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Targeted Sequencing Informs the Evaluation of Normal Karyotype Cytopenic Patients for Low-Grade Myelodysplastic Syndrome

Eric J. Duncavage[1], **Jennifer O'Brien**[1], **Kiran Vij**[2], **Christopher A. Miller**[3], **Gue Su Chang**[3], **Jin Shao**[2], **Meagan A. Jacoby**[2], **Sharon Heath**[2], **Megan R. Janke**[2], **Kevin Elliott**[2], **Robert S. Fulton**[3,4], **Catrina Fronick**[3], **Michelle O'Laughlin**[3], **Peter Westervelt**[2], **Timothy J. Ley**[2,3,4], **Richard K. Wilson**[3,4,5], and **Matthew J. Walter**[2,4]

[1] Department of Pathology and Immunology, Washington University, St. Louis, MO

[2] Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, Missouri

[3] The McDonnell Genome Institute, Washington University, St Louis, Missouri

[4] Department of Genetics, Washington University School of Medicine, St Louis, Missouri

[5] Division of Genomics and Bioinformatics, Department of Medicine, Washington University in St Louis, St Louis, MO

The diagnosis of myelodysplastic syndrome (MDS) requires persistent cytopenias, not otherwise explained, and evidence of morphologic dysplasia in the bone marrow. Low-grade MDS (bone marrow blasts <5%) has morphologic dysplasia in at least 10% of cells in one or more cell lineages.(1) Low-grade MDS is particularly challenging to diagnose, as no definitive criteria for morphologic dysplasia exist and evaluation may be subject to high inter-observer variability.(1-3) The ability to diagnose low-grade MDS can be improved by incorporating cytogenetic evaluation of the bone marrow, especially in the setting of equivocal morphologic dysplasia. However, many MDS cases (up to 60%) lack cytogenetic abnormalities, limiting the overall utility of cytogenetics as a diagnostic adjunct.(4)

Multiple studies have demonstrated that the majority of MDS patients (~80% in some studies) harbor recurrent somatic mutations in a group of 20-30 genes.(5-7) Further, some gene mutations confer an adverse prognosis independent of clinical scoring systems.(5, 6, 8) We sought to determine whether targeted DNA sequencing of recurrently mutated MDS genes could be a useful adjunct in the diagnostically challenging subgroup of cytopenic

Corresponding Author: Eric J. Duncavage, MD, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8118, St. Louis, MO, 63110, EDuncavage@path.wustl.edu, Phone: 314-747-2007.

Protection of Human Subjects:
All patients sequenced as part of this study were fully consented for sequencing studies.

Conflicts of Interest:
E.D. is a technical consultant for PierianDx, Cofactor Genomics, and Molecular Health, none of which were involved in this study. The remaining authors have no conflicts of interest.

patients with low blast counts and a normal karyotype, thereby identifying a subset of patients that may potentially be at a higher risk of developing MDS or AML.

We screened 599 patients who presented between 1/2002 and 11/2015, consented for sequencing studies on a protocol approved by the Human Research Protection Office at Washington University, and had banked bone marrow and control tissue (skin). Forty-three patients were selected based on 1) stringent cytopenia criteria (WBC <1,800/μL, hemoglobin <10g/dL, platelets <100k/μL) in at least one lineage, 2) bone marrow blasts <5% by flow cytometry and/or morphologic evaluation (and had slides available for review) 3) WBC <14k/uL, 4) non-clonal metaphase cytogenetics, and 5) absence of prior therapy for MDS (**Table 1**). Bone marrow specimens were independently reviewed (blinded) for blast count and dysplasia by two board-certified hematopathologists (ED and KV) and the percentage of dysplastic cells in the myeloid, erythroid, and megakaryocytic lineages enumerated. Dysplasia was binned into categories of <10%, 10-20%, 21-50%, and >50%. Definitive dysplasia was established when both pathologists identified dysplasia in ≥10% of cells in at least one lineage. Equivocal dysplasia was rendered when there was disagreement over the identification of ≥10% dysplasia in at least one lineage. No dysplasia was rendered when both pathologists agreed that dysplasia was <10% in all lineages. Genomic DNA was extracted from bone marrow and skin (as a source of normal DNA) and enriched for the coding exons of a panel of 284 commonly mutated myeloid genes (**Supplementary Table 1**).(5-8) DNA was extracted from aspirate coverslips for follow-up cases when cryopreserved cells were not available. Libraries were sequenced on a HiSeq 2500 (Illumina, San Diego, CA) instrument with 2×101 bp reads. The resulting data was analyzed for single nucleotide variants (SNVs) and insertions/deletions (indels), using standard analysis pipelines in paired normal mode, as previously reported.(9) To reduce false positive calls, only variants with ≥5 variant reads, ≥50x total coverage in marrow and skin samples, ≥5% variant allele fraction (VAF, variant reads/total reads) in the marrow, not present in dbSNP (unless known canonical somatic hotspot mutations), and that resulted in protein coding changes were conservatively included in the analysis. Copy number alterations (CNAs) and loss of heterozygosity were called using the CopyCAT2 package.

Mean unique coverage depth was 265x for primary bone marrows, 252x for skin, and 388x for follow-up coverslips. Of the 43 sequenced cases, 29 had a coding-region somatic mutation in at least one gene (mean 2.8 mutations/case, range 1-8 mutations/case). The most commonly mutated gene was *SRSF2* (8 cases), followed by *TET2* (7 cases), *SF3B1* (6 cases), and *U2AF1* (6 cases), (**Figure 1a**). Of the 284 sequenced genes, 40 were mutated in at least one case, and 12 were mutated in 2 or more cases. The mean VAF of SNV mutations was 29.9% (range 5-98%). Co-occurrence data is presented in **Supplementary Figure 1**.

Morphologic review of bone marrow demonstrated definitive dysplasia (≥10% of cells in at least one lineage) made by two pathologists in 28 cases, establishing the diagnosis of MDS. No significant dysplasia (<10% in any lineage) was seen in 8 cases, and equivocal dysplasia (where hematopathologists did not agree that dysplasia was present in ≥10% cells in at least one lineage) in 7 cases. Twenty-one of 28 cases (75%) with definitive dysplasia (i.e., MDS) and normal cytogenetics had a somatic coding region mutation in at least one gene. Three of 8 cases (37.5%) without dysplasia had mutations and 5 of 7 (71%) cases with equivocal

dysplasia harbored somatic mutations (**Figure 1b**). There was no significant difference in mutation VAFs or maximum VAF per patient between the dysplasia and no dysplasia groups (**Figure 1c**). Cases with dysplasia or equivocal dysplasia had more mutations per case than those without dysplasia (p=0.018 and p=0.036, respectively) (**Figure 1d**). The fraction of cases with mutations tended to be higher for the dysplasia versus no dysplasia group (p=0.086) (**Figure 1b**). No copy number altered regions were detected, although UPN609948 showed copy-neutral loss-of-heterozygosity on chromosome 7 (**Supplementary Figure 2)**.

Mutations were detected in 8 patients with equivocal (n=5) or no dysplasia (n=3) and 6 of these 8 patients developed high-grade MDS or had persistent cytopenias requiring pharmacologic treatment. Follow-up data from 5 patients with equivocal dysplasia and somatic mutations showed that 2 developed blast counts >5% (UPN568547, UPN976842) with persistence of mutations and 3 received MDS treatment. UPN701797 had persistent anemia responsive to erythropoietin, UPN724989 was responsive to filgrastim, and UPN728125 had cytopenia improvement following decitabine treatment (**Figure 1e**). Of the 3 patients with no dysplasia who had somatic mutations, UPN204802 was treated with erythropoietin, UPN859688 subsequently died due to multiple comorbidities without MDS, and UPN529198 had severe iron deficiency anemia secondary to short bowel syndrome (responsive to intravenous iron) and a persistent *TET2* mutation without MDS (**Figure 1e**).

No mutations were detected in 7 patients with equivocal (n=2) or no dysplasia (n=5) and only 2 of these 7 patients were empirically treated as MDS or diagnosed as MDS, and none progressed to high-grade MDS. The 2 patients with equivocal dysplasia and no mutations were diagnosed with hypereosinophilic syndrome (UPN786953) and anemia secondary to end stage renal disease without progression to MDS (UPN610864) (**Figure 1e**). Of the 5 patients with no dysplasia or somatic mutations, UPN577914 developed MDS with a non-clonal deletion on chromosome 7 after presenting with an autoimmune anemia. No mutations were identified on subsequent sequencing. UPN976020 was diagnosed with an autoimmune cytopenia that fully recovered and UPN163943 had count recovery with erythropoietin treatment. The remaining 2 patients were diagnosed with severe aplastic anemia and treated with an allogeneic bone marrow transplant and cyclosporin (UPN769282, UPN332207, respectively) (**Figure 1e**).

In this cohort, 5 of 7 (71%) cytopenic patients with blasts <5% and equivocal dysplasia had a somatic mutation in their bone marrow cells, similar to the frequency for cytogenetically normal MDS patients with blasts <5% (21/28, 75%). In contrast, somatic mutations were detected in 3 of 8 cases (37.5%) without definitive dysplasia (**Figure 1b**). Patients with cytopenias and somatic MDS-associated mutations, but without definitive dysplasia, fit the newly described category of clonal cytopenia of undetermined significance (CCUS).(10) Kwok and colleagues showed that CCUS patients have a similar spectrum of mutated genes and VAFs as patients with bona fide MDS, similar to our findings.(11) Cargo and colleagues recently showed that 91% of ‘pre-diagnostic’ marrows from cytopenic patients who went on to MDS or AML harbored driver gene mutations, suggesting they progressed from an antecedent CCUS.(12) In contrast, the spectrum of mutations in our cohort differs from individuals with clonal hematopoiesis with indeterminate potential (CHIP) - defined by

mutations but no cytopenias - where 50% of cases have a *DNTM3A* mutation.(13, 14) In our study, *DNMT3A* was mutated in <5% of patients and *JAK2* and *TP53* were not mutated (genes observed in CHIP). The persistent *TET2* mutation in UPN529198 may represent a CHIP mutation.

In contrast to prior work by Cargo *et al* and Kwok *et al*, this study focused solely on the diagnostically challenging group of patients with cytopenias and normal cytogenetics (i.e., no evidence of clonal disease) and sequenced a larger number of myeloid associated genes using paired normal tissue to definitively call somatic mutations. Similar to Kwok *et al,* we show that while the mean VAF and maximum VAF is similar between patients with dysplasia and no-dysplasia, patients with dysplasia have an increased number of mutations per case. Further, using follow-up clinical data and subsequent bone marrow biopsies we show that it is more common for cytopenic patients with equivocal/no dysplasia and a gene mutation to be subsequently diagnosed or empirically treated for MDS compared to patients without a mutation (6/8 versus 2/7, respectively). The data suggest that the presence of a gene mutation in a cytopenic patient may be associated with increased risk of developing MDS and provide a rationale for future prospective studies.

Ultimately, sequencing-based evaluation may also provide a means for tracking tumor burden and monitoring patients for subsequent clonal expansion or development of definitive MDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. Jul 30; 2009 114(5):937–51. [PubMed: 19357394]
2. Malcovati L, Hellstrom-Lindberg E, Bowen D, Ades L, Cermak J, Del Canizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. Oct 24; 2013 122(17):2943–64. [PubMed: 23980065]
3. Senent L, Arenillas L, Luno E, Ruiz JC, Sanz G, Florensa L. Reproducibility of the World Health Organization 2008 criteria for myelodysplastic syndromes. Haematologica. Apr; 2013 98(4):568–75. [PubMed: 23065505]
4. Nybakken GE, Bagg A. The genetic basis and expanding role of molecular analysis in the diagnosis, prognosis, and therapeutic design for myelodysplastic syndromes. The Journal of molecular diagnostics : JMD. Mar; 2014 16(2):145–58. [PubMed: 24457119]

5. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. Feb; 2014 28(2):241–7. [PubMed: 24220272]

6. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. Sep 12.2013

7. Walter MJ, Shen D, Shao J, Ding L, White BS, Kandoth C, et al. Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. Leukemia. Jun; 2013 27(6):1275–82. [PubMed: 23443460]

8. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. The New England journal of medicine. Jun 30; 2011 364(26):2496–506. [PubMed: 21714648]

9. Cancer Genome Atlas Research N. Comprehensive molecular profiling of lung adenocarcinoma. Nature. Jul 31; 2014 511(7511):543–50. [PubMed: 25079552]

10. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. Jul 2; 2015 126(1):9–16. [PubMed: 25931582]

11. Kwok B, Hall JM, Witte JS, Xu Y, Reddy P, Lin K, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. Nov 19; 2015 126(21):2355–61. [PubMed: 26429975]

12. Cargo CA, Rowbotham N, Evans PA, Barrans SL, Bowen DT, Crouch S, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. Nov 19; 2015 126(21):2362–5. [PubMed: 26392596]

13. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. The New England journal of medicine. Dec 25; 2014 371(26):2477–87. [PubMed: 25426838]

14. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. The New England journal of medicine. Dec 25; 2014 371(26):2488–98. [PubMed: 25426837]

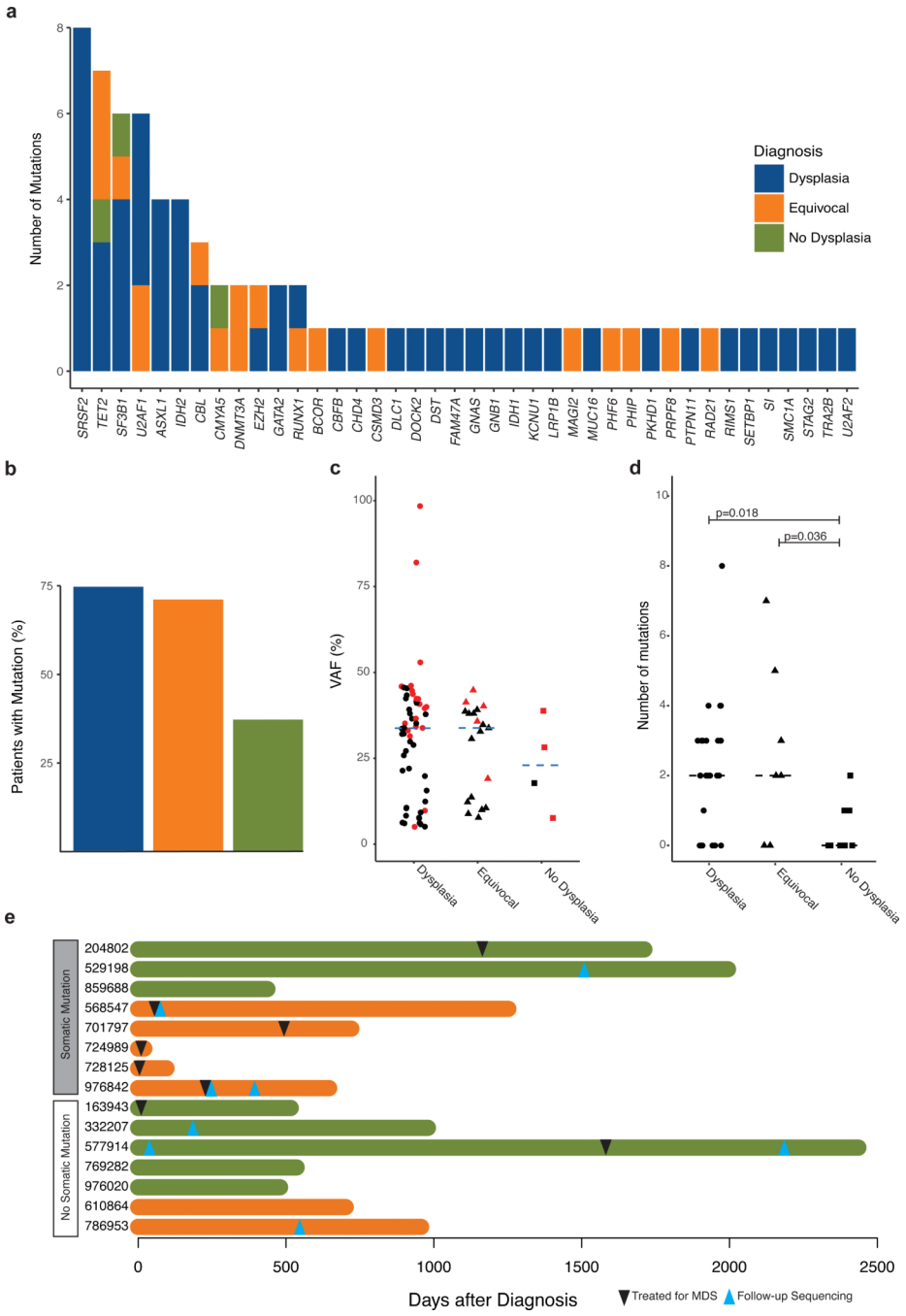

Figure 1. Gene mutations present in cytopenic patients and subsequent clinical outcome

(a) Distribution of recurrent gene mutations by morphologic category in cytopenic patients. A total of 40 genes were mutated at least once. Colors indicate the morphologic classification: dysplasia (blue), equivocal dysplasia (orange), no dysplasia (green). (b) Frequency of cases in each diagnostic category with at least one somatic mutation. (c) Somatic mutation variant allele fractions (VAFs) by morphologic category. Red points indicate the maximum VAF for each case; blue bars indicate the median VAF for each category. (d) Number of somatic mutations per case for each diagnostic category. There was

a significant difference between the number of mutations per case in the dysplasia vs. no dysplasia and equivocal dysplasia vs. no dysplasia categories (student's t-test); blue bars indicate median values. (e) Follow-up data for patients in the equivocal or no dysplasia categories grouped by mutational status. The length of the bar indicates the duration of follow-up; black triangles indicate when patients were treated for MDS; blue triangles indicate when follow-up sequencing was performed.

Table 1

Clinical Characteristics of Study Patients

Consensus Morphologic Diagnosis	UPN	Age	Sex	WBC	Hgb	Plts	Myeloid Dysplasia*	Erythroid Dysplasia*	Megakarocytic Dysplasia*	Blasts*	Genes with Somatic Mutations	Follow-Up interval (days)	Treated for MDS
Dysplasia	178791	72	M	2.9	9.5	131	10-20%/<10%	<10%/10-20%	21%-50%	1-3%	*SF3B1, DOCK2*	NA	NA
Dysplasia	215695	75	M	2.7	11.9	49	<10%	<10%	>50%	1-3%/4-<5%	*IDH2, SRSF2*	NA	NA
Dysplasia	233604	76	F	5.7	12.1	17	<10%	<10%	10-20%	1-3%	*ASXL1, U2AF1*	NA	NA
Dysplasia	357137	62	F	2.3	9.1	127	<10%	10-20%	10-20%	1-3%	*CBL, IDH1, SRSF2*	NA	NA
Dysplasia	364510	47	M	5.2	9.7	138	<10%	<10%/10-20%	10-20%/21-50%	1-3%	*None*	NA	NA
Dysplasia	375001	80	F	3.7	6.9	38	<10%	<10%	>50%	1-3%	*GNB1, U2AF2, U2AF1, FAM47A, ASXL1*	NA	NA
Dysplasia	397410	82	M	10.7	9.5	157	<10%	<10%	10-20%/>50%	1-3%	*SRSF2*	NA	NA
Dysplasia	400904	71	F	4	9	43	<10%	<10%/10-20%	10-20%	1-3%	*ASXL1, DLC1, GATA2*	NA	NA
Dysplasia	445082	76	F	2	10.4	82	<10%/10-20%	<10%	10-20%	1-3%	*None*	NA	NA
Dysplasia	469650	75	F	3.2	9.5	381	<10%	10-20%	10-20%/>50%	1-3%	*PTPN11, SF3B1, TET2*	NA	NA
Dysplasia	479751	63	F	3.4	9	240	<10%/10-20%	<10%	10-20%/21-50%	1-3%	*ASXL1, SETBP1, U2AF1*	NA	NA
Dysplasia	501812	70	M	4.2	8.4	92	<10%	10-20%	21-50%	1-3%	*CBL, IDH2, SRSF2*	NA	NA
Dysplasia	567350	55	M	3.1	12.4	70	<10%	10-20%/<10%	<10%/21-50%	1-3%	*None*	NA	NA
Dysplasia	583362	51	M	3.5	11.5	83	<10%/10-20%	<10%	>50%	1-3%/4-<5%	*KCNU1, IDH2, SRSF2, ASXL1*	NA	NA
Dysplasia	584690	59	F	2.5	8.7	196	21-50%/<10%	10-20%	10-20%	1-3%	*SF3B1*	NA	NA
Dysplasia	589769	76	F	9.9	7.8	84	<10%	<10%	21-50%/10-20%	1-3%	*GATA2/SRSF2*	NA	NA
Dysplasia	609948	83	F	3.2	8.1	136	<10%	<10%	10-20%/21-50%	1-3%	*ASXL1, RIMS1, U2AF1*	NA	NA
Dysplasia	658726	66	F	6	9.6	587	10-20%/<10%	<10%	>50%	1-3%	*None*	NA	NA
Dysplasia	668295	79	F	8.3	8.3	379	<10%	<10%/10-20%	21%-50%	1-3%	*SF3B1, MUC16*	NA	NA
Dysplasia	680719	29	M	9.7	9.7	146	10-20%	<10%	21%-50%	1-3%	*DST*	NA	NA
Dysplasia	755644	65	M	6	9.6	197	<10%/10-20%	<10%	10-20%/21-50%	1-3%	*None*	NA	NA
Dysplasia	796695	75	M	6.6	11.5	45	<10%	<10%	21%-50%/10-20%	1-3%	*LRP1B, TET2, SRSF2*	NA	NA
Dysplasia	831900	77	M	1.7	9.6	21	21-50%/10-20%	<10%	<10%/10-20%	1-3%	*ASXL1,CBFB, EZH2, SI, STAG2, TET2*	NA	NA
Dysplasia	858330	89	M	2.5	8.6	79	10-20%/<10%	<10%/21-50%	10-20%	1-3%	*CDH4, TRA2B*	NA	NA
Dysplasia	884180	67	F	2	10.8	72	<10%	10-20%	<10%	1-3%	*None*	NA	NA
Dysplasia	932838	69	M	13.4	11.9	60	>50%/10-20%	<10%	10-20%/>50%	1-3%	*SMC1A*	NA	NA

Consensus Morphologic Diagnosis	UPN	Age	Sex	WBC	Hgb	Plts	Myeloid Dysplasia*	Erythroid Dysplasia*	Megakarocytic Dysplasia*	Blasts*	Genes with Somatic Mutations	Follow-Up interval (days)	Treated for MDS
Dysplasia	977120	54	M	1.6	6.9	129	<10%	10-20%	10-20%/21-50%	1-3%	*IDH2, SRSF2*	NA	NA
Dysplasia	983847	55	F	6.5	7.9	404	<10%	10-20%	21%-50%	1-3%	*None*	NA	NA
Equivocal Dysplasia	568547	71	F	2.5	11.9	31	<10%	<10%/10-20%	<10%	1-3%	*BCOR, DNMT3A, EZH2, PHIP, PRBF8 RUNX1, TET2*	1252	Yes
Equivocal Dysplasia	610864	55	F	2.9	9.3	34	<10%	<10%	<10%/10-20%	1-3%	*None*	702	No
Equivocal Dysplasia	701797	82	M	3.9	9.2	174	<10%/21-50%	<10%	<10%/21-50%	1-3%	*PHF6, TET2*	721	Yes
Equivocal Dysplasia	724989	84	M	1	12.7	22	<10%	10-20%/<10%	<10%	1-3%	*PKHD1, MAGI2, CSMD3, DNMT3A, RAD21*	21	Yes
Equivocal Dysplasia	728125	54	M	1.7	7.4	46	<10%	<10%	>50%/<10%	1-3%	*SF3B1, U2AF1*	96	Yes
Equivocal Dysplasia	786953	81	F	13.6	9.9	242	<10%	10-20%/<10%	10-20%/<10%	1-3%	*None*	957	No
Equivocal Dysplasia	976842	50	F	2.1	8.7	41	<10%	<10%	<10%/>50%	1-3%	*CBL, TET2, U2AF1*	646	Yes
No Dysplasia	163943	63	F	3.7	11.7	36	<10%	<10%	<10%	1-3%	*None*	516	Yes
No Dysplasia	204802	77	F	7	9.6	449	<10%	<10%	<10%	1-3%	*CMYA5*	1712	yes
No Dysplasia	332207	65	F	2.6	10	25	<10%	<10%	<10%	1-3%	*None*	980	No
No Dysplasia	529198	61	F	3.4	8.2	327	<10%	<10%	<10%	1-3%	*TET2*	1995	No
No Dysplasia	577914	48	F	1.8	6.1	237	<10%	<10%	<10%	1-3%	*None*	2436	Yes
No Dysplasia	769282	19	M	1.4	8.5	24	<10%	<10%	<10%	1-3%	*None*	536	No
No Dysplasia	859688	54	F	2.6	10.9	40	<10%	<10%	<10%	1-3%	*SF3B1*	473	No
No Dysplasia	976020	63	F	0.9	10.3	288	<10%	<10%	<10%	1-3%	*None*	480	No

White blood cell counts (WBC) reported in 10^3 cells/mcl

Hemoglobin (Hgb) reported in g/dl

Platelets (Pelts) reported in 10^3/mcl

NA, Not Applicable

*For discordant cases, dysplasia data is listed as reviewer 1 findings/reviewer 2 findings