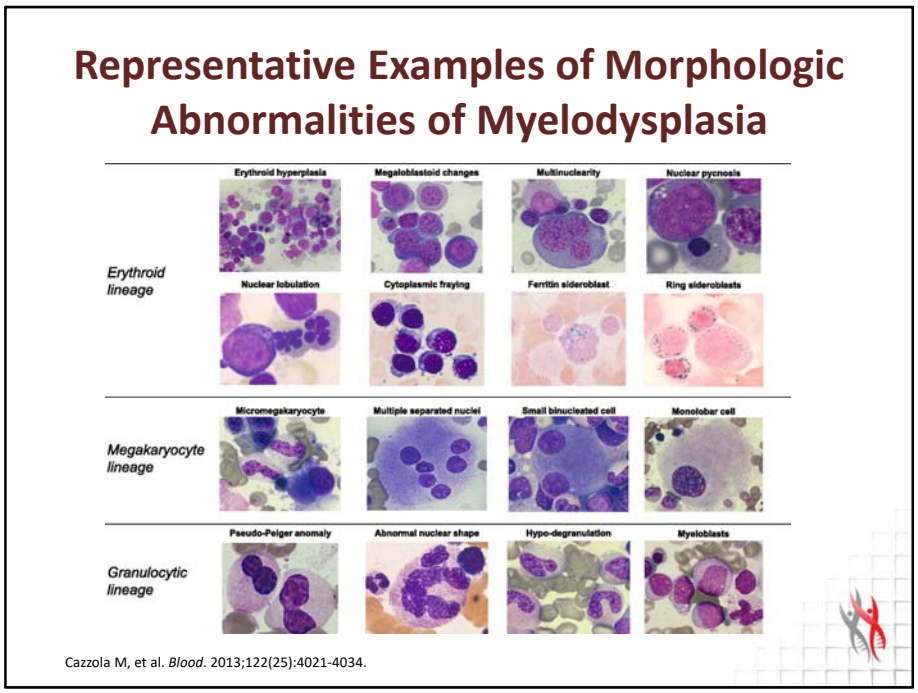

Here is a slide reminding us of the various types of morphologic or microscopic findings we can observe in myelodysplasia. This is the basis for bone marrow evaluation and microscopic evaluation of each of the three hematopoietic cell lineages including the erythroid, megakaryocyte, and granulocyte lineages. You will note that some of these findings are quite subtle and involve assessment of fine nuclear or cytoplasmic cellular details.

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Limits of Morphology

- Dysplasia may not necessarily = MDS
- Patients with MDS may not show definitive morphologic evidence of dysplasia
- Dysplasia is not entirely reproducible among pathologists
- Sample quality

Cazzola M, et al. *Blood*. 2013;122(25):4021-4034.

Morphology has significant limitations. In one way, we can think of morphology as being very limited in terms of sensitivity and specificity, so this is to say that dysplasia may not necessarily mean that a patient has MDS. The reason for this is that we know that patients with secondary cytopenias such as hemolytic anemia or autoimmune thrombocytopenia may show morphologic findings which are very similar or, in fact, identical to those which can be observed in myelodysplastic syndrome. Conversely, patients with MDS may not show morphologic evidence of dysplasia, so there is a real lack of potential sensitivity and specificity to morphology in the diagnosis of MDS. Third, and this is a frequent source of frustration among treating physicians, is that dysplasia is not entirely reproducible among pathologists, and there are several reasons for this. One of the major ones includes sample quality variations. Hemodiluted bone marrow aspirate smears, for example, are very difficult to evaluate and may lead to interobserver lack of reproducibility in the diagnosis.

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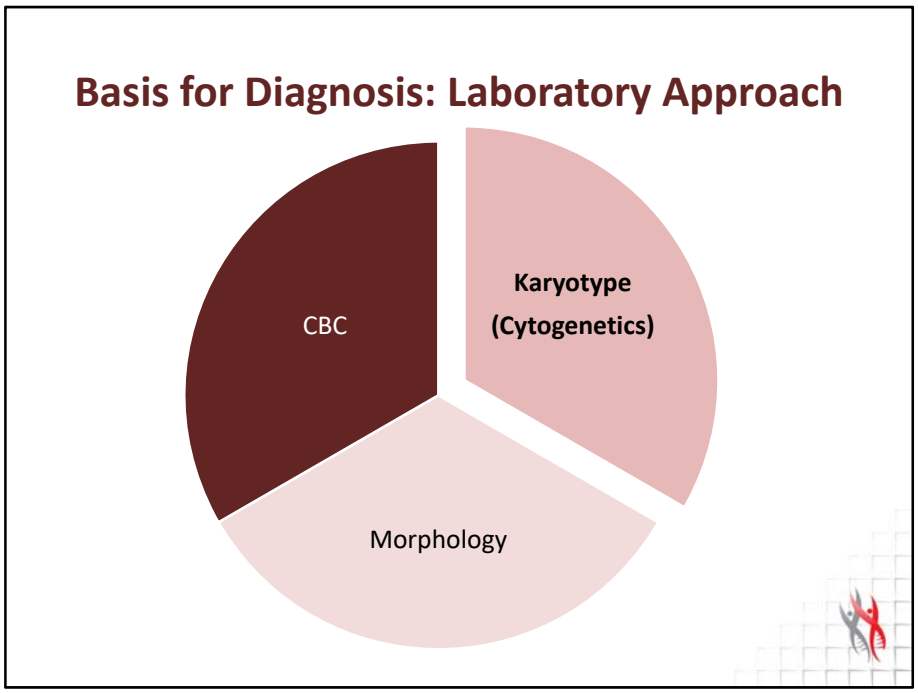

The third mainstay in the diagnosis of MDS has been a conventional karyotype.

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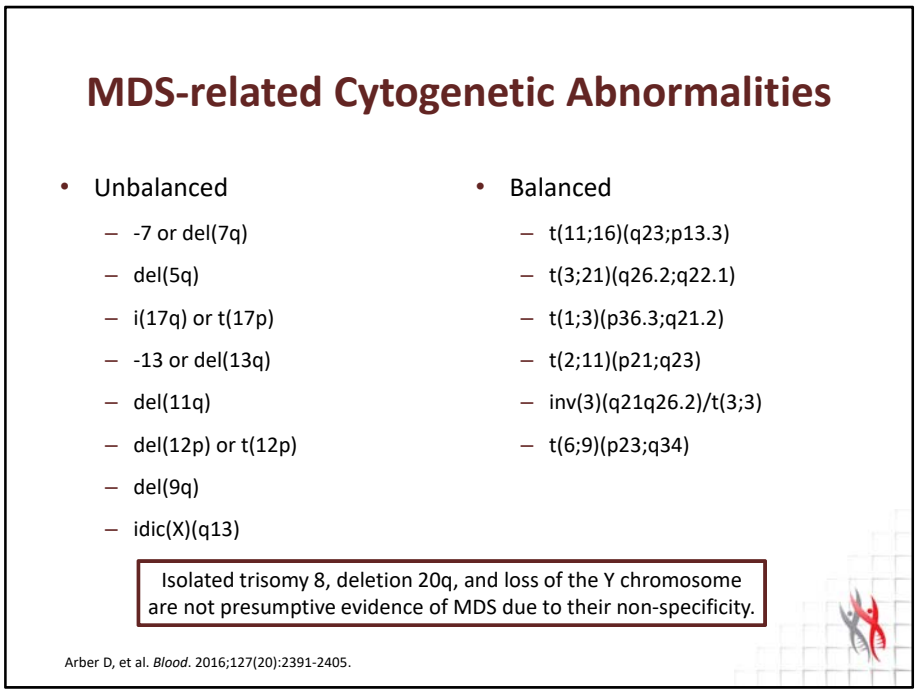

This is because there are a number of cytogenetic abnormalities which can be detected by a routine karyotype which can serve as a presumptive diagnosis of MDS even in the absence of morphologic criteria. These can be balanced rearrangements or unbalanced abnormalities, mainly loss of chromosomal material. However, and this is a theme with ancillary testing, there are limitations here which are important to note. First and foremost is that up to 50% of patients with true MDS may have a normal karyotype. In addition, there are karyotypic findings which are not necessarily specific for myelodysplastic syndrome. These include trisomy 8, del(20q), and loss of the Y chromosome in older males, which cannot be used as presumptive diagnosis of MDS in the way that those listed here in this table can be.

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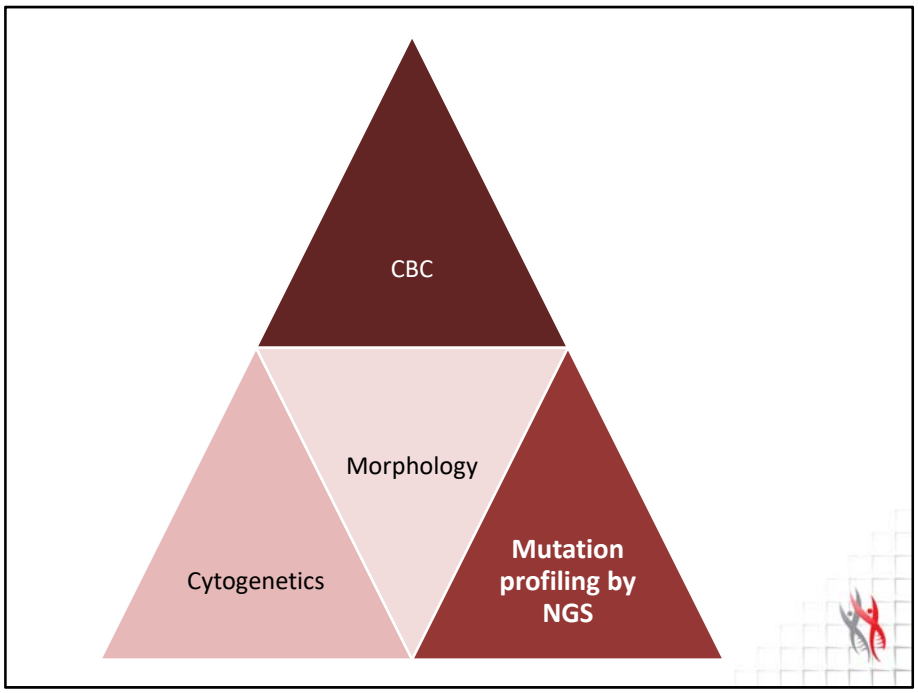

With that being said, there is considerable excitement about the availability of mutation profiling by NGS which has increased considerably in the recent years. There have been huge advances in genomics which have allowed us to sequence nucleic acids in a very high throughput fashion, which allows us to make tests which sequence a large number of genes at the same time using the same assay for reasonable costs. You may think this is a magic bullet for MDS diagnosis, and I will show you that the picture is a little bit more complicated; but mutation profiling by NGS nevertheless is a very useful diagnostic tool in assessing patients with suspicion for myelodysplastic syndrome.

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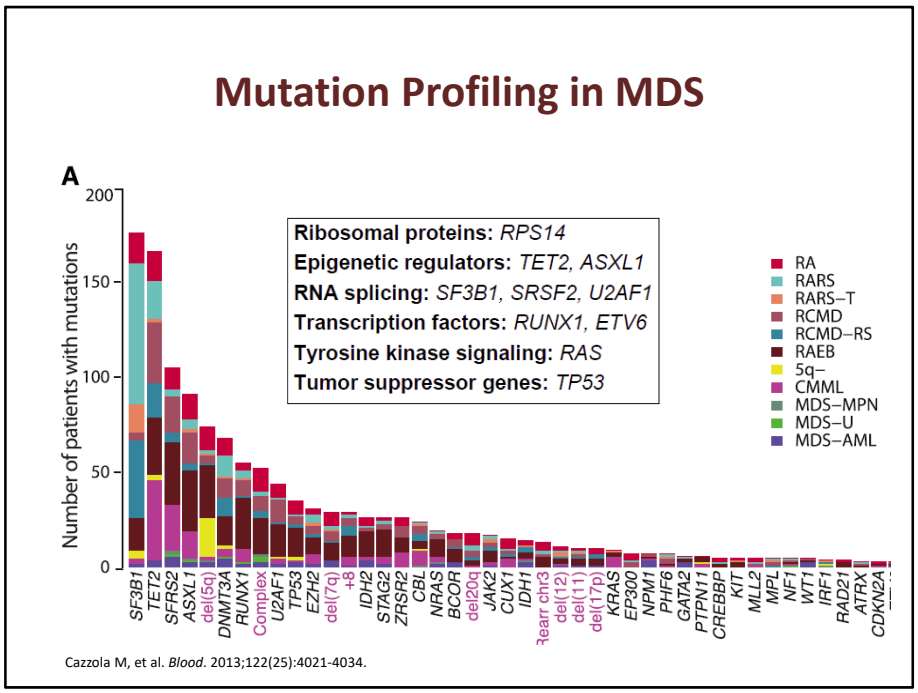

This is largely due to what we know are the variety of somatic mutations which are present in patients with myelodysplastic syndrome. This figure shows that there is a large amount of genetic heterogeneity in myelodysplastic syndrome; but there are recurrent mutations in genes involved in epigenetic regulation, RNA splicing, transcription factors, and tumor suppressor genes. These commonly include genes such as TET2, ASXL1, SF3B1, SRSF2 and TP53, for example.

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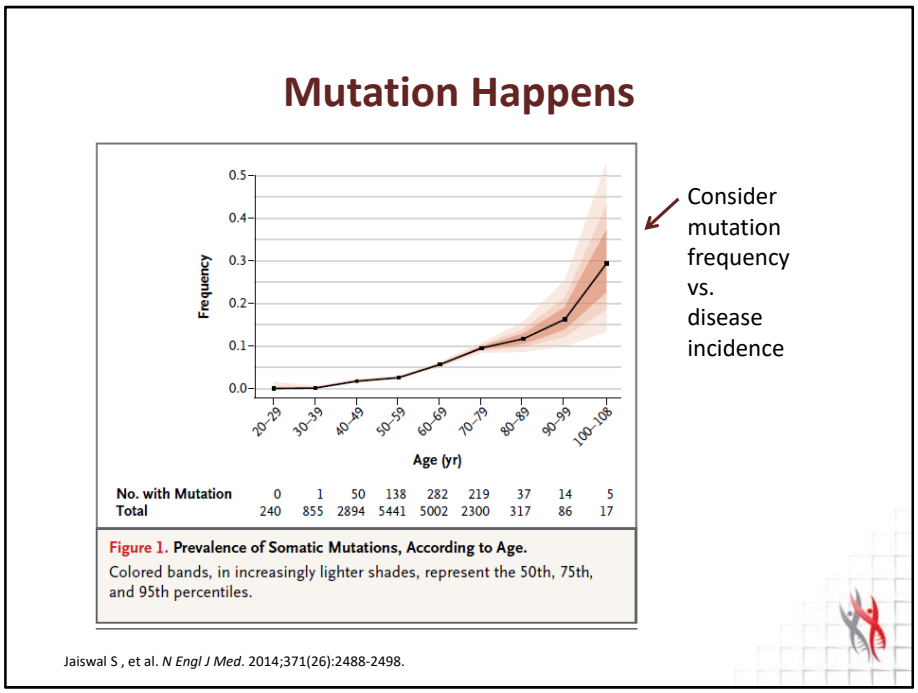

However, we should note that these gene mutations can occur and are detectable in patients with normal hematopoiesis, who lack cytopenias, and yet show clonal hematopoiesis. We know this as a result of two large population cohort-based studies in which somatic mutations were detected in normal individuals as a function of age. As we look at older individuals in the age range of 70 to 80 years, for example, we observe that up to 10% of them will demonstrate a detectable somatic mutation identical to that which we might observe in a patient with MDS. If we consider the mutation frequency as a function of age, versus the disease incidence of myelodysplastic syndrome, we see that the incidence of mutation is greater than that of the disease incidence.

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Clonal Hematopoiesis of Indeterminate Potential (CHIP)

- No morphologic evidence of malignancy
- Exclude PNH, MGUS, MBL
- Presence of a somatic mutation associated with myeloid malignancies
  - ***DNMT3A, TET2, ASXL1,*** *SF3B1, TP53, JAK2, CBL, BCOR, BCORL1, SRSF2*
  - Variant frequency at least 2%, median = 9%
  - Most patients with one gene mutation, <10% with two
- Risk of progression ~1% per year

Steensma DP, et al. *Blood.* 2015;126(1):9-16.

This has led us to a new term in hematology called CHIP, or clonal hematopoiesis of indeterminate potential. This is a preclinical state which we can think of as analogous to monoclonal B cell lymphocytosis and CLL, or monoclonal gammopathy of undetermined significance and plasma cell neoplasms such as myeloma. The criteria for making a so-called diagnosis (again this is a preclinical state without symptoms, so it should not really be thought of as a pathologic state) are that there is no morphologic evidence of malignancy, yet there is a detectable somatic mutation in the gene which is commonly mutated in myeloid malignancies such as MDS. Most commonly this will involve genes related to epigenetic regulation such as DNMT3A, TET2, or ASXL1. Less commonly, other genes including SF3B1 (a spliceosome-related gene), TP53 (the tumor suppressor), and others may be observed in CHIP. Now the mutations in CHIP are generally low-level mutations, which is to say that they demonstrate variant allele frequencies less than 10%. More on variant allele frequencies in a bit. In addition, most patients with CHIP will demonstrate only a single gene mutation. A few, approximately less than 10% based on those population cohort studies, will show two mutations. However, having greater than two somatic mutations is extremely rare in CHIP; and we can use this in diagnosis as I will discuss later. Importantly, the risk of progression to overt hematologic malignancy in CHIP is greater than the normal population and is approximately 1% per year, similar to the other preclinical states which I described previously. In addition – perhaps surprisingly – there was observed an overall increased risk of mortality as a result of CHIP; and this is likely due to cardiovascular events and may be related to cytokine-related inflammation.

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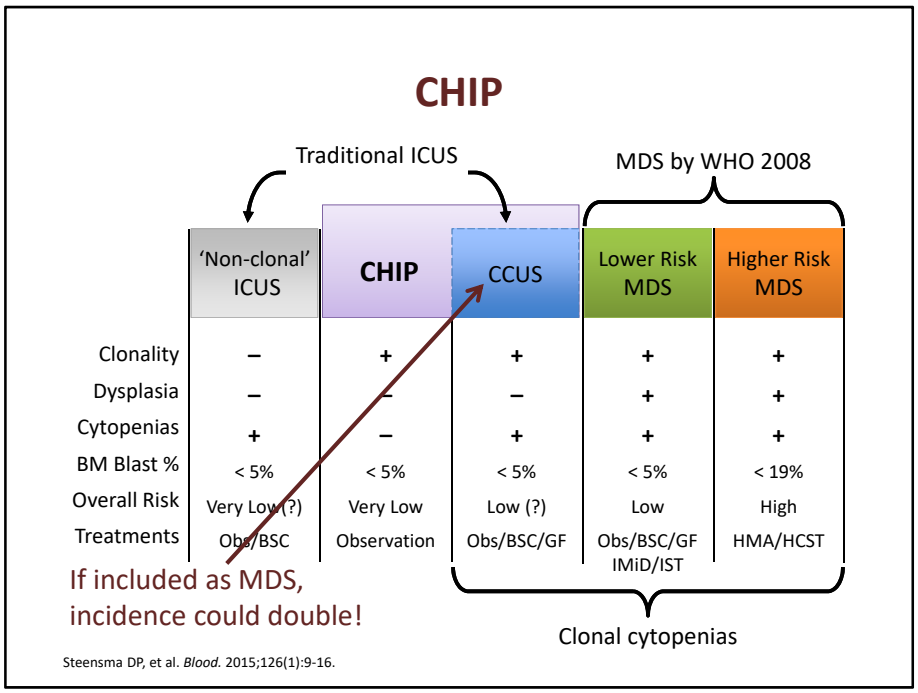

While CHIP is easy enough to distinguish from MDS because it lacks the cytopenias which are the diagnostic hallmark of MDS, there is a closely related but distinct condition called CCUS (clonal cytopenias of undetermined significance) which is a real diagnostic challenge and needs to be differentiated from myelodysplastic syndromes. I will go on to discuss a few features which can help us make a diagnosis of MDS in that setting. CCUS is a setting in which a patient has significant cytopenias but does not show morphologic dysplasia, and typically one or more somatic mutations is detected by next-generation sequencing. This is a situation in which if dysplasia was present, diagnosis of MDS could be made but there is insufficient morphologic evidence of dysplasia for definitive diagnosis. Note that if patients with CCUS were considered to be MDS patients, the incidence could double, so this is clearly a gray area in which there are some subtle issues that need to be considered, and I will discuss a few of these.

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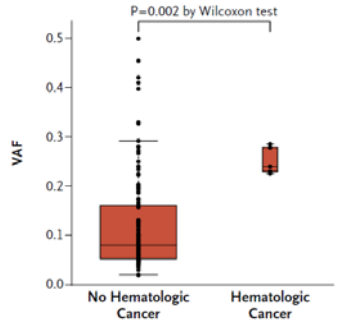
How Do Variant Allele Fractions (VAFs) Help?

- Somatic vs. germline
- Cutoff for clinical relevancy?
- VAF >10% appears less common in CHIP



Jaiswal S, et al. *N Engl J Med*. 2014;371(26):2488-2498.

One is the whole concept of variant allele frequencies, which will be included on your next-generation sequencing panel results in most cases. Variant allele fraction (VAF) is simply the number of sequencing reads in which the mutation of interest is present, as a percentage or a fraction of the total number of sequencing reads at that region of interest. VAFs can tell us a few things. One is that it can give us a hint about whether the mutation in question is somatic or germline. Somatic mutations tend to be less than 50%, assuming they are autosomal chromosomes; whereas germline mutations will be present at a variant allele frequency of approximately 50% or, if they are on an X chromosome in a male, 100%. VAFs can be helpful in the context of differentiating clonal hematopoiesis from myelodysplastic syndrome because in patients with CHIP and CCUS, the VAFs tend to be lower. Particularly in patients with simple CHIP, the variant allele fraction is usually less than 10%; whereas in patients with MDS, the variant allele fractions tend to be higher.

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Negative Predictive Value

- Greater than 85% of patients with MDS have one or more somatic mutations
- If diagnosing MDS, a negative NGS panel result should prompt re-evaluation of other causes of cytopenia(s)

Papaemmanuil E, et al. *Blood.* 2013;122(22):3616-3627.; Steensma DP, et al. *Blood.* 2015;126(1):9-16.

Next generation sequencing panel testing allows us additional information in the diagnostic workup of patients with possible myelodysplastic syndrome, even when the results are negative or no variants were detected. This is because the vast majority of MDS patients, approximately 85%, will show one or more somatic mutations using one of these gene panels. Therefore, a negative result has negative predictive value for a diagnosis of myelodysplastic syndrome. In these cases in which a patient suspected of having MDS is worked up and is negative by mutation panel testing as well as conventional cytogenetics, there should be an assessment of possible secondary causes of cytopenias, and those should be excluded clinically.

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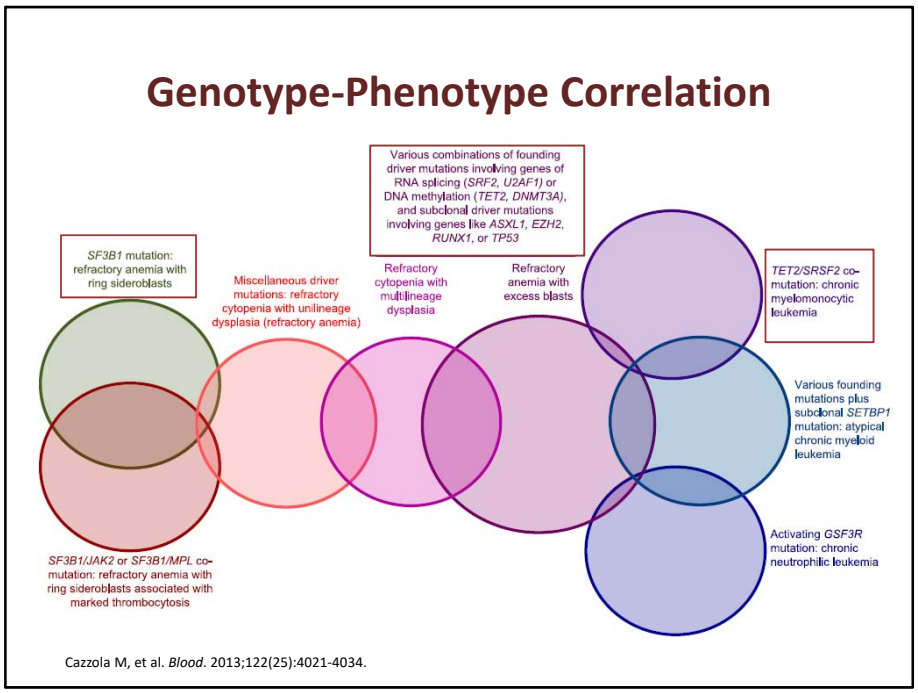

One of the most specific findings in panel testing which can be used to make a diagnosis of MDS are patterns of mutations which have high specificity. For example, co-expression of TET2 and SRSF2 has a high positive predictive value for diagnosis of chronic myelocytic leukemia and MDS/MPN overlap syndrome. SF3B1, a spliceosome gene, is closely related to the presence of ring sideroblasts in myelodysplastic syndrome. In fact, co-mutation of specific patterns of genes, typically CHIP-related genes such as DNMT3A, TET2, or ASXL1 along with spliceosome genes such as SRSF2, U2AF1, or ZRSR2, for example, has a high positive predictive value for a diagnosis of myelodysplastic syndrome. While single-gene mutations may be observed in CHIP or CCUS, there are specific combinations and specific patterns of gene mutations which we can look for which have higher positive predictive value for a true diagnosis of MDS.

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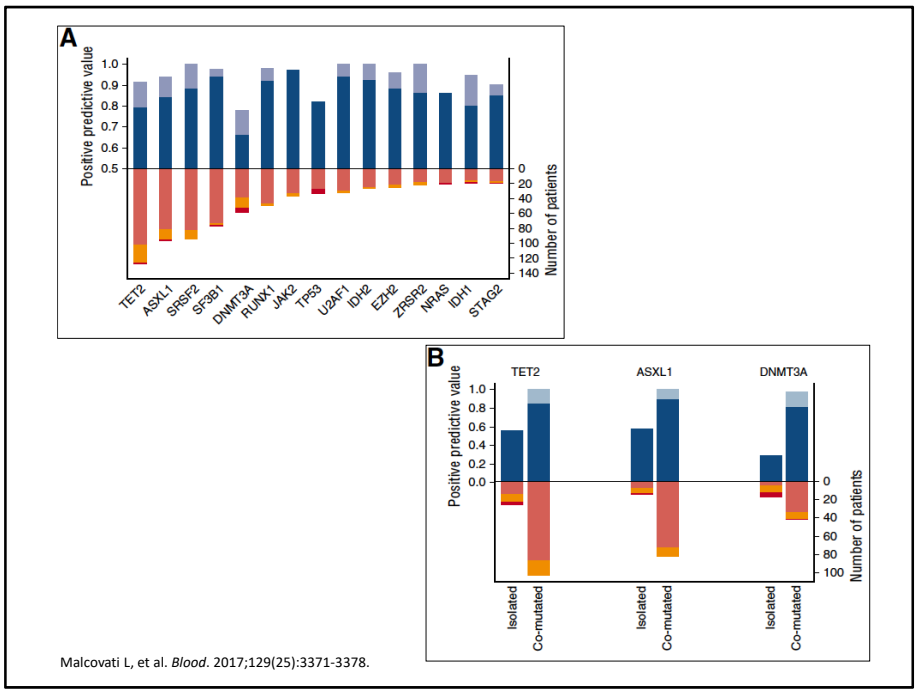

Malcovati L, et al. *Blood*. 2017;129(25):3371-3378.

Recent studies have clarified this point. This figure shows that certain genes have higher positive predictive values for a definitive diagnosis of myeloid neoplasm, in this case MDS, than others. Genes related to the spliceosome machinery (of which we have mentioned several but most commonly would involve SRSF2, SF3B,1 and U2AF1) have a generally high positive predictive value for a diagnosis of MDS. Especially when they are seen in combination with TET2, ASXL1, or DNMT3A, the positive predictive value is in the high 90% range. A result such as that would be highly suspicious for a diagnosis of MDS, even in the absence of cytogenetic evidence, for example.

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"Rule-out MDS"

- 68-year-old female, no significant PMH
- CBC: Hb 9.8 g/dL, ANC 1.7 k/mcL, Plts 150 k/mcL
- PB: normocytic, rare Pelgeroid neutrophil
- BM: 40-50% cellularity, mild dyserythropoiesis (~10%), rare hypogranular neutrophils (<10%), normal megakaryocytes
- Karyotype and MDS FISH panel: normal
- **Myeloid malignancies NGS panel…**

With all that being said, I would like to run through a clinical scenario in which we can apply some of this information in a diagnosis of MDS. This is a 68-year-old woman without a significant past medical history, and she presents with cytopenias. In particular, she has a moderate normocytic anemia and a hemoglobin of 9.8 g/dL, absolute neutrophil count of 1.7 thousand per microliter, and a platelet count of 150,000 per microliter. A bone marrow biopsy was performed which showed some mild hypercellularity of 40% to 50%, as well as mild dyserythropoiesis estimated by the pathologist as 10%, and some rare hypogranular neutrophils less than 10%, as well as normal megakaryocytes. We will note that these dysplastic morphologic features are really at or below the threshold defined by the WHO for a definitive diagnosis of myelodysplastic syndrome; greater than 10% dysplastic cells in one or more lineages. I would summarize these findings so far as being a little bit ambiguous, so they are not definitive. Prior to myeloid malignancies NGS panel testing, the next step would be to look for karyotypic or MDS FISH abnormalities. In this case, they are normal so here we get a chance to use NGS in a diagnostic scenario.

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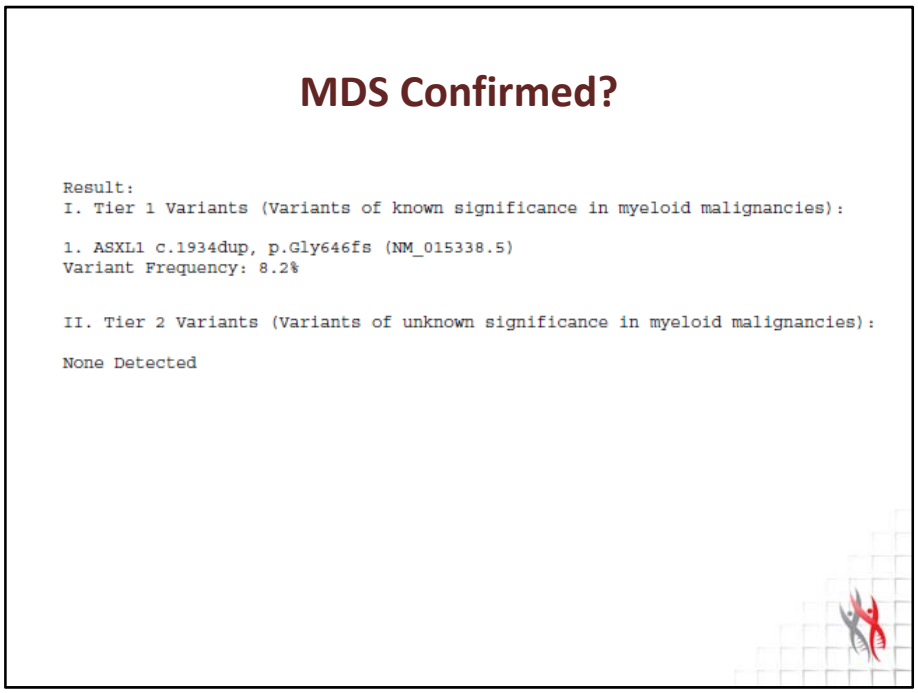

Now suppose that our NGS panel results were as follows: a single ASXL1 mutation is detected. This is a typical exon 12 frameshift mutation which is seen in myeloid malignancies, but note that the variant allele frequency is relatively low here; approximately 8%. The ASXL1 mutation is the only mutation that was observed in this assay. The question would be: do these results confirm a diagnosis of MDS? Given what we have learned today, I think the answer would be: not necessarily. This patient has cytopenias, has some mildly dysplastic morphologic features, and has a somatic mutation detected by NGS; but all of these things could be compatible with a diagnosis of clonal cytopenia of undetermined significance. The differential here still includes MDS as well as preclinical state such as CCUS. Patients like this should be followed clinically and are at an increased risk for development of overt MDS.

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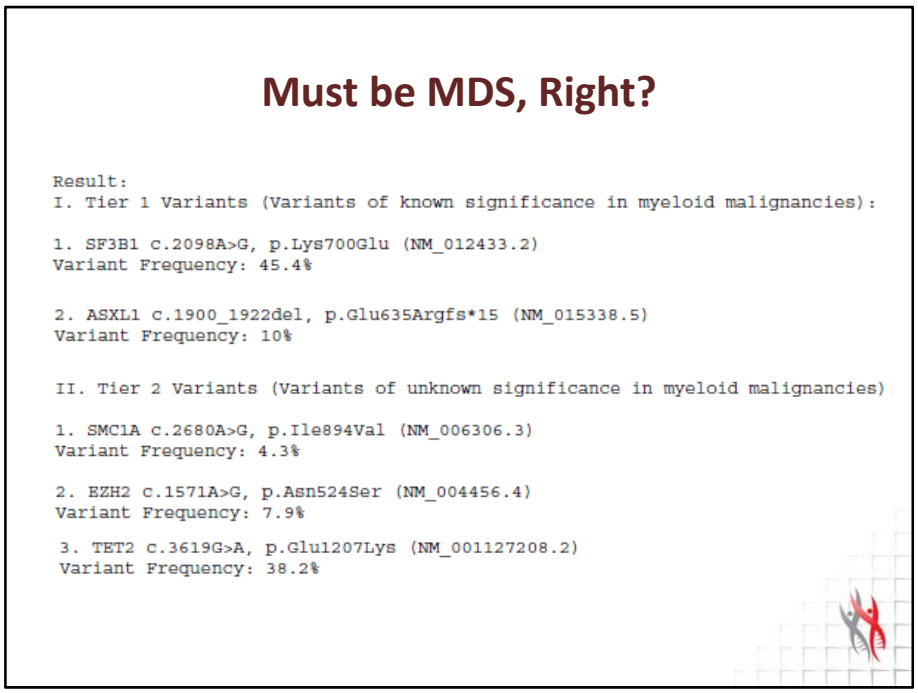

Let us imagine that this set of NGS results was obtained for our patient. In this scenario, we have mutation of a spliceosome gene, SF3B1. This is a recurrent lysine 700 residue which is mutated; it is commonly mutated in patients with MDS as well as other myeloid malignancies. The variant allele fraction is high: it is 45%, which suggests that nearly all of the hematopoietic precursors that the patient has are SF3B1 mutated. In addition, there is another mutation of ASXL1, again a typical exon 12 frameshift mutation which has a lower variant allele frequency at 10%. In addition, there are three variants of uncertain significance at various allele frequencies which are not informative in this scenario. This patient must have MDS, right? I think in this case, the NGS findings are very helpful and are highly suggestive of a diagnosis of MDS. Here, we have co-mutation of a spliceosome gene and DNA methylation or chromatin modification gene ASXL1. Again, that combination is highly specific for a diagnosis of MDS and the patient can be managed as such.

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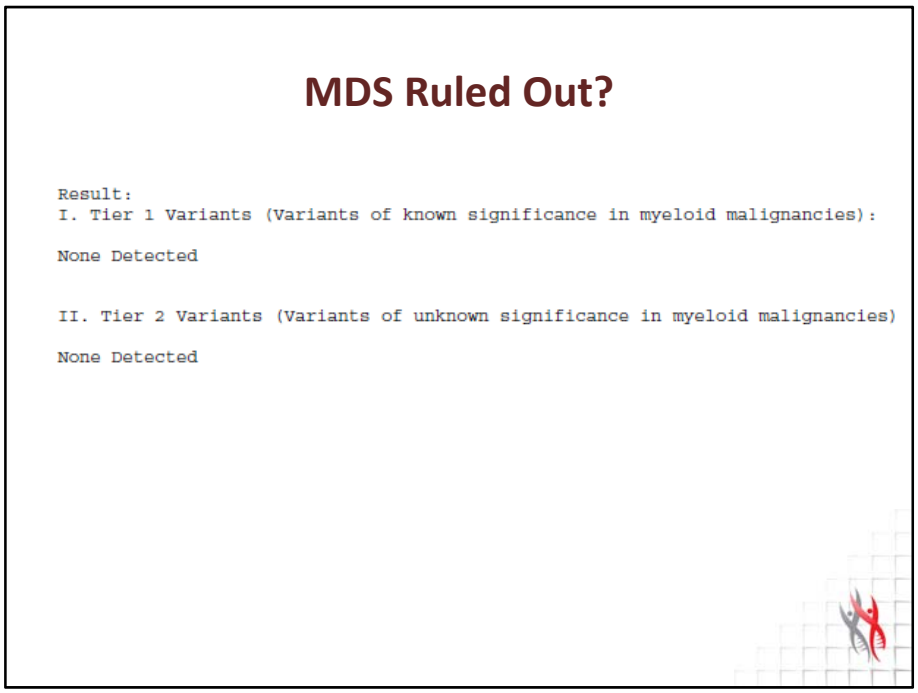

Let us imagine that our NGS test results were as follows: there were no variants detected. This is likely showing us that there is a high negative predictive value for a diagnosis of MDS in the context of this patient with a normal karyotype, negative FISH studies, and no morphologic findings that were definitive. This should prompt us to think about other secondary causes of MDS and rule them out using clinical and laboratory means. These patients should be followed for subsequent resolution of their cytopenias and treated supportively.

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Key Points

- MDS remains a genetically heterogeneous, challenging to diagnose hematologic cancer
- Most MDS patients demonstrate somatic mutation(s) by NGS, but these alone may not be diagnostic (ie, CHIP or CCUS)
- Specific gene mutations or patterns of co-mutation have high positive predictive value for MDS
- Data is accumulating to support the incorporation of NGS findings in MDS diagnostic criteria and prognostic scoring systems – stay tuned

To conclude, I would like to leave you with these key takeaway points. MDS remains a genetically heterogenic, challenging-to-diagnose hematologic cancer. Most MDS patients demonstrate somatic mutation by NGS, but these alone may not be diagnostic unto themselves. This is because of known preclinical states such as CCUS and CHIP in which somatic mutations can be detected in patients without bona fide disease. Specific gene mutations or patterns of co-mutation have high positive predictive value for MDS and can be used to make a diagnosis in select patients. Lastly, data is accumulating to support the incorporation of NGS findings in MDS diagnostic criteria, such as the WHO criteria in the future and prognostic scoring systems such as the International Prognostic Scoring System or IPSS, so stay tuned.