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Expanding the Spectrum of CSF3R Mutated Myeloid Neoplasm Beyond Chronic Neutrophilic Leukemia and Atypical Chronic Myeloid Leukemia: A Comprehensive Analysis of 13 Cases

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Abstract

Background: Genetic alterations of *CSF3R*, typically associated with chronic neutrophilic leukemia (CNL) and atypical chronic myeloid leukemia (aCML), rarely occur in other myeloid neoplasms. **Methods:** This study characterized the clinical, morphologic, cytogenetic, and molecular features of 13 patients with non-CNL non-aCML myeloid neoplasms with *CSF3R* alterations. Patients (median age 77 years) were categorized into myelodysplastic/myeloproliferative neoplasm (MDS/MPN) (n=5), acute leukemia (n=4), and other myeloid neoplasms (n=4) based on WHO 2022 and ICC criteria. **Results:** The *CSF3R* p.*Thr*618*Ile* mutation was most frequent (11/13), with additional pathogenic variants including p.*Gln*743*Ter* and frameshift mutations affecting the cytoplasmic tail. Variant allele frequencies (VAFs) ranged from 2% to 49%, with the highest median VAF in the MDS/MPN group. Co-mutations varied by subtype; MDS/MPN, NOS and CMML cases frequently harbored mutations in epigenetic regulators (*ASXL1*, *TET2*) and splicing factors (*SF3B1*, *SRSF2*, *ZRSR2*), while acute leukemia cases showed alterations in *JAK3*, *STAT3*, and *NRAS*. Survival analysis revealed distinct patterns across the three diagnostic groups, with MDS/MPN having the poorest prognosis. **Conclusion:** This study expands the recognized spectrum of *CSF3R*-related myeloid neoplasms and highlights the clinical and molecular heterogeneity associated with these mutations, emphasizing the need for comprehensive molecular profiling and potential for targeted therapies.

Keywords: CSF3R; Myeloid neoplasm; Myelodysplastic/myeloproliferative neoplasm; Acute leukemia; Myelodysplastic neoplasm

1. Introduction

The colony-stimulating factor 3 receptor (CSF3R) is a key member of the hematopoietic receptor super-family. The protein CSF3R, encoded by the eponymous gene (*CSF3R*), plays a crucial role in proliferation, differentiation, and survival of granulocytes [1, 2]. Upon binding its ligand granulocyte colony stimulating factor (G-CSF), CSF3R triggers various downstream pathways including JAK-STAT pathway, leading to its biological effects [3]. Due to this key biological function in hematopoiesis, genetic alterations of *CSF3R* are highly linked to granulocytic dysfunction. The T618I mutation of *CSF3R*, in particular, is the most frequent genetic alteration that leads to constitutive receptor activation, resulting in aberrant JAK-STAT, SRC family kinase, and RAS-MAPK signaling, driving granulocytic proliferation and cytokine-independent cell survival [4, 5].

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Somatic activating *CSF3R* mutations are highly associated with chronic neutrophilic leukemia (CNL), which present with neutrophilia, hypercellular bone marrow with granulocytic proliferation, and absence of dysplasia or increased blasts. This rare myeloproliferative neoplasm (MPN) usually shows an indolent clinical course, although the outcome may vary [6, 7]. CNL may acquire additional mutations and progress to blast crisis with or without acquisition of dysplasia; however, the specific alteration of *CSF3R* persists in the progressing disease, often with increased allele frequency, suggesting a linear molecular progression of this disease [8, 9]. In addition to CNL, *CSF3R* alterations are frequently seen in atypical chronic myeloid leukemia (aCML), also known as myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N), and severe congenital neutropenia (SCN) [10, 11]. Alterations of *CSF3R* are also infrequently associated with other myeloid neoplasms such as chronic myelomonocytic leukemia (CMML), myelodysplastic neoplasms (MDS) or acute myeloid leukemia (AML) [12].

Although *CSF3R* genetic alteration may belong to three different classes, the point mutation T618I, affecting the transmembrane proximal domain, is the most common pathogenic alteration in myeloid neoplasms. This mutation often acts as the driver mutation in CNL, persisting throughout the disease progression [8]. Importantly, CNL patients, with T618I mutation, show significantly worse prognosis compared to those with other *CSF3R* mutations [13]. While this mutation may not independently drive leukemogenesis, it may play a synergistic role in disease pathogenesis. Its ability to constitutively activate JAK-STAT pathway, has made it a rational therapeutic target, with JAK inhibitors such as ruxolitinib which has been successfully used to treat neoplasms driven by this alteration [11, 14, 15]. All these reasons make identification of myeloid neoplasms with *CSF3R* alteration clinically relevant.

In this study, we have examined the clinical, hematological, histomorphological cytogenetic, and molecular characteristics of 13 cases of non-CNL, non-aCML myeloid, and non-SCN myeloid neoplasms. We have further documented the mainstay therapy they have received and their outcome. By investigating these rare cases we explored if there is any underlying unifying characteristics in these neoplasms.

2. Materials and Methods

2.1. Patient Selection and Data Collection

We searched our institutional database for all pathology diagnostic reports for "*CSF3R*". All retrieved cases were manually reviewed independently by two board certified hematopathologists. Patients were included if they harbored a *CSF3R* mutation confirmed by next-generation sequencing (NGS), had a clinical and hematopathology diagnosis that did not meet criteria for CNL, aCML, or SCN, and had comprehensive molecular, cytogenetic, and morphologic data available. Of all such cases, only newly diagnosed myeloid neoplasms were included in this study. Demographic and clinical data, including age, sex, clinical presentation, and laboratory findings, were extracted from the institutional electronic medical record system manually.

2.2. Hematologic and Morphologic Analysis

Complete blood counts, peripheral blood smears, and bone marrow aspirates/core biopsies were reviewed. Dysplasia across erythroid, myeloid, and megakaryocytic lineages were documented.

2.3. Cytogenetic and FISH Analysis

Conventional karyotyping and fluorescence in situ hybridization (FISH) were performed for all cases as part of standard diagnostic evaluation.

2.4. Molecular Studies

Targeted NGS was performed through OnkoSight myeloid panel (BioReference Health™) comprising up to 50 genes which include: ABL1, ANKRD26, ASXL1, ATRX, BCOR, BCORL1, BRAF,

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CALR, CBL, CCND2, CDKN2A, CEBPA, CSF3R, CUX1, DDX41, DNMT3A, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA2, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KMT2A, KRAS, MAP2K1, MPL, MYD88, NF1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2.

2.5. Statistical Analysis

Descriptive analysis were employed to summarize clinical and laboratory data. All statistical analyses including Kaplan-Meier survival analysis and data visualizations were performed using Python 3.14™. Tables, and all visualizations with histograms, box-plots, bar charts, heatmap, lollipop plots were generated with libraries such as Matplotlib, Seaborn, and Pandas.

2.6. Ethical Considerations

This study was conducted in accordance with institutional review board (IRB) policies for the use of patient data in research. Patient identifiers were anonymized to ensure confidentiality.

3. Results

3.1. Clinical Findings

We have examined the molecular profile of 1400 patients with myeloid neoplasms diagnosed at our institute during a seven-year period. We identified thirteen (0.9%) with CSF3R-mutated myeloid neoplasms which were categorized into three diagnostic groups. The MDS/MPN group includes three cases of MDS/MPN-unclassified (MDS/MPN-U) and two cases of CMML, the acute leukemia group includes two cases of AML, one case of mixed phenotype acute leukemia (MPAL)-M/T and one case of myeloid sarcoma (MS). The third group includes three cases of de novo MDS and one case of MPN with progression to MDS. The median age for the whole cohort was 77 years (range, 22 to 89 years), with a male predominance (9 males, 4 females). Patients in the MDS/MPN group were predominantly elderly (median age 84 years; range, 78 to 89 years). The acute leukemia cases spanned a wide age range (22 to 69 years; median, 56 years). Patients in the other myeloid neoplasm group had a median age of 68 years (range: 57 to 79 years). Elevated lactate dehydrogenase (LDH) level (≥242 U/L) was present in 11 out of 13 patients, and splenomegaly was seen in two patients. Clinical history was significant for previous malignancy (4 cases), autoimmune disease (3 cases), and hepatitis (1 case) (Table 1).

Table 1. Comprehensive Clinicopathologic, Cytogenetic, and Outcome Profile of the 13-Patient Myeloid Neoplasm Cohort**.**

	Case1	Case2	Case3	Case4	Case5	Case6	Case 7	Case8	Case9	Case 10	Case 11	Case 12	Case 13
Age	89	81	87	84	78	22	69	55	56	57	77	63	79
Gender	F	M	F	M	M	M	F	M	M	F	M	M	M
WBC	58.5	14.6	44	16.2	51.9	25.5	4.2	6.1	1.4	10.4	2.6	25.3	2.5
Hb	10.6	7.3	7.4	9.2	11.7	11.7	12	11	7.6	9.4	10.6	7.7	11.2
MCV	99.7	100.4	103.9	85.5	105.5	94.6	81	86.2	92.6	90.9	84.3	98.7	96.3
Plt	216	27	141	32	158	47	184	95	44	117	280	77	65
Mono(%)	3	6	NA	10	12.1	0	9	1	4	5.2	NA	2.9	9
Mono#	1.2	0.9	2.3	1.8	6.3	0	0.4	0	0.1	0.54	NA	0.7	0.22
PB blasts (%)	0	0	0	0	0	85	0	63	14	0	NA	0	0
LDH	385	220	531	418	888	406	337	314	666	416	1269	1176	ND
Cellularity	90	85-90	95	70-85	100	70-85	Limited	95-100	70	95	90	95-100	30
M:E	10:1	MP	MP	1:1	2:1	0.8:1	3:1	MP	5.2:1	6.5:1	1.6:1	10.8:1	MP
Erythroid	Dys +	Dys +	Dec	Dys +	Dec	Dys+	Dec	Dec	Dec	Dys+	Dys+	Inc	Mild dec
Myeloid	NA	NA	Inc	Dys+	Inc	Minimal	Dec	Dec	Dec	Dys+	Dys+	Rare	Dys+

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Megakaryocytes	NA	Dys +	Dec	Dys+	Inc, Dys+	No	Rare	Dec	Present	Dys+	Dys+	Dys+	Dec
BM blasts(%)	< 3	< 3	< 3	7	1	66	4	83	56	8	8	8	5
Fibrosis	0	Gr 2-3	Gr 2-3	0	0	0	0	0	0	0	0	Gr 1-2	0
Karyotype	46, XX	45,XY,-7	46, XX	46,XY del(7)(q22)	46, XX	46, XY	46, XX	46,XY	Complex	46,XX	46,XY	46,XY,der(13;14)9q10;q10)	45, -X,-Y
FISH	Normal	Monosomy 7	NA	Del(7q)	Normal	RUNX1 (3 copies)	Normal	Normal	RUNX1T1/RUNX1	Normal	Normal	NA	Normal
Diagnosis	MDS/MPN-U	MDS/MPN-U	MDS/MPN-U	CMML	CMML	AML	MS	MPAL	AML	MDS-EB1	MDS-EB1	ET/MPN transformed to AML	MDS-EB1
Treatment	Hy	HA	NA	Supportive	Hy	7+3, HIDAC, +GO	Chemo 7+3	Chemo	Chemo	No Rx	Chemo	Chemo ASCT	No Rx
Prognosis	Died, 33 months	Died, 2 months	2 months	Died, 19 days	Alive, 28 months	CR, 4 years	Relapse; 14 months	Died, 9months	19months, relapsed	1 year, alive	Transform into AML, Alive, 2 years	CR, 1 year	5 months, alive
Clinical	None	Hepatitis, Cirrhosis	Breast Ca, Colon Ca	Prostate Ca	B-ALL, remission	None	RA	DVT	None	OA knee	MG	None	Prostate Ca
Splenomegaly	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No

Abbreviations: MDS/MPN-U: Myelodysplastic syndrome/Myeloproliferative neoplasm-Unclassifiable; HA-Hypomethylating agents; Hy: Hydroxyurea; Dys: Dysplasia; Inc: Increased; Dec: Decreased; MS: Myeloid sarcoma; RA: Rheumatoid arthritis; MG: Myasthenia gravis; OA: Osteoarthritis; DVT: Deep vein thrombosis; MP: Myeloid predominant.

3.2. Peripheral Blood Findings

For the whole cohort, the median white blood cell (WBC) count was 14.6 × 10^9/L (range, 1.4 × 10^9/L to 58.5 × 10^9/L), with both leukopenia and leukocytosis observed. Hemoglobin (Hb) levels ranged from 7.3 g/dL to 12.0 g/dL, (median, 9.4 g/dL), with severe anemia (Hb < 8.0 g/dL) noted in four patients. Platelet counts ranged from 27 × 10^9/L to 216 × 10^9/L (median, 95 × 10^9/L). Patients in the MDS/MPN group presented with leukocytosis and varying degrees of anemia, and thrombocytopenia (3/5 cases). Peripheral blood smears revealed neutrophilia (>80% of leukocytes), with immature granulocytes comprising <10% of the WBC count in all the cases. Absolute monocytosis (>1x10^9/L and ≥10% of cells) were noted only in the CMML patients. There was no increase in basophil or blast in all cases. Peripheral smears in the acute leukemia group exhibited varying degrees of leukocytosis and cytopenia, with circulating blasts ranging from 1% to 77%. Peripheral smears in the other myeloid neoplasm group revealed normocytic anemia, anisopoikilocytosis, thrombocytopenia (3/4 cases), and dysgranulopoiesis.

3.3. Histopathological Findings in Bone Marrow

Bone marrow biopsies in the MDS/MPN group consistently revealed marked hypercellularity (70 to 100% cellularity) with myeloid predominance in three cases and erythroid predominance or normal M:E ratio in each of the remaining two cases. Dysplasia is noted in all the cases. All but one case (patient 4) had less than 5% blasts. Ring sideroblasts exceeding 15% were observed in two cases (case 1 and case 4). Moderate to marked reticulin fibrosis (grade 2-3) was seen in two patients (case 2 and case 3).

Bone marrow aspirates and biopsies in the acute leukemia patients revealed hypercellularity (70% to 85% cellularity) with extensive blast infiltration and decreased normal hematopoiesis in three

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cases. The bone marrow of the myeloid sarcoma case showed myeloid hyperplasia, left-shifted granulopoiesis, and dyserythropoiesis and the involved extramedullary site (lymph node) showed effacement of the architecture by a diffuse proliferation of immature myeloid cells, consistent with MS.

Bone marrow findings in the other myeloid neoplasm group ranged from normocellular to markedly hypercellular marrow (30 to100% cellularity), with myeloid predominance in 3 out of 4 patients, and dysplasia in at least one lineage in all patients. Increased blasts (5 to 8%) were noted in the bone marrow in all patients.

The histomorphological and immunophenotypic findings of the bone marrow biopsies of representative cases are depicted in Figure 1.

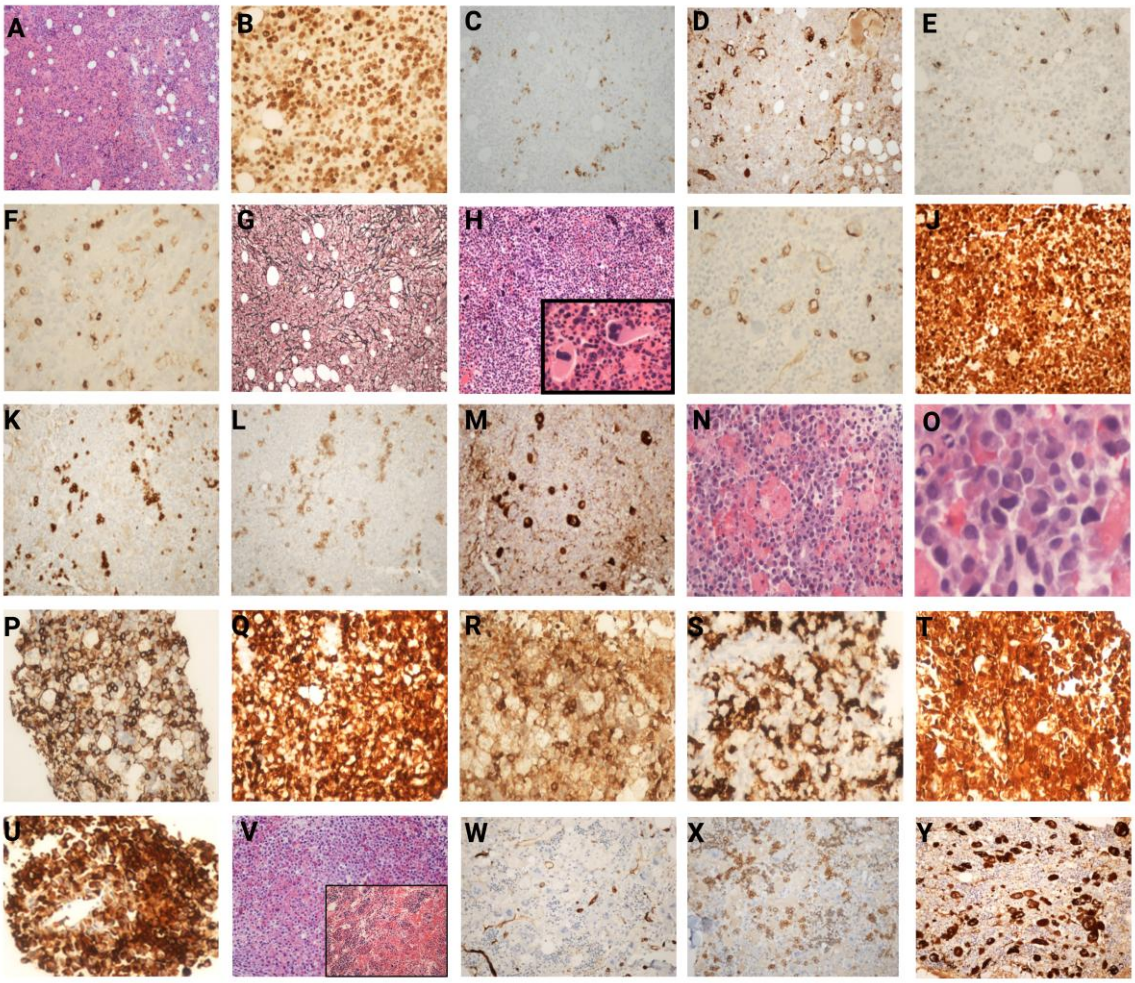

Figure 1. Histomorphological and immunohistochemical features of myelodysplastic/myeloproliferative neoplasms. (A-G) Photomicrographs showing features of the bone marrow of an MDS/MPN-U patient with CSF3R alteration. A) Photomicrograph showing bone marrow with marked hypercellularity, myeloid predominance, and decreased erythroid and megakaryocytic populations. B) Myeloperoxidase and C) CD71 confirm myeloid predominance. D) Factor VIII IHC showing megakaryocytes with atypia. E) CD34 IHC demonstrating <5% CD34-positive blasts. F) CD14 IHC highlighting an increased monocytic population. G) Reticulin stain showing grade 2-3 myelofibrosis. (H-M) Photomicrographs showing features of the bone marrow of a CMML patient with CSF3R alteration. H) Photomicrograph of bone marrow biopsy showing markedly hypercellular marrow with significant granulocytic hyperplasia and a prominent left shift in myeloid maturation (H&E, 40X, inset 100X). I) CD34 IHC demonstrating less than 5% blasts and megakaryocytes with aberrant CD34 expression. J) Myeloperoxidase, K) CD71, and L) E-cadherin confirm myeloid predominance and left-shift in the erythroid compartment. M) Factor VIII highlights dysplastic megakaryocytes. N-U): Photomicrographs showing

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features of myeloid sarcoma with CSF3R alteration: N) Photomicrograph showing atypical proliferation of pleomorphic hematopoietic cells with necrosis and apoptosis (H&E, 40X), O) H&E, 400X). Neoplastic cells are positive for P) CD45, Q) CD4, R) CD33, S) MPO, T) CD163, and U) Muramidase (IHC, 40X). V- Y) Photomicrographs showing features of bone marrow of patient with essential thrombocythemia gaining secondary CSF3R alteration. V) Photomicrograph showing a markedly hypercellular bone marrow (H&E, 20X) with left-shifted myeloid maturation and inset showing increased atypical megakaryocytic forms (H&E, 400X). W) CD34 immunohistochemical stain is highlighting the sinusoids and few megakaryocytic forms indicative of dyspoiesis (IHC, 100X). X) E-cadherin immunohistochemical stain shows prominence of pronormoblasts (IHC, 100X). Y) Factor VIII marks the increased megakaryocytes with atypia and occasional clustering ((IHC, 100X, 400X).

3.4. Cytogenetic and FISH Analysis

Among the 13 cases, normal karyotypes were observed in 8 cases. Abnormalities included monosomy 7 (Case 2), deletion 7q (Case 4), a complex karyotype in Case 9 (44-45,X,-Y,der(4)t(4;6)(p12;p11.2)der(4)(q31),-8,-9,+mar1,+mar2[6]/46,XY [cp14]), and a derivative chromosome involving chromosomes 13 and 14 in Case 12. Case 13 demonstrated a 45,-X,-Y karyotype. FISH studies identified monosomy 7 in Case 2 and deletion 7q in Case 4, confirming karyotype findings. Amplification of the RUNX1 gene was detected in Case 6 (three copies), and a RUNX1T1/RUNX1 rearrangement was identified in Case 9. FISH was normal in 7 cases; results were unavailable in 2 cases. One patient with MDS/MPN-U had an isolated deletion of the Y chromosome, likely an age-related finding.

3.5. Molecular Findings

The mutational landscape across the 13 patients of *CSF3R*-mutated myeloid neoplasms was highly heterogeneous. All patients harbored *CSF3R* alterations, with variant allele frequencies (VAFs) ranging from 2% to 49%, indicating varying degrees of clonal involvement.

Grouped analysis revealed that patients with MDS/MPN-U and CMML demonstrated the highest CSF3R VAFs (median, 39%; range, 26%–49%), consistent with a predominant clonal driver role. In contrast, cases with acute leukemia exhibited the lowest VAFs (median, 10%; range, 10%–15%), suggesting a possible subclonal or secondary role of CSF3R in leukemogenesis. Other myeloid neoplasms displayed intermediate VAFs (median, 12%; range, 2%–24%), indicating a smaller contribution of CSF3R to disease pathogenesis. These differences are visualized in Figures 2A and 2B, which display VAF distributions across diagnostic groups using both bar and box plots, respectively.

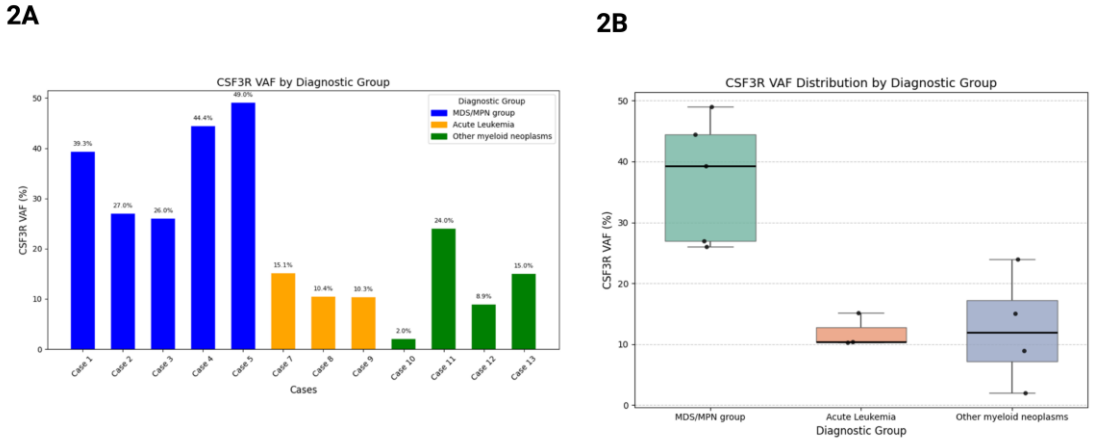

Figure 2. (A) This bar chart illustrates the VAF of CSF3R mutations across individual cases, categorized by diagnostic groups. Blue bars represent cases classified as MDS/MPN group, which demonstrate intermediate to high CSF3R VAF values (median, 39%; range, 26%–49%). Orange bars represent cases diagnosed as acute

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leukemia (median, 10%; range, 10%–15%), which tend to exhibit lower CSF3R VAF values compared to the MDS/MPN group. Green bars represent cases diagnosed for other myeloid neoplasms, which show varying levels of CSF3R VAF (median, 12%; range, 2%–24%). (B) Box plot showing CSF3R Variant Allele Frequencies (VAF%) across three diagnostic groups: MDS/MPN group, acute leukemia, and other myeloid neoplasms.

Co-mutations varied by disease subtype and are depicted in the categorical mutation heatmap (Figure 3). The most common co-mutations were *ASXL1* and mutations in RAS signaling pathway (*NRAS*, *KRAS* and *PTPN11*). MDS/MPN-U and CMML patients frequently carried mutations in epigenetic regulators (*ASXL1*, *TET2*) and RNA splicing genes (*SF3B1*, *SRSF2*, *ZRSR2*). A *SETBP1* mutation was identified in one patient with CMML. In the acute leukemia group, *CSF3R* mutations co-occurred with alterations in signaling pathway genes, including *JAK3*, *STAT3*, and *NRAS*. For instance, case 6 (AML with biallelic *CEBPA* mutations) also harbored *STAT3* mutation. Other myeloid neoplasms, including MDS, showed low-frequency *CSF3R* mutations (≤10%), suggesting a smaller subclonal role. Interestingly, *KRAS* mutations were observed in two of three MDS cases, possibly indicating an underlying MDS/MPN-like biology.

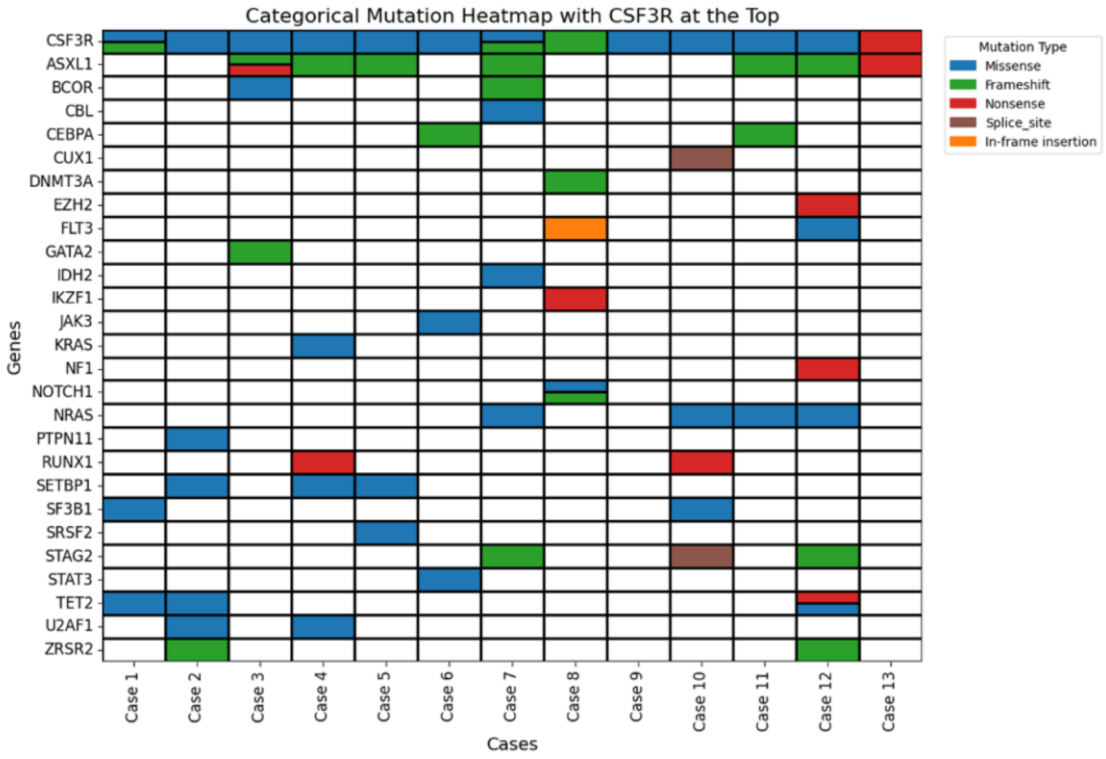

Figure 3. The heatmap highlights the mutation patterns and recurrently altered genes seen across 13 cases of myeloid neoplasms. Colored boxes represent mutation types: blue for missense, green for frameshift, red for nonsense, brown for splice site, and orange for in-frame insertion. White boxes indicate no mutation.

The most frequent *CSF3R* mutation was the canonical p.Thr618Ile missense mutation in exon 14, identified in 11 cases. Three additional pathogenic mutations were detected: a nonsense mutation (p.Gln743Ter) in two cases and two frameshift mutations (p.Lys785SerfsTer26 and p.S810Qfs) affecting the cytoplasmic tail of the protein in three cases. Case 1 carried both p.Thr618Ile and p.S810Qfs mutations. Only two patients had an isolated *CSF3R* mutation; one of these was of AML with a *RUNX1*::*RUNX1T1* translocation, suggesting that *CSF3R* was not the driver mutation in these two cases.

The mutation landscape was visualized using a stacked bar chart showing mutation percentages per case (Figure 4). Across the cohort, the most frequently mutated genes included ASXL1, NRAS,

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SETBP1, and TET2. Cases 4 and 5 demonstrated the highest mutation burden, with CSF3R mutations comprising 44.4% and 49% of total mutations, respectively. In contrast, cases 6–9 (acute leukemia) exhibited lower overall mutation percentages, with no single gene exceeding 30% VAF. The stacked bars also reveal patterns of co-mutations in individual cases, such as the presence of multiple gene mutations in cases 1, 4, and 5 (MDS/MPN-U and CMML), compared to a more restricted mutational profile in some acute leukemia cases. This diversity in mutation percentages highlights the complex clonal architecture of CSF3R-mutated myeloid neoplasms and emphasizes the importance of considering the broader mutational context when interpreting the role of CSF3R in disease pathogenesis and therapeutic decision-making.

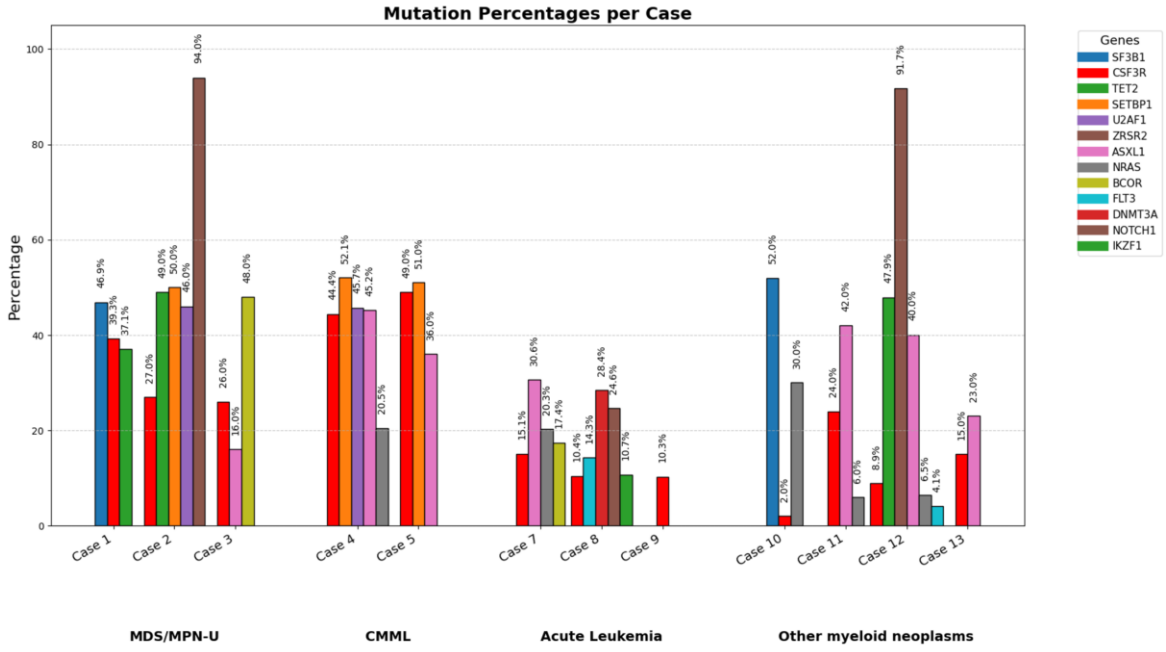

Figure 4. Genetic Mutation Frequencies in CSF3R-Driven Myeloid Neoplasms. This bar chart illustrates the percentage frequencies of key representative genetic mutations identified in various cases of CSF3R-driven myeloid neoplasms showcasing the heterogeneity of these disorders. Case 6, which harbors CSF3R, STAT3, CEBPA, and JAK3 mutations, is excluded from this graph as percentage data for these mutations is not available.

3.6. Clinical Outcomes

Survival data were available for all patients across the three diagnostic categories: MDS/MPN, acute leukemia, and other myeloid neoplasms. Kaplan-Meier analysis demonstrated distinct survival patterns among the groups. The median survival for the entire cohort was approximately 12 months. The MDS/MPN group exhibited the steepest decline in survival, with most patients succumbing to the disease early in its course. The acute leukemia group had a more gradual decline, reflecting variable clinical trajectories. In contrast, the other myeloid neoplasms group, comprised mainly of MDS-IB1, had a more stable survival curve, consistent with slower disease progression. Survival curves for each group are shown in Figure 5.

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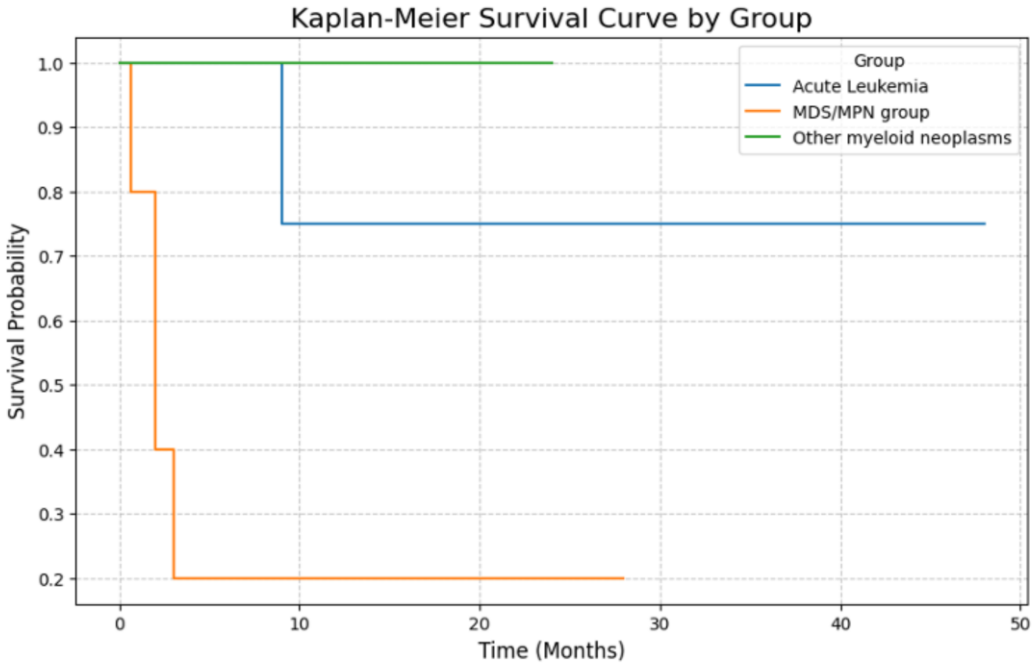

Figure 5. The Kaplan-Meier survival curve illustrates the survival probabilities of three diagnostic groups: MDS/MPN group (orange), Acute Leukemia (blue), and other myeloid neoplasms (green), over time in months. The MDS/MPN group shows a steep decline in survival, reflecting the poor prognosis of disorders like MDS/MPN-U and CMML. The Acute Leukemia group exhibits a more gradual decrease, indicating variable outcomes in aggressive diseases like AML, MPAL, and myeloid sarcoma. The other myeloid neoplasms demonstrate relatively stable survival, consistent with its indolent nature. Vertical ticks on the curves mark censoring, representing patients still alive or lost to follow-up.

4. Discussion

Our study highlights the significant presence of *CSF3R* mutation across a diverse range of myeloid neoplasms, expanding their known spectrum beyond the traditional associations with CNL, aCML, and severe congenital neutropenia (SCN) [16]. In our cohort of thirteen patients, we identified *CSF3R* alterations across a variety of myeloid neoplasms, including MDS/MPN-U, AML, MPN with disease progression, MPAL, CMML, and MDS-IB1, suggesting a broader role for *CSF3R* in pathogenesis of myeloid neoplasms and clonal evolution.

Functionally, these mutations affect distinct receptor domains. The membrane-proximal missense mutation p.*Thr*618*Ile*, impairs self O-glycosylation, resulting in ligand-independent receptor dimerization and continuous signaling of JAK-STAT signaling pathway [17]. In contrast, truncation mutations (p.*Gln*743*Ter*, p.*Lys*785*Ser*fsTer26) and frameshift mutations (p.S810Qfs) cluster within the cytoplasmic tail and disrupt negative regulatory motifs responsible for receptor internalization and degradation, thereby prolonging receptor activity, and preferentially activating the SRC/TNK2 signaling pathway and enhanced cell proliferation [11, 18, 19]. The lollipop plot shown in Figure 6, illustrate their distribution across different functional domains.

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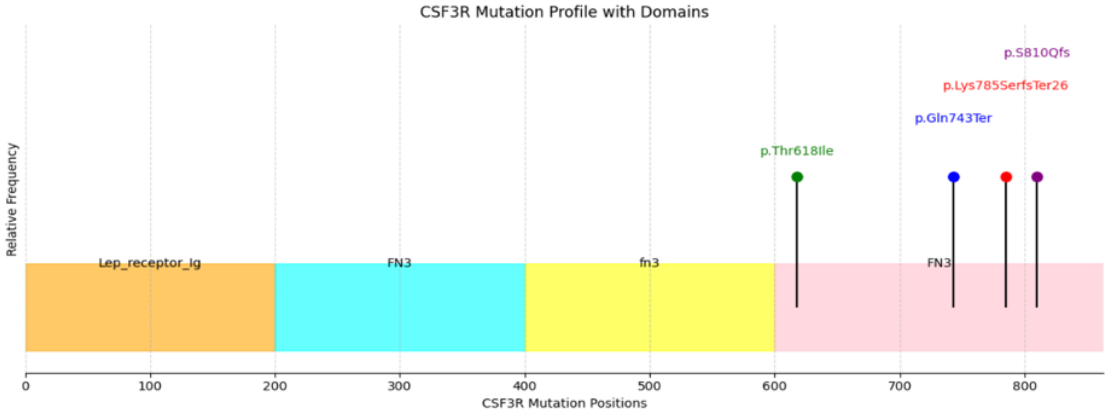

Figure 6. Lollipop plot conveying CSF3R Mutation Profile with Functional Domains and Hotspot Mutations.

While the *CSF3R* T618I mutation remains a hallmark of CNL (reported in up to 83% of cases) and is also present in aCML [17], our findings reinstate that this mutation is not restricted to these entities. This functional divergence emphasizes their distinct roles in myeloid pathogenesis and align with prior studies showing that *CSF3R* truncation mutations are frequently associated with severe disease phenotypes and resistance to therapy [5, 20].

We also found frequent co-occurrences of *CSF3R* mutations with alterations involving other genes including, but not limited to, *ASXL1*, *NRAS*, *KRAS*, and *SETBP1* in the MDS/MPN-U and CMML patients. These co-mutations likely contribute to the phenotypic heterogeneity and clinical variability observed in these *CSF3R*-driven neoplasms [21]. Among these, *ASXL1* mutations were the most frequent co-mutations in our cohort, consistent with their known association with poor prognosis and disease progression in myeloid malignancies [13, 22]. This suggests that *ASXL1* co-mutations might exacerbate the effects of *CSF3R* mutations by promoting epigenetic dysregulation. *ASXL1* mutation, in combination with proliferative mutations such as *ETNK1* and *SETBP1* bring about the MDS/MPN phenotype in aCML. It is likely that the combination of *ASXL1* and *CSF3R* creates similar phenotype with the same mechanism. *NRAS* and *KRAS* mutations, observed in a few cases, highlight the role of aberrant RAS-MAPK signaling in driving clonal proliferation and leukemic transformation in these neoplasms [23].

In their study of *CSF3R* alteration in CNL and CMML, Ouyang and colleagues found *SRSF2* and *SETBP1* to be associated with worse prognosis, whereas *CSF3R* alteration did not affect outcome [24]. Other studies have suggested the role of *SETBP1* alteration as secondary drivers of leukemic transformation and resistance to therapy [25]. In our dataset, both *SETBP1* (case 2, 3, and 5) and *SRSF2* (case 5) were noted predominantly in the MDS/MPN subcategory and was associated with poorer outcomes.

Amongst acute leukemia patients in our cohort, one patient had *RUNX1::RUNX1T1* translocation, while another had biallelic *CEBPA* mutation, both of which are known to co-occur with *CSF3R* alterations [26, 27]. This pattern implies that in these settings, *CSF3R* mutations may contribute to disease progression.

Clinically, the identification of *CSF3R* mutations has profound therapeutic implications, emphasizing the need for routine molecular profiling in myeloid malignancies to identify *CSF3R* mutations and associated co-mutations. This is particularly relevant in patients without clearly defined category (MDS/MPN-U), where the presence of p.Thr618Ile or truncation mutations could provide a therapeutic target. The association of *ASXL1*, *SRSF2*, and *SETBP1* mutations with poor prognosis, as evidenced by other studies, underscore the need for comprehensive molecular profiling [28]. As previously discussed, the constitutive activation of signaling pathways like JAK-STAT and SRC/TNK2 in *CSF3R*-altered neoplasms presents opportunities for targeted interventions with documented efficacy of JAK-inhibitors such as

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ruxolitinib in these neoplasms [11, 29]. Additionally, inhibitors targeting downstream pathways, such as SRC family kinases, could complement JAK inhibitors in cases where alternative signaling predominates. These therapeutic approaches could potentially be combined with other emerging therapeutic approaches such as MEK-inhibitors to target RAS pathway or DNA methyltransferase inhibitors in *ASXL1* mutated neoplasms.

5. Conclusions

Our study provides valuable insights into the genetic landscape of *CSF3R* mutated myeloid neoplasm and adds to the growing body of evidence that *CSF3R* mutations, especially T618I, are a critical component of the molecular landscape in MDS/MPN. *CSF3R* mutations should not be considered exclusive to CNL, aCML, or SCN, but rather as an important targetable genetic alteration found in a broader spectrum of diseases, including MDS/MPN, AML, and other myeloid disorders. Given the genetic heterogeneity of MDS/MPN, *CSF3R* mutations could serve as an important marker for diagnostic stratification and prognostic prediction.

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