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Somatic Mutations in STAG2 Are Associated with Separated Megakaryocyte Nuclear Lobes in Myelodysplastic Syndromes

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Waihay Wong (Northwestern University, United States) Rebecca Zon (Dana Farber Cancer Institute, United States) Christopher Gibson (Novartis Biomedical Research, United States) Caleb Ho (Foundation Medicine, Inc., United States) Olga Pozdnyakova (Brigham and Women's Hospital, United States) Donna Neuberg (Dana-Farber Cancer Institute, United States) Elisabeth Battinelli (Brigham and Women's Hospital, United States) Marlise Luskin (Dana-Farber Cancer Institute, United States) Zuzana Tothova (Dana-Farber Cancer Institute, United States) Elizabeth Morgan (Brigham and Women's Hospital, United States) Benjamin Ebert (Dana-Farber Cancer Institute, United States)

Abstract:
Myelodysplastic syndrome (MDS) is driven by genetic mutations, but diagnosis relies on morphologic evaluation of bone marrow hematopoiesis. Only a small number of genetic abnormalities define specific bone marrow morphologic features in MDS, such as those harboring SF3B1 mutations and deletions of chromosome 5q. We hypothesized that additional genetic alterations are associated with specific dysplastic morphologic features in MDS. We assessed genetic-morphologic associations between commonly mutated genes and 10 morphologic features in a cohort of MDS bone marrows with a high degree of dysplasia. We replicated the association of SF3B1 mutations with ring sideroblasts and found that dysplastic megakaryocytes with separated nuclei were independently associated with STAG2 and/or ASXL1 mutations. In addition, STAG2 mutations were associated with abnormal myeloid nuclear segmentation and myeloid cell hypogranulation. These findings demonstrate that STAG2 and ASXL1 mutations are associated with specific morphologic abnormalities in MDS.

Conflict of interest: COI declared - see note

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Somatic Mutations in *STAG2* Are Associated with Separated Megakaryocyte Nuclear Lobes in Myelodysplastic Syndromes

Waihay J Wong[1,2,#,+], Rebecca L Zon[1,3,#], Christopher J Gibson[1,Δ], Caleb Ho[4], Olga Pozdnyakova[3,∩], Donna Neuberg[5], Elisabeth M Battinelli[2], Marlise R Luskin[1,2], Zuzana Tothova[1,6], Elizabeth A Morgan[3], Benjamin L Ebert[1,6,*]

1) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA
2) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
3) Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
4) Foundation Medicine, Cambridge, MA 02141, USA
5) Department of Data Science, Dana-Farber Cancer Institute, Boston, MA 02215, USA
6) The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

# Contributed equally
+ Current address: Department of Pathology, Northwestern University, Chicago, IL 60611
∩ Current address: Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104

* Corresponding author:

Benjamin Ebert, MD PhD
Dana-Farber Cancer Institute
450 Brookline Avenue, D1628
Boston, MA 02215
Phone: 617-632-4266
E-mail: benjamin_ebert@dfci.harvard.edu

Sequencing and morphologic data may be found in a data supplement available with the online version of this article.

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Key Points

- Unbiased identification of genetic-morphologic associations demonstrates a significant association between separated megakaryocyte nuclear lobe morphology with *STAG2* and *ASXL1* mutations
- *STAG2* mutations are additionally associated with abnormal myeloid nuclear segmentation and myeloid cell hypogranulation

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Abstract

Myelodysplastic syndrome (MDS) is driven by genetic mutations, but diagnosis relies on morphologic evaluation of bone marrow hematopoiesis. Only a small number of genetic abnormalities define specific bone marrow morphologic features in MDS, such as those harboring *SF3B1* mutations and deletions of chromosome 5q. We hypothesized that additional genetic alterations are associated with specific dysplastic morphologic features in MDS. We assessed genetic-morphologic associations between commonly mutated genes and 10 morphologic features in a cohort of MDS bone marrows with a high degree of dysplasia. We replicated the association of *SF3B1* mutations with ring sideroblasts and found that dysplastic megakaryocytes with separated nuclei were independently associated with *STAG2* and/or *ASXL1* mutations. In addition, *STAG2* mutations were associated with abnormal myeloid nuclear segmentation and myeloid cell hypogranulation. These findings demonstrate that *STAG2* and *ASXL1* mutations are associated with specific morphologic abnormalities in MDS.

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Introduction

Our understanding of the genetic landscape in myelodysplastic syndrome (MDS) has grown rapidly over the past decade. Large-scale sequencing studies have demonstrated that specific genetic mutations are associated with patient outcomes.[1-4] As a result, MDS subtypes are increasingly defined by genetic features.[5,6]

Nevertheless, histologic evaluation of bone marrow blasts and myelodysplasia remain a key component of MDS diagnosis. Only a few genetic aberrations have an established association with specific bone marrow features. MDS with isolated 5q deletion is associated with micromegakaryocytes, and MDS with *SF3B*1 mutations is associated with ring sideroblasts.[7-9] We hypothesized that additional genetic-morphologic associations exist that can inform the role of these mutations in abnormal hematopoiesis. We rigorously evaluated two independent myelodysplastic cohorts to uncover genetic-morphologic associations between common myeloid gene mutations and bone marrow dysplastic features.

Methods

Cohort selection

All studies involving human participants were conducted in accordance with ethical guidelines established by the Declaration of Helsinki and were approved by the relevant IRB. 272 MDS patients diagnosed according to WHO criteria at Brigham and Women's Hospital (BWH) from 2013 to 2014 via bone marrow biopsy were screened for the presence of prominent dysplastic features and 89 bone marrow core biopsies and aspirates were selected for further analysis. Blinded pathology review (see below) confirmed that 80 of 89 cases showed at least one dysplastic feature (out of 10 evaluated features) which was rated as 3 (high) or 2 (moderate).

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Nine cases were downgraded to mild dysplasia (grade 1 in at least one dysplastic feature) after pathology review. Subsequently, we identified 1047 non-overlapping patients diagnosed with a myeloid malignancy at BWH between 2014 and 2018, all of whom had available DNA next-generation sequencing data including mutational status of five genes of interest identified in the discovery cohort: *STAG2*, *RUNX1*, *SRSF2*, *ASXL1*, and *SETBP1*. This enrichment cohort of 155 bone marrow biopsies and aspirates were selected on the basis of mutational profile to represent all combinations of mutations in *STAG2*, *RUNX1*, *SRSF2*, *ASXL1*, and *SETBP1* that were present within eligible cases. The demographic and hematologic characteristics of this cohort were compared with the initial cohort (Table S5).

DNA sequencing

In the discovery cohort, targeted next generation sequencing was performed using a 50 gene panel (Fig. S1 and Table S1). Samples in the enrichment cohort underwent clinical next generation sequencing of 95 genes (Table S3).[10] In both cohorts, pathogenic variants were classified as described previously.[11] The minimum VAF cutoff for mutation calling was 2%.

Pathology evaluation

Each bone marrow biopsy and aspirate smear in the discovery cohort was evaluated by two hematopathologists blinded to diagnosis and genotype and scored for ten dysplastic features (small hypolobated megakaryocytes, widely separated megakaryocyte nuclei, abnormal myeloid nuclear segmentation, myeloid hypogranulation, Auer rods, ring sideroblasts, erythroid binucleation, irregular erythrocyte nuclei, erythroid nuclear cytoplasmic dyssynchrony, erythroid karyorrhexis) by two independent hematopathologists on a scale of 0 (absent) to 3 (prominent) (Fig. 1A). In the enrichment cohort, widely separated megakaryocyte nuclei compared to normal mononucleated megakaryocytes (Fig. S2) were graded absent or present by three independent

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hematopathologists blinded to the diagnosis and genotype of each case. Myeloid nuclear segmentation and hypogranulation were scored by two independent hematopathologists on a scale of 0 (absent) to 3 (prominent). The median score was used except in difficult to ascertain cases (n=18), which underwent consensus review.

Statistical analysis

Statistical analysis was performed in R (version 3.5) using *Corrplot* package to derive Spearman's correlation coefficients between dysplastic features and the variant allele frequency (VAF) of each gene that was mutated in three or more patients. *Stats* package was used for odds ratio analysis. Wald's confidence interval and Fisher's exact P value were used in univariate analyses. For each dysplastic feature, genes identified in univariate analysis were tested by multivariable logistic regression using backwards elimination by Akaike (*MASS* package). The discovery cohort was resampled 100X into train (80%) and test (20%) sets to determine the VAF that best predicted specific dysplastic features with at least 75% accuracy (*Rpart* package). A default VAF cutoff of 0.1 was used for genes without splits.

Results

We surveyed 272 patients diagnosed with MDS according to WHO criteria and selected 89 bone marrow core biopsies and aspirate smears on the basis of having a high degree of dysplasia of any type. In pairwise analyses of dysplastic phenotypes (Fig. 1A) and somatic mutations (Table S2) *ASXL1*, *SETBP1*, *SRSF2*, *STAG2*, and *RUNX1* mutations were associated with myeloid and megakaryocytic dysplasia (Fig. 1B). Somatic mutations in *TP53* and *SF3B1* were associated with a distinct morphologic phenotype including erythroid dysplasia, consistent with known roles for these genes in erythropoiesis.[12,13]

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Univariate analysis demonstrated that megakaryocytes with widely separated nuclei were significantly associated with mutations in *STAG2* (OR = 22.2; 95% CI [4.59, 107.8], *P* = 3.59 x 10^{-6}), *SRSF2* (OR = 14.85; 95% CI [3.02, 72.9], *P* = 0.0001), *RUNX1* (OR = 7.15; 95% CI [2.04, 25.1], *P* = 0.002), and *ASXL1* (OR = 4.33; 95% CI [1.54, 12.2], *P* = 0.008) (Fig. S3A). In multivariable analysis, only *STAG2* and *SRSF2* mutations were significantly and independently associated with widely separated nuclei in megakaryocytes (*STAG2*, OR = 32.4; 95% CI [5.45, 624.0], *P* = 0.001; *SRSF2*, OR = 8.87, 95% CI [1.68, 67.6], *P* = 0.015) (Fig. S3A). We observed that *STAG2*, *SRSF2*, *RUNX1* and *ASXL1* mutations demonstrated some gene dosage effects on separated megakaryocyte nuclei incidence, although these effects were likely confounded by the presence of co-mutations (Fig. S4). We iteratively partitioned each gene of interest based on VAF to determine the threshold that best predicted megakaryocyte nuclear lobe separation. With the exception of *RUNX1* (allele frequency cutoff = 0.40), this analysis identified VAF cutoffs for *STAG2*, *SRSF2*, and *ASXL1* ranging from 0.03 to 0.10 for detection of megakaryocyte nuclear lobe separation (Fig. S5).

STAG2 gene mutations were associated not only with separated megakaryocyte nuclei but also with myeloid dysplasia. In univariate analysis, granulocytes with hypolobated or hypersegmented nuclei were significantly associated with mutations in *STAG2* (OR = 3.68; 95% CI [1.21, 11.3], *P* = 0.03), *NRAS* (OR = 10.4; 95% CI [1.02, 106.0], *P* = 0.045), *SETBP1* (OR = 19.4; 95% CI [2.12, 177.3], *P* = 0.003), *RUNX1* (OR = 3.44; 95% CI [1.08, 11.0], *P* = 0.047) and *ASXL1* (OR = 3.82; 95% CI [1.34, 10.9], *P* = 0.02) (Fig. S3B). Multivariable analysis showed independent significant associations of both *STAG2* and *SETBP1* mutations with abnormal granulocytic nuclear segmentation (*STAG2*, OR = 7.08, 95% CI [1.94, 28.0], *P* = 0.004; *SETBP1*, OR = 12.2, 95% CI [1.29, 267.4], *P* = 0.042) (Fig. S3B). Furthermore, *STAG2*

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mutations were significantly associated with myeloid cell hypogranulation in both univariate (OR = 5.45, 95% CI [1.71, 17.4], $P = 0.004$) and multivariable (OR = 12.7, 95% CI [3.10, 86.3], $P = 0.002$) analyses (Fig. S3C).

Since co-occurrence of mutations in the discovery cohort potentially confounded the association of individual gene mutations with particular morphologic abnormalities, we compiled a group of 155 myeloid neoplasms with myelodysplastic features that were enriched in co-mutations of *ASXL1*, *RUNX1*, *SETBP1, SRSF2*, and *STAG2* (Fig. S6 and Table S4). In this enrichment cohort, *STAG2* mutations were significantly associated with separated megakaryocyte nuclei (univariate OR = 5.95, 95% CI [2.82, 12.6], $P = 6.0 \times 10^{-6}$; multivariable OR = 5.54, 95% CI [2.62, 12.1], $P = 1.41 \times 10^{-5}$), abnormal myeloid nuclear segmentation (univariate OR = 3.73, 95% CI [1.55, 8.98], $P = 0.005$; multivariable OR = 3.04, 95% CI [1.23, 7.65], $P = 0.02$), and myeloid cell hypogranulation (univariate OR = 7.02, 95% CI [2.85, 17.3], $P = 1.85 \times 10^{-6}$; multivariable OR = 6.48, 95% CI [2.63, 16.7], $P = 5.68 \times 10^{-6}$) (Fig. 2A-C). Inclusion of samples with *SRSF2* mutations only, *ASXL1* mutations only, and samples with *ASXL1* and *SRSF2* co-mutations enabled the effect of these genes to be disambiguated. For example, the association of *SRSF2* mutations with separated megakaryocyte nuclei in the discovery cohort was likely due to co-mutation with *STAG2* and was not confirmed in enrichment analysis (Fig. S3A and Fig. 2A). Consistent with findings from univariate analysis in the discovery cohort, *ASXL1* mutations were associated with separated megakaryocyte nuclei in the enrichment cohort (univariate OR = 2.88, 95% CI [1.39, 5.94], $P = 0.004$; multivariable OR = 2.55, 95% CI [1.19, 5.67] $P = 0.018$).

Discussion

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STAG2 encodes a member of the ring-shaped cohesin complex that is required for DNA loop extrusion, transcriptional regulation, DNA damage repair and sister chromatid cohesion. *STAG2* is often mutated in cancer, including in the germline leading to Cornelia de Lange syndrome, a childhood cohesinopathy characterized by well-defined facial features, limb malformations and low platelet count, although a link to dysmegakaryopoiesis remains unproven.[14,15] Among myeloid malignancies, *STAG2* mutations are particularly associated with MDS and define a subset of AML that arise out of an antecedent myeloid neoplasm.[16,17] Although a prior study of "de novo" AML also described an association between *STAG2* mutations and megakaryocyte dysplasia in a small sample size[18], our analysis of 17 and 50 individuals with mutated *STAG2* in two independent cohorts – one unbiased and one enriched for *STAG2* mutations – demonstrated a robust association between *STAG2* mutation and separated megakaryocyte nuclei as a feature of myelodysplasia. Nevertheless, the precise role of *STAG2* in megakaryopoiesis and MDS pathogenesis remains unclear. A recent study demonstrated that inactivation of cohesin-mediated loop extrusion in myeloid cells results in nuclear hypersegmentation and neutrophil differentiation, suggesting a potential mechanism for *STAG2* and other cohesin components in regulating nuclear segmentation and shape.[19] Dysplastic megakaryocytes demonstrate uneven chromosome ploidy within each separate nuclei, but it is unknown whether this phenomenon is predicated on loss of cohesin complex function.[20]

In conclusion, we report that the presence of separated megakaryocyte nuclei in MDS is strongly associated with mutations in *STAG2* (69% of mutated vs 22% of unmutated, $P < 0.0001$) and *ASXL1* (47% of mutated vs 24% of unmutated, $P = 0.0002$). Additionally, *STAG2* mutations in MDS are significantly associated with abnormal myeloid nuclear segmentation (58% vs 19%, $P < 0.0001$) and myeloid cell hypogranulation (74% vs 21%, $P < 0.0001$). These

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associations show similar strength to previously observed associations between *SF3B1* mutation and ring sideroblasts.[9] Further investigation is required to determine whether *STAG2* and *ASXL1* mutations confer specific functional deficits in megakaryocyte and myeloid cell differentiation in MDS.

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Authorship Contributions

B.L.E., E.M.B., E.A.M., M.R.L., C.J.G. and Z.T. conceived of and designed the project and secured funding. W.W., E.A.M., M.R.L., C.J.G. and Z.T. assembled patient cohorts; E.A.M. and C.J.G. interpreted sequencing data; W.W., C.H., O.P. and E.A.M. reviewed pathology; W.W.,

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R.L.Z. and D.N. analyzed results; W.W., R.L.Z. and B.L.E. wrote the manuscript. All authors revised the manuscript for intellectual content.

Data Sharing Statement

Sequencing and morphologic data may be found in a data supplement available with the online version of this article.

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Disclosure of Conflicts of Interest

B.L.E. has received research funding from Novartis and Calico. He has received consulting fees from Abbvie. He is a member of the scientific advisory board and shareholder for Neomorph Inc., Big Sur Bio, Skyhawk Therapeutics, and Exo Therapeutics. R.L.Z. is a consultant for and stockholder in Triveni Bio. D.N. has stock ownership in Madrigal Phamaceuticals. Z.T. has received research funding from Novartis, not related to this work. C.H. is a full-time employee of Foundation Medicine, Inc., which was not involved in this study. All other authors declare no conflict of interests.

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References

1. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *The New England Journal of Medicine*. 2011;364(26):2496-2506.

2. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627; quiz 3699.

3. Lindsley RC, Saber W, Mar BG, et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. *The New England Journal of Medicine*. 2017;376(6):536-547.

4. Bernard E, Tuechler H, Greenberg PL, et al. Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. *NEJM evidence*. 2022;1(7):EVIDoa2200008.

5. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703-1719.

6. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200-1228.

7. Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G. Distinct haematological disorder with deletion of long arm of no. 5 chromosome. *Nature*. 1974;251(5474):437-438.

8. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64-69.

9. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. *Blood*. 2015;126(2):233-241.

10. Kluk MJ, Lindsley RC, Aster JC, et al. Validation and Implementation of a Custom Next-Generation Sequencing Clinical Assay for Hematologic Malignancies. *The Journal of molecular diagnostics: JMD*. 2016;18(4):507-515.

11. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal Hematopoiesis Associated With Adverse Outcomes After Autologous Stem-Cell Transplantation for Lymphoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2017;35(14):1598-1605.

12. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *The New England Journal of Medicine*. 2011;365(15):1384-1395.

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13. Dutt S, Narla A, Lin K, et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. *Blood*. 2011;117(9):2567-2576.

14. Lambert MP, Jackson LG, Clark D, Kaur M, Krantz ID, Deardorff MA. The incidence of thrombocytopenia in children with Cornelia de Lange syndrome. *American Journal of Medical Genetics Part A*. 2011;155A(1):33-37.

15. Cavalleri V, Bettini LR, Barboni C, et al. Thrombocytopenia and Cornelia de Lange syndrome: Still an enigma? *American Journal of Medical Genetics Part A*. 2016;170A(1):130-134.

16. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367-1376.

17. Jann J-C, Hergott CB, Winkler M, et al. Subunit-specific analysis of cohesin-mutant myeloid malignancies reveals distinct ontogeny and outcomes. *Leukemia*. 2024;38(9):1992-2002.

18. Weinberg OK, Gibson CJ, Blonquist TM, et al. Association of mutations with morphological dysplasia in de novo acute myeloid leukemia without 2016 WHO Classification-defined cytogenetic abnormalities. *Haematologica*. 2018;103(4):626-633.

19. Patta I, Zand M, Lee L, et al. Nuclear morphology is shaped by loop-extrusion programs. *Nature*. 2024;627(8002):196-203.

20. Münch C, May AM, Hauschke D, Roth J, Lassmann S, Werner M. Internuclear chromosome distribution of dysplastic megakaryocytes in myelodysplastic syndromes is dependent on the level of ploidy. *Chromosoma*. 2011;120(3):265-273.

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Figure legends

Figure 1. Genetic morphologic associations in MDS. (**A**) Dysplastic features evaluated include (*clockwise from top left*) erythroid binucleation, erythroid nuclear irregularity, erythroid karyorrhexis, erythroid mitosis, ring sideroblast, Auer rod, abnormal myeloid nuclear segmentation, myeloid hypogranulation, hypolobated megakaryocyte, and separated megakaryocyte nuclei. (**B**) Correlation matrix showing association between evaluated dysplastic features and gene mutations in the discovery cohort. Only associations with adjusted P-value < 0.05 are shown. The area of each circle represents the absolute Spearman's correlation coefficient (ρ). *N*, number of samples.

Figure 2. Specific gene mutations are associated with dysplastic features. Forest plots of univariate (white) and multivariable (black) analyses for gene mutations associated with separated megakaryocyte nuclei (**A**), abnormal myeloid nuclear segmentation (**B**), and myeloid hypogranulation (**C**) in the enrichment cohort. **D-G**, Bone marrow biopsy micrographs demonstrate megakaryocytes with widely separated nuclei in a patient with *STAG2*, *SRSF2*, *ASXL1*, *IDH2*, *KRAS*, *PTPN11* and subclonal *CUX1* and *RUNX1* mutations (**D**) and a patient with germline *GATA2* and secondary somatic *STAG2* mutations (**E**). In contrast, dysplastic megakaryocytes are present but do not display separated nuclei in a patient with *ASXL1*, *RUNX1*, *SRSF2* and *TET2* mutations (**F**) or a patient with *ASXL1* and *SRSF2* mutations (**G**).

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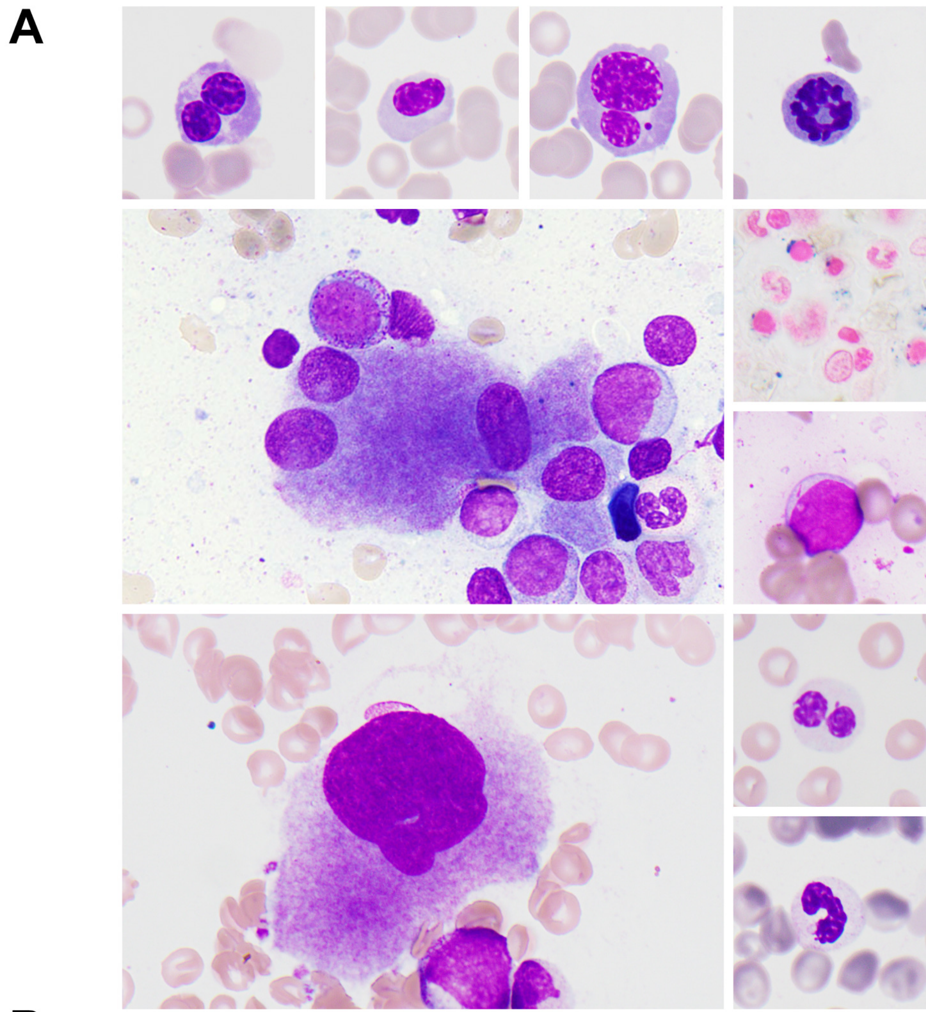
Figure 1

Fig. 1



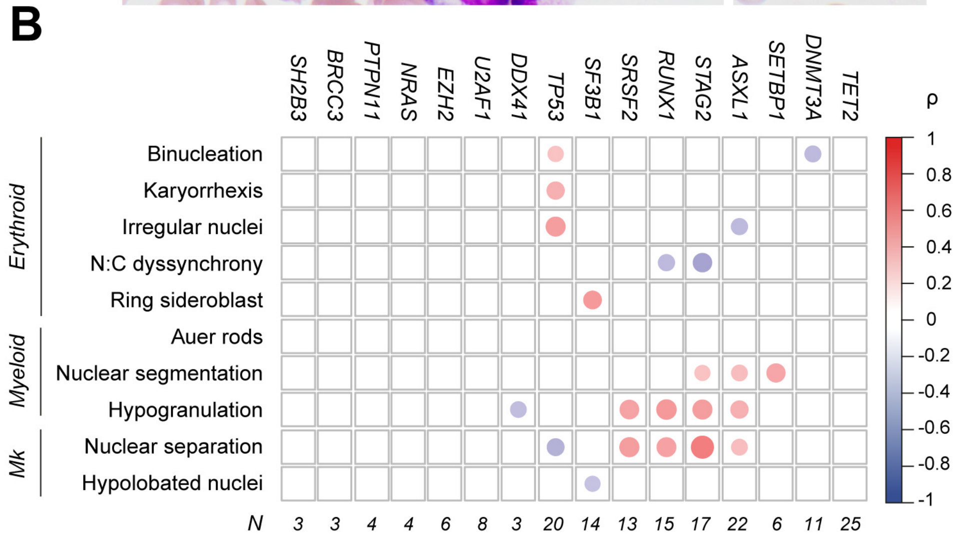


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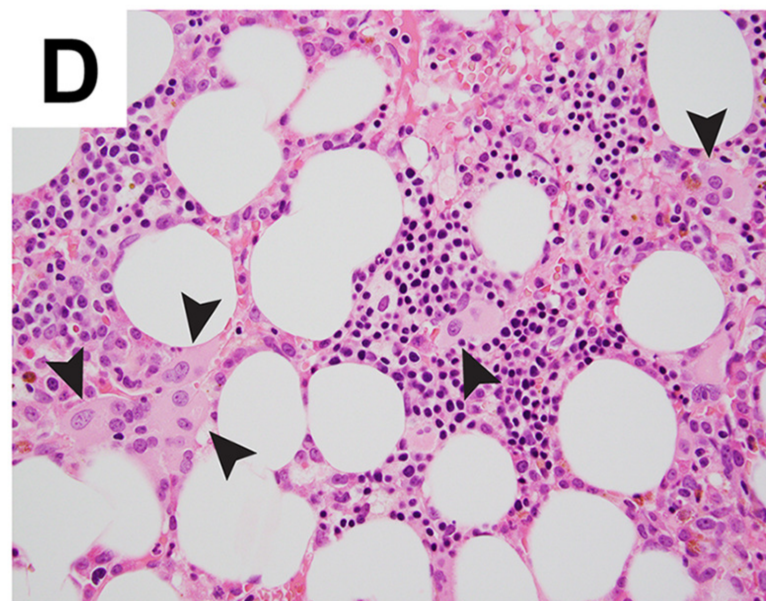
Figure 2

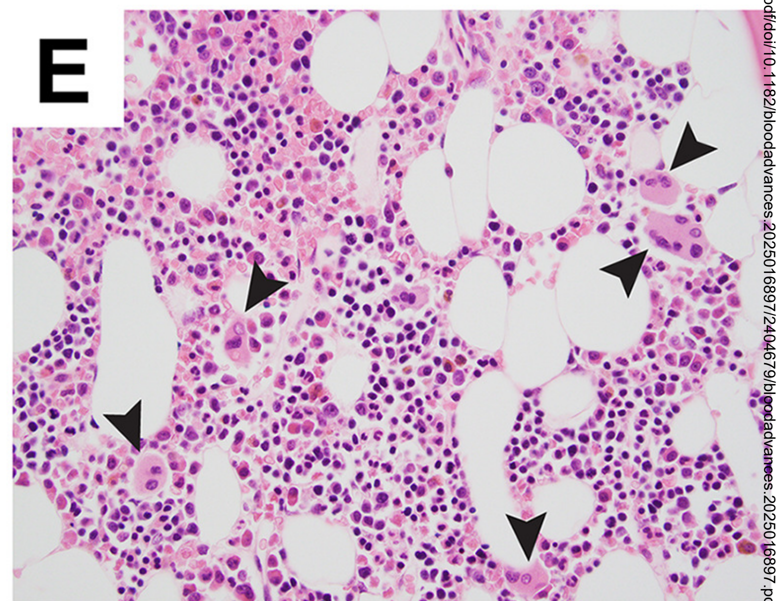
Fig. 2

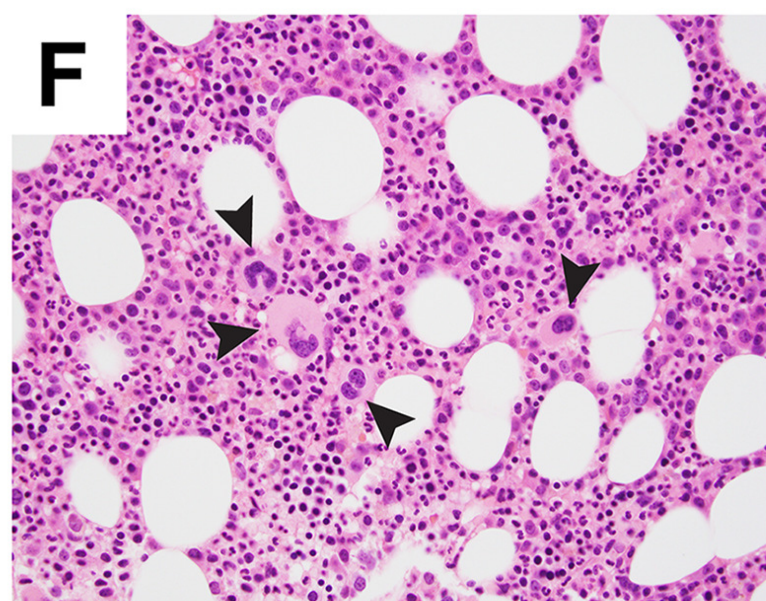
	Univariate Odds (95% CI)	*P* value	Multivariable Odds (95% CI)	*P* value
Enrichment cohort				
A. Separated megakaryocyte nuclei				
STAG2	5.95 (2.82–12.6)	<0.001*	5.54 (2.62–12.1)	<0.001*
ASXL1	2.88 (1.39–5.94)	0.004*	2.55 (1.19–5.67)	0.018*
SRSF2	1.74 (0.88–3.44)	0.118		
RUNX1	0.72 (0.34–1.49)	0.465		
TP53	0.42 (0.09–2.00)	0.337		
B. Abnormal myeloid nuclear segmentation				
STAG2	3.73 (1.55–8.98)	0.005*	3.04 (1.23–7.65)	0.016*
ASXL1	2.63 (1.08–6.41)	0.035*		
SETBP1	0.95 (0.35–2.55)	1.000		
NRAS	0.94 (0.28–3.22)	1.000		
RUNX1	0.28 (0.09–0.88)	0.034*	0.21 (0.04–0.74)	0.026*
C. Myeloid cell hypogranulation				
STAG2	7.02 (2.85–17.3)	<0.001*	6.48 (2.63–16.7)	<0.001*
ASXL1	2.74 (1.13–6.63)	0.035*		
RUNX1	0.72 (0.28–1.89)	0.640		

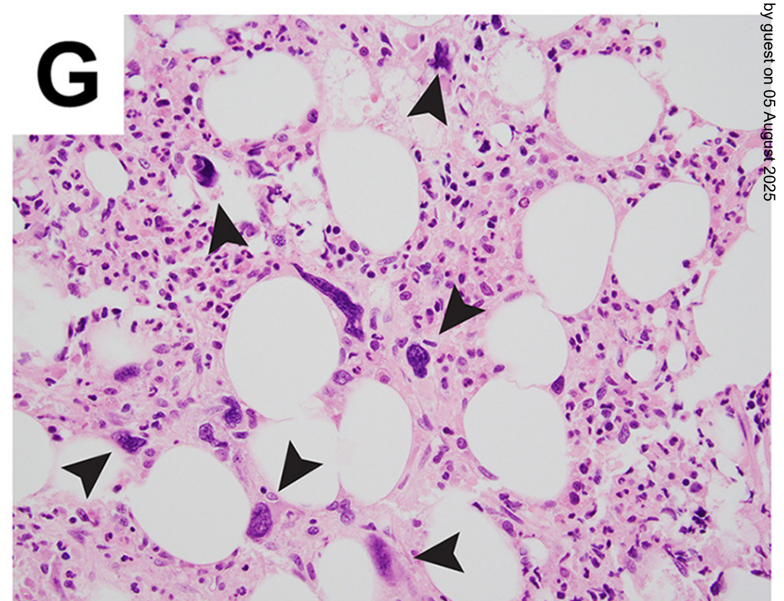
Univariate Odds (*vs unmutated*, 95% CI): 0.1 1.0 10

Multivariable Odds (*vs unmutated*, 95% CI): 0.1 1.0 10 100









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