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Mast Cell Leukemia: Clinical and Molecular Features and Survival Outcomes of Patients in the ECNM Registry

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Abstract:
Mast cell leukemia (MCL) is a rare subtype of systemic mastocytosis (SM) defined by >20% mast cells (MC) on a bone marrow aspirate. We evaluated 92 patients with MCL from the European Competence Network on Mastocytosis registry. Thirty-one (34%) patients had a diagnosis of MCL with an associated hematologic neoplasm (MCL-AHN). Chronic MCL (lack of C-findings) comprised 14% of patients, and only 4.5% had 'leukemic MCL' ({greater than or equal to} 10% circulating MCs). *KIT* D816V was found in 62/85 (73%) evaluable patients; 9 (11%) individuals exhibited alternative KIT mutations, and no KIT variants were detected in 14 (17%) subjects. Ten evaluable patients (17%) had an abnormal karyotype and the poor-risk *SRSF2*, *ASXL1*, and *RUNX1* (*S/A/R*) mutations were identified in 16/36 (44%) patients who underwent next-generation sequencing. Midostaurin was the most common therapy, administered to 65% of patients, and 45% as first-line therapy. The median overall survival (OS) was 1.6 years. In multivariate analysis (*S/A/R* mutations excluded due to low event rates), a diagnosis of MCL-AHN (HR 4.7, 95% CI 1.7 - 13.0, p = 0.001) and abnormal karyotype (HR 5.6, 95% CI 1.4 - 13.3, p = 0.02) were associated with inferior OS; *KIT* D816V positivity (HR 0.33, 95% CI 0.11 - 0.98, p = 0.04) and midostaurin treatment (HR 0.32, 95% CI 0.08 - 0.72, p = 0.008) were associated with superior OS. These data provide the most comprehensive snapshot of the clinicopathologic, molecular, and treatment landscape of MCL to date, and should help further inform subtyping and prognostication of MCL.

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Abstract

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Key Points

- The median OS of MCL patients was 1.58 years; a diagnosis of MCL-AHN and an abnormal karyotype were each associated with inferior outcomes
- Midostaurin was the most commonly used agent in MCL and was associated with improved OS in a multivariate analysis

Introduction

Systemic mastocytosis (SM) is a myeloid neoplasm characterized by expansion and accumulation of clonal mast cells (MC) in the bone marrow (BM) and other organs. Non-advanced subtypes of SM include indolent SM (ISM) and smoldering SM (SSM); advanced SM (AdvSM) subtypes include aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN), and mast cell leukemia (MCL).[1-4] MCL is rare, comprising <5% of all SM cases, and is defined by World Health Organization (WHO) diagnostic criteria for SM plus the criterion of ≥ 20% MCs on a BM aspirate smear.[5] Although one or more C-findings (SM-related organ damage) is a prerequisite for a diagnosis of ASM, it is not required for a diagnosis of SM-AHN or MCL, despite being commonly encountered in these diseases. The prognosis of MCL is often grim, with a median overall survival (OS) of < 2 years.[5-7]

MCL can be further subdivided into variants based on clinicopathologic features. These subtypes include: primary (*de novo*) vs. secondary MCL (arising from another SM variant); acute (with C-findings) vs. chronic (without C-findings) MCL; MCL with or without an AHN; and leukemic (≥ 10% circulating MC) vs. aleukemic MCL (< 10% circulating MC).[4,5,8-12]

More recently, cytogenetic and molecular data have provided additional insight into the biology and prognosis of MCL. As with other AdvSM subtypes, patients with MCL frequently exhibit the *KIT* D816V driver mutation, albeit at a generally lower frequency than ASM and SM-AHN.[13-15] Other commonly mutated genes include *TET2, SRSF2, ASXL1,* and *RUNX1*.[6,16,17] The presence of *SRSF2*, *ASXL1,*and/or *RUNX1* (*S/A/R*) mutations predict inferior survival, and *S/A/R*

mutations (in addition to other mutations such as *NRAS* and *DNMT3A*) have been incorporated into prognostic scoring systems for patients with AdvSM.[6,17-21]

Historically, MCL has been treated with cytoreductive chemotherapy, most commonly single agent cladribine (2-CdA), and in some cases multi-agent acute myeloid leukemia (AML)-type induction regimens.[5] A possible salvage treatment option for these patients is allogeneic hematopoietic cell transplantation (allo-HCT), especially when a response to 2-CdA and/or chemotherapy is obtained. However, a multicenter, retrospective series reported that MCL patients (n = 12) exhibited worse overall survival (OS) among all AdvSM patients undergoing allo-HCT, with a 3-year OS of only 17%.[22]

More recently, KIT-targeting drugs have demonstrated encouraging activity in patients with AdvSM, including MCL. Reduction in objective measures of MC burden (percentage of BM MC, serum tryptase level, splenomegaly, *KIT* D816V variant allele frequency), as well as reversion of C-findings and symptoms have been consistent findings. Based on a global, non-randomized phase II trial, the multikinase/KIT inhibitor midostaurin was approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2017 for patients with AdvSM.[17] More recently, the selective KIT D816V inhibitor avapritinib was also approved by the FDA in 2021 as first line therapy and by the EMA in 2022 as second line therapy based on safety and efficacy results from the phase I EXPLORER trial and an interim analysis of the phase II PATHFINDER trial in patients with AdvSM.[23,24]

To date, MCL has been systematically characterized in few reports, including a review of 51 patients from various centers, a series of 28 cases from Germany, and a series of 13 patients from the US.[5-7] However, many features of this rare disorder remain incompletely understood. In this study, we describe the clinical characteristics, molecular features, current treatment patterns, and survival outcomes of a well-characterized, multi-institutional cohort of patients with MCL from the European Competence Network on Mastocytosis (ECNM) registry. To our knowledge, this is the largest cohort of patients with this rare advanced myeloid neoplasm.

Methods

ECNM Registry Database and Patients

Data were abstracted from the fifth data wave of the ECNM registry, which contains clinical, laboratory, pathologic, and molecular information on patients with SM from 30 European centers and one center in the United States (Stanford Cancer Institute).[25] All patients with a diagnosis of MCL were included in this analysis. All diagnoses of MCL were made between 1994 – 2019 and data were abstracted through 7/1/2020. The diagnosis of MCL was established according to diagnostic criteria provided by the WHO and the consensus group.[1-4,12] The study design adhered to the tenets of the Declaration of Helsinki and was approved by the participating centers' institutional review boards. Prior to inclusion in the ECNM registry, all patients provided written informed consent or, if deceased or inactive at the treating center, were included according to IRB standards at the treating center.

The following parameters were captured for this study: age, sex, date of diagnosis (histology based), diagnosis according to the WHO classification[26], any SM diagnoses prior to diagnosis of MCL, laboratory values at time of MCL diagnosis, percentage of MC in BM aspirate smears and blood films, molecular and cytogenetic data, presence of hepatosplenomegaly, weight loss (defined as >10% loss during the last 12 months prior to diagnosis), skeletal involvement of SM (defined as an osteolytic lesion(s) ≥ 2 cm) , treatment courses, and death or last follow-up. The mutation-adjusted risk score (MARS), the global prognostic score for mastocytosis (GPSM), and international prognostic scoring system for mastocytosis (IPSM) for AdvSM prognosis were each calculated from the above relevant variables.[19,21,27]

All cytogenetic analysis was performed at local laboratories using conventional methods. *KIT* D816V mutation status was measured at local laboratories using either single-gene assays or next-generation sequencing multi-gene panels on BM or peripheral blood, following ECNM recommendations.[14] If a *KIT* D816V mutation was absent, laboratories were encouraged to search for alternative *KIT* mutations whenever feasible.

Statistical Analysis

Continuous variables were summarized by median and range and binary outcomes were summarized by proportion. For secondary MCL, progression was defined as transformation from one WHO SM category (ISM, SSM, ASM, or SM-AHN) to MCL. OS was defined as time from MCL diagnosis to death from any cause. For patients who underwent allo-HCT, progression-free survival (PFS) was defined as time from allo-HCT to disease progression or death from any cause. OS was estimated using the Kaplan-Meier method and compared using log-rank tests.

Univariate covariate effects on OS were evaluated using linear or logistic regression. Covariates with a *p*-value of < 0.05 were included in a multivariate cox proportional hazard model. *P* values < 0.05 were considered significant. Analyses were performed using R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical and Laboratory Characteristics

At time of data abstraction, the ECNM contained 2,931 patients with SM, of which 576 (19.7%) had AdvSM and 92 (3.1%) had MCL and were included in this analysis. Fifty-seven (62%) MCL patients had follow-up records available; of those, the median follow-up time was 1.1 years (range 0.1 – 10.2).

Clinical and laboratory characteristics at time of MCL diagnosis are described in **Table 1 (**stratification by MCL vs MCL-AHN in **Supplemental Table 1;** stratification by acute vs. chronic MCL in **Supplemental Table 2)**. The median age at diagnosis was 60.4 years (range 25.4 – 90.8). Most patients (70.7%) had *de novo* MCL while 29.3% had secondary MCL. Sixty-one (66.3%) patients had a diagnosis of MCL only and 31 (33.7%) had a diagnosis of MCL-AHN; of those, the most common AHN was myelodysplastic syndrome/myeloproliferative neoplasm-unclassifiable (MDS/MPN-U; 45.2%), followed by chronic myelomonocytic leukemia (CMML; 25.8%), chronic eosinophilic leukemia (CEL; 9.7%), and AML (9.7%). Concurrently identified lymphoid neoplasms included multiple myeloma and non-Hodgkin lymphoma (both 3.2%).

The median serum tryptase at diagnosis was 333.5 μg/L (range 50.9 – 7,490; normal level < 11). The majority of patients (81.5%) had a tryptase of ≥ 200 μg/L. Of the 89 patients with a complete blood count with differential available, 11 (12.4%) had circulating MCs, while only 4 (4.5%) met criteria for 'leukemic MCL' with ≥ 10% circulating MCs. Of the 60 patients with karyotype information available, 10 (17%) had an abnormal karyotype.

The majority of patients (86%) had acute MCL, with at least one C-finding present. Most patients (68%) had both hematologic and non-hematologic C-findings, 25% of patients had non-hematologic C-findings only, and only 5 patients (6%) had hematologic C-findings only (see **Table 1** for listing of C-findings). Of the 13 patients with chronic MCL, 8/13 (62%) had *de novo* MCL, 10/13 (77%) had MCL without an AHN, and 10/13 (77%) were *KIT* D816V positive; no chronic MCL patients had circulating peripheral blood mast cells (0/13) or an abnormal karyotype (0/9 evaluable). Follow-up data was available in 7 patients with chronic MCL; of these, 3 progressed to acute MCL (median time to progression 5 months, range 4.7 – 27).

Progression to Secondary MCL

Of the 576 patients with AdvSM, 24 (4.2%) progressed to secondary MCL, including 11/147 (7.5%) patients with ASM and 13/337 (3.9%) with SM-AHN. Of the patients with SM-AHN, 11/13 progressed to MCL-AHN and 2/13 progressed to MCL. By contrast, in patients with non-AdvSM (n = 2,208), only 5 (0.2%) ultimately progressed to secondary MCL, including 5/2132 (0.2%) patients with ISM and 2/76 (2.6%) with SSM (both of which had proceeding ISM). Two patients progressed from ISM to ASM to MCL, one patient progressed from ASM to SM-AHN

to MCL, and the two patients progressed from ISM to SSM to MCL (these individuals were counted among the aforementioned patients who progressed to MCL) **(Table 2).**

Of the 27 patients with secondary MCL, the most common diagnosis at initial presentation was SM-AHN (14 patients, 52%), ASM (7 patients; 26%), and ISM (6 patients; 22%); no patients with secondary MCL were diagnosed with SSM at initial presentation. The median time to progression from initial SM diagnosis was 1.8 years (range 0.1 – 13.5) (**Figure 1A)**. Progression to MCL occurred most quickly in patients with an initial diagnosis of SM-AHN, with a median time to progression of 0.7 years (range 0.1 – 7.1 years), followed by ASM (3.9 years; range 0.8 – 13.5 years), and ISM (5.3 years; range 4.4 – 10.3 years). The most common diagnosis immediately prior to MCL progression was SM-AHN (13 patients; 48%) followed by ASM, (11 patients; 41%), SSM (2 patients; 7%), and ISM (1 patient; 4%) (**Figure 1B)**. Three patients did not progress to MCL until ≥ 5 years. Of those 3 patients, 2 had therapy information available and both were treated with interferon-alfa.

Overall Survival in Subgroups of Patients with MCL

The median OS of the entire cohort was 1.6 years (95% confidence interval [CI] 1.27 – 3.16 years) **(Figure 2A)**. Notably, select patients in this cohort demonstrated long-term survival with 11 patients alive ≥ 5 years from MCL diagnosis and 2 patients alive ≥ 10 years. Of the 11 patients alive ≥ 5 years from MCL diagnosis, 10 patients (91%) had MCL (vs MCL-AHN), all patients were *KIT* D816V positive, 5 patients had karyotype information available (all of whom had normal cytogenetics), and only 2 (18%) patients had chronic MCL (**Supplemental Table 3)**.

Of the 54 patients with cause of death data available, 41 (76%) died from their disease, 3 (6%) died from treatment complications, and 10 (18%) had other causes of death.

Compared to patients with MCL alone (n = 61), patients with MCL-AHN (n = 31) had an inferior OS (median OS 1.3 vs 2.3 years, p = 0.02) (**Figure 2B)**. Patients with acute MCL (n = 79) also had inferior OS compared to patients with chronic MCL (n = 13) (median OS 1.5 years vs not reached, p = 0.04) (**Figure 2C**). There was no difference in OS for patients with *de novo* (n = 65) vs secondary MCL (n = 27) (median OS 1.8 vs 1.4 years, p = 0.95) (**Figure D)**. An abnormal karyotype (n = 10) was associated with inferior OS compared to MCL cases with a normal karyotype (n = 50) (1.4 vs 1.72 years, p = 0.025) (**Figure 2E)**. Compared to patients with aleukemic MCL (n = 85), patients with leukemic MCL (n = 4) exhibited inferior OS (0.4 vs 1.9 years, p = 0.0064). Similarly, patients with any circulating MCs (n = 11) had inferior OS compared to patients with no circulating MCs (n = 78) (0.5 vs 3.2 years, p = 0.0013) (**Figure 2F**).

Comparison of OS in Patients with MCL versus other subtypes of AdvSM

Of the 576 patients with AdvSM, patients with MCL (n = 92) had significantly inferior OS compared to patients with ASM (n = 147) and SM-AHN (n = 337) (1.6 vs 6.2 vs 2.8 years, respectively, p < 0.001) (**Supplemental Figure 1A)**. This relationship was preserved when the patients with SM-AHN were restricted to patients with ASM-AHN (n = 197) (1.6 vs 6.2 vs 2.1 years, respectively, p < 0.001) (**Supplemental Figure 1B**). Similarly, when compared to patients with ASM-AHN (n = 197), patients with MCL-AHN (n = 31) had inferior OS (2.1 vs 1.3 years, respectively, p = 0.03) **(Supplemental Figure 1C**).

Impact of Mutational Profiles in Patients with MCL

Of the 85 patients with *KIT* mutational status available, 71 (84%) had a *KIT* mutation (**Figure 3A**). The most common *KIT* mutation was D816V (n = 62, 72.9%) while 9 patients (10%) had an alternative *KIT* mutation. Of the 9 patients with an alternative *KIT* mutation, 8 patients had an alternative substitution at the D816 locus (e.g. D816Y, D816H, D816T) while 1 patient had a *KIT* S501_A502 duplication which has been reported as an activating mutation[28]. Fourteen patients (16.5%) had no *KIT* mutation detected, although it is unknown whether full *KIT* sequencing was obtained in these patients.

Compared to *KIT* D816V-negative patients (n = 23), *KIT* D816V positive patients (n = 62) had superior OS (median OS 3.2 vs 0.9 years, $p < 0.001$) (**Figure 3B**). Patients with alternative *KIT* mutations, however, had inferior OS compared to *KIT* D816V-positive patients, with OS resembling that of patients without *KIT* mutations detected, with median OS 3.2 vs 1.1 vs. 0.65 years for *KIT* D816V positive (n = 71) vs. alternative *KIT* mutation (n = 9) vs. no detectable *KIT* mutation (n = 14) ($p = 0.004$) (**Figure 3C)**.

Data from multi-gene myeloid panels was available for 36 patients (**Figure 4**). The most commonly mutated genes aside from *KIT* were *SRSF2* and *ASXL1 (*11 patients each, 30.6%), followed by *TET2* (7 patients, 19.4%), *SF3B1* (5 patients, 13.9%), *RUNX1* (4 patients, 11.1%), and *N/KRAS* (3 patients, 8.3%). The number of mutations besides *KIT* was higher in patients with MCL-AHN, with a median of 2 mutations per patient compared to 0.5 mutations per patient in patients with MCL alone ($p = 0.005$). Of the 36 patients with multi-gene myeloid panels available, 16 (44.4%) had mutations in *SRSF2, ASXL1*, or *RUNX1* (*S/A/R*). Patients with *S/A/R*

mutations had inferior OS compared to patients without *S/A/R* mutations (median OS 0.5 years vs not reached, p = 0.005) (**Supplemental Figure 2A)**.

Prognostic Scores

MARS and GPSM prognostic scores were calculated for the 36 patients with molecular data available. Patients with low MARS had superior OS compared to patients with intermediate and high MARS (median OS NR vs 1.3 vs 0.7 years, p = 0.022) (**Supplemental Figure 2B**). Similarly, patients with low or intermediate GPSM had superior OS compared to patients with high GPSM (median OS 0.7 vs 1.7 years, p = 0.04) (**Supplemental Figure 2C**). All patients had IPSM scores calculated with lower scores associated with significantly improved OS (OS for AdvSM-1 vs AdvSM-2 vs AdvSM-3 vs AdvSM-4: 6.6 vs 7.4 vs 1.4 vs 1.3 years, p = 0.004) (**Supplemental Figure 2D**).

Treatment Outcomes in Patients with MCL

Treatment data following MCL diagnosis was available for 75 patients, including 75 patients receiving first-line treatment, 46 second-line, and 28 third-line or greater (**Figure 5A, B**). Midostaurin was the most common therapy, administered to 49 patients (65.3%) at any point during their treatment course and 34 patients (45.3%) as first-line therapy. The next most common therapy was cladribine, administered to 42 patients (56%) at some point during their treatment course and 21 patients (28%) as first-line therapy. Cladribine was also the most common therapy given in the second and third line or greater settings, administered to 12 (26%) and 9 (32%) patients, respectively.

Patients who received midostaurin at any point during their treatment course had superior OS compared to patients who did not (median OS 2.3 vs 1.1 years, p = 0.01) (**Supplemental Figure 3A)**. In the first line setting, patients who received midostaurin had a median OS of 3.2 years vs 1.3 years for patients who received a different first line therapy (p = 0.08) (**Supplemental Figure 3B)**.

Of the 92 patients in our cohort, 8 patients (4 with MCL and 4 with MCL-AHN) received an allo-HCT, which occurred at a median of 6.8 months following MCL diagnosis. Following allo-HCT, the median PFS was 0.4 years and median OS 0.8 years (**Supplemental Figure 4**). The median duration of follow-up was 0.7 years (range 0 – 2.3). At time of last follow-up, 3 of the 8 patients who received allo-HCT were alive; 4 patients died from relapsed/progressive disease and 1 from treatment complications.

Comparative Treatment Patterns between MCL versus other subtypes of AdvSM

Compared to patients with MCL, a significantly smaller proportion of patients with ASM received treatment with midostaurin at any point during their treatment course (35% vs 53%, p = 0.006) or as first-line therapy (23% vs 37%, p = 0.02). Similarly, compared to patients with MCL, a smaller proportion of patients with SM-AHN received midostaurin at any point during their treatment course (32% vs 53%, p = 0.0002) or as first-line therapy (24% vs 37%, p = 0.02) (**Supplemental Figure 5A**).

There was no difference in the proportion of patients with ASM vs MCL who received treatment with cladribine at any point during their treatment course (35% vs 46%, p = 0.1) or as first-line

therapy (18% vs 23%, p = 0.41). By contrast, a smaller proportion of patients with SM-AHN received treatment with cladribine at any point during their treatment course (16% vs 46%, <0.0001) or as first-line therapy (9% vs 23%, p = 0.0008) (**Supplemental Figure 5B**).

Factors Influencing Survival in Patients with MCL

Uni- and multivariate analyses of the association between baseline patient, disease, and treatment characteristics on OS are shown in **Table 3**. In univariate analysis, final diagnosis of MCL-AHN (Hazard Ratio [HR] 1.9, 95% CI 1.1 – 3.2, p = 0.02), the presence of any circulating MCs (HR 3.9, 95% CI (1.6 – 9.4, p = 0.003), an abnormal karyotype (HR 2.5, 95% CI 1.1 – 5.7, p = 0.03), and the presence of *S/A/R* mutations (HR 5.8, 95% CI 1.9 – 17.0, p = 0.002) were associated with inferior OS; chronic MCL (HR 0.3, 95% CI 0.1 – 0.9, p = 0.04), *KIT* D816V positivity (HR 0.3, 95% CI 0.1 – 0.5, p < 0.001) and treatment with midostaurin at any point following MCL diagnosis (HR 0.5, 95% CI 0.3 – 0.9, p = 0.01) were associated with superior OS.

The aforementioned variables were entered into a multivariate analysis. Final diagnosis of MCL-AHN (HR 4.7, 95% CI 1.7 – 13.0, p = 0.001) and abnormal karyotype (HR 5.6, 95% CI 1.4 – 13.3, p = 0.02) were associated with inferior OS; *KIT* D816V positivity (HR 0.3, 95% CI 0.1 – 0.98, p = 0.04) and midostaurin treatment (HR 0.3, 95% CI 0.08 – 0.7, p = 0.008) were associated with superior OS. *S/A/R* mutations were not included in the multivariate analysis due to low event rate.

Discussion

In this study, we describe the clinical features, molecular characteristics, treatment patterns, and survival outcomes in a cohort of 92 patients with MCL collected in the ECNM registry. We confirm that leukemic and chronic MCL are rare subtypes, comprising 4.5% and 14% of our cohort, respectively, consistent with historical reports.[6]

In our cohort, the median OS of patients with MCL was 1.6 years, although a few patients survived ≥ 5 or even ≥10 years following their MCL diagnosis. This is similar to previously published reports, in which OS ranges from 0.5 – 2.6 years (**Supplemental Table 4**).[5-7] MCL patients exhibited the worst survival of all AdvSM patients in the registry, including individuals with ASM as well as SM-AHN (irrespective of whether all SM-AHN patients were analyzed [OS=2.8 years] or just those with ASM-AHN [OS=2.1 years]).

We demonstrate that a diagnosis of MCL-AHN, abnormal karyotype, any circulating MCs, *KIT* D816V negativity, and treatment status (not receiving midostaurin) were all significantly associated with inferior OS in a multivariate analysis. The observation that any number of circulating MCs found in a peripheral blood smear is associated with inferior OS should prompt reconsideration of the current threshold of ≥10% as the definition of 'leukemic' MCL.[2] In addition, no difference in survival outcomes was observed between *de novo* and secondary MCL, consistent with prior reports.[6] Given this, the prognostic value of subdividing MCL into *de novo* vs secondary variants may be limited. Indeed, the similar survival curves between the two groups may suggest that "*de novo*" MCL could reflect some patients who lacked a prior BM biopsy demonstrating an antecedent variant of SM. By contrast, the presence vs. absence of C-findings,

which respectively define acute and chronic MCL, demonstrates a statistically significant difference in OS between these two groups. The use of prognostic scoring systems such as MARS or GPSM, which incorporate the *S/A/R* panel, or the IPSM, provide complementary methods for risk stratifying MCL patients, as demonstrated by the differences in OS in our cohort.

The *KIT* D816V mutation drives the proliferation of neoplastic MC. In patients with AdvSM, *KIT* D816V positivity ranges from 84 – 95%.[19,23,29,30] Alternative *KIT* mutations are less common, at ≤3%.[19,30] In our cohort, *KIT* D816V positivity was lower (73%) than that described in the broader AdvSM population. This has also been described in other MCL studies, where *KIT* D816V positivity ranged from 23% to 68%.[5-7] In addition, we found that alternative *KIT* mutations were more common than in the AdvSM population, at 10%, consistent with prior reports of 15% - 21%,[5-7] and that alternate or lack of *KIT* mutations were associated with less favorable outcomes compared to cases with *KIT* D816V. Since not all patients in our cohort received sequencing for alternate *KIT* mutations, it is possible patients with "no" *KIT* mutation in fact had an alternative *KIT* mutation, either within or outside of exon 17. The true prevalence of alternate *KIT* mutations and their impact on clinical outcomes should be further evaluated in a larger cohort of *KIT* D816V-negative patients using uniform sequencing techniques.

Besides *KIT,* MCL cases demonstrated a variety of myeloid-associated gene mutations, with *SRSF2, ASXL1, TET2, SF3B1,* and *RUNX1* being the most common. Nearly half (44%) of the patients had *S/A/R* mutations, and these were associated with inferior prognosis, consistent with prior studies of AdvSM.[6,30] These additional alterations were more common in patients with

MCL-AHN vs MCL alone, suggesting that the presence of additional somatic myeloid mutations should prompt evaluation for a co-existing AHN. As these alterations were detected via bulk sequencing, it is unclear whether the increased mutational burden in patients with MCL-AHN was due to increased mutations in the MCL clone, in the AHN clone, or in both, reflecting a common myeloid progenitor affecting both populations. Prior studies have indicated that the *KIT* D816V mutation can be identified in cells derived from the AHN clone as well as in MCL.[15,31] Additional studies, including the use of single-cell sequencing, should help delineate the clonal landscape of SM-AHN.

In our cohort, midostaurin was the most common treatment, administered to over half of patients at some point following MCL diagnosis, and largely in the frontline setting. Patients who received midostaurin had superior OS compared to those who did not. Avapritinib was the second KIT-targeting agent approved for AdvSM in 2021 and 2022; however, only 4 patients in our cohort received avapritinib, and the impact of this KIT-targeting agent on MCL-specific outcomes requires further evaluation. Although no head-to-head comparison has been undertaken, the 24-month overall survival rates of MCL patients treated with midotaurin and avapritinib were 26% and 92%, respectively, from registrational trials of these drugs.[23,29] In the current series, 8 patients received allo HCT, but OS was not improved in these individuals, consistent with a prior retrospective report of transplanted patients with AdvSM, where patients with MCL had the worst outcomes, with a 3-year OS of only 17%.[22]

As expected from the nature of a registry analysis, our study has several limitations. While the ECNM cohort is well characterized, patients were treated across multiple sites, and data for all

pertinent variables for the 92-patient cohort was not always available. For example, assays undertaken for alternative *KIT* mutations were only available for a subset of patients, as was the use of multigene NGS panels. Nevertheless, our study represents the largest described cohort of patients with MCL and provides valuable insight into both disease histopathology, molecular genetics, and clinical outcomes. These data should help inform subtyping of MCL in the context of new updates to the classification of SM. Further evaluation regarding the prognostic significance of *KIT* D816V vs alternative or no *KIT* mutation is warranted, as is further investigation of long-term disease outcomes following treatment with midostaurin and avapritinib.

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Table 1. Clinical and Laboratory Characteristics of Patients with Mast Cell Leukemia at Diagnosis

Variable	All Patients (n = 92)	*De novo* MCL (n = 65)	Secondary MCL (n = 27)
Age at MCL diagnosis, years; median (range)	60.4 (25.4 – 90.8)	60.4 (27.1 – 90.8)	60.0 (25.4 – 73.4)
Males, n (%)	59 (64.1%)	43 (66.2%)	16 (59.3%)
Diagnosis, n (%)			
MCL	61 (66.3%)	46 (70.7%)	15 (55.6%)
MCL-AHN	31 (33.7%)	19 (29.2%)	12 (44.4%)
MDS/MPN-U	14 (45.2%)	12 (18.5%)	2 (7.4%)
CMML	8 (25.8%)	2 (3.1%)	6 (22.2%)
CEL/Eosinophilia	3 (9.7%)	2 (3.1%)	1 (3.7%)
AML	3 (9.7%)	1 (1.5%)	2 (7.4%)
Multiple Myeloma	1 (3.2%)	1 (1.5%)	0
NHL	1 (3.2%)	1 (1.5%)	0
Not specified	1 (3.2%)	0	1 (3.7%)
C-Findings, n/N (%)*			
No C-findings present	13 (14%)	8 (12.3%)	5 (18.5%)
Hemoglobin < 10 g/dL	43 (46.7%)	31 (47.7%)	12 (44.4%)
Platelets < 100 x 10^9/L	46 (50.0%)	30 (46.2%)	16 (59.3%)
Absolute Neutrophil Count < 1 x 10^9/L	2 (2.2%)	2 (3.1%)	0
Weight Loss (> 10% in 6 months)	41/85 (41.8%)	37/61 (60.7%)	4/24 (16.7%)
Albumin < 3.5 g/dL	26/83 (31.3%)	21/57 (36.8%)	5/26 (19.2%)
Hepatomegaly with ascites or portal hypertension	19/84 (22.6%)	13/59 (22.0%)	6/25 (24.0%)
Alkaline Phosphatase > 150 U/L	47/87 (54.0%)	31/60 (51.7%)	16/27 (59.3%)
Osteolytic lesion(s) ≥ 2 cm	4/70 (5.7%)	2/51 (3.9%)	2/19 (10.5%)
Other Relevant Findings, n/N (%)*			
Serum tryptase, µg/L median (range)	333.5 (50.9 – 7490)	308 (57.2 – 7490)	396 (50.9 – 1820)
≥ 200 µg/L, n (%)	75 (81.5%)	53 (81.5%)	22 (81.5%)
Any peripheral blood MCs detectable, n/N (%)	11/89 (12.4%)	8/63 (12.7%)	3/26 (11.5%)
≥ 3% peripheral blood MCs	7/89 (7.9%)	6/63 (9.5%)	1/26 (3.8%)
≥ 10% peripheral blood MCs	4/89 (4.5%)	4/63 (6.3%)	0/26 (0%)
MC infiltration in BM aspirate smear, %; median (range)**	30 (20 – 100)	30 (20 – 100)	30 (20 – 88)
Abnormal Karyotype, n/N (%)	10/60 (17%)	7/38 (18.4%)	3/22 (13.6%)
S/A/R mutations present, n/N (%)	16/36 (44%)	9/24 (37.5%)	7/12 (58.3%)

*Denominator is total number of patients (92, 65, or 27 for full cohort, de novo, or secondary MCL, respectively) unless otherwise specified

** 1 patient did not have a bone marrow biopsy, but had >10% mast cells in the peripheral blood

AML, acute myeloid leukemia; BM, bone marrow; CEL, chronic eosinophilic leukemia; CMML, chronic myelomonocytic leukemia; MCs, mast cells; MCL, mast cell leukemia; MCL with an associated hematologic neoplasm; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin lymphoma; *S/A/R*, *SRSF2/ASXL1/RUNX1*

Table 2. Progression to Secondary Mast Cell Leukemia by Preceding Diagnosis

Diagnosis prior to MCL	Number of patients, n/N (%)		Time to MCL progression from initial diagnosis at presentation, years; median (range)
	Initial diagnosis at presentation	Diagnosis immediately prior to MCL	
ISM	6/27 (22%)	1/27 (4%)	5.3 (4.4 – 10.3)
SSM	0/27 (0%)	2/27 (7%)	Not applicable
ASM	7/27 (26%)	11/27 (41%)	3.9 (0.8 – 13.5)
SM-AHN	14/27 (52%)	13/27 (48%)	0.7 (0.1 – 7.1)

ASM, aggressive systemic mastocytosis; ISM, indolent systemic mastocytosis; MCL, mast cell leukemia; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis

Table 3. Univariate and Multivariate Analyses of Factors Associated with Overall Survival in Mast Cell Leukemia

	Univariate		Multivariate	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Patient Characteristics				
Male Sex	0.92 (0.52 – 1.6)	0.77		
Age ≥ 60	1.5 (0.87 – 2.5)	0.15		
Disease Characteristics				
Chronic MCL (No C-Findings)	**0.34 (0.12 – 0.94)**	**0.04**	0.91 (0.19 – 4.3)	0.90
Secondary MCL	1.0 (0.57 – 1.8)	0.95		
Diagnosis of MCL-AHN	**1.9 (1.1 – 3.2)**	**0.02**	**5.22 (1.9– 14.2)**	**0.001**
Tryptase ≥200 ug/L	1.1 (0.45 – 2.5)	0.88		
Any PB MCs	**3.9 (1.6 – 9.4)**	**0.003**	1.71 (0.42 – 7.0)	0.46
Percent MCs on BM aspirate smear (continuous variable)	1.0 (0.99 – 1.0)	0.93		
Abnormal Karyotype	**2.5 (1.1 – 5.7)**	**0.03**	**3.56 (1.21 – 14.9)**	**0.02**
KIT* D816V positive	**0.27 (0.13 – 0.53)**	**<0.001**	**0.43 (0.16 – 0.96)**	**0.04**
S/A/R* mutations present*	**5.8 (1.9 – 17.0)**	**0.002**		
Treatment Characteristics				
First line midostaurin	0.61 (0.34 – 1.1)	0.09		
Ever-treatment with midostaurin	**0.49 (0.28 – 0.86)**	**0.01**	**0.45 (0.07 – 0.63)**	**0.002**

*From PB or BM
**Not included in multivariate analysis due to low number of evaluable patients
Bolded patient characteristics indicate statistical significance in either univariate or multivariate analysis

AHN, associated hematologic neoplasm; BM, bone marrow; MC, mast cell; MCL, mast cell leukemia; PB, peripheral blood

Figure 1. Progression to Secondary Mast Cell Leukemia

Progression to secondary mast cell leukemia (MCL) (n = 27). (A) Time to progression to secondary MCL from initial SM diagnosis (B) Diagnoses immediately prior to secondary MCL (left bar graph) and at initial presentation (right bar graph);

Figure 2. Overall Survival of Patients by Subtype of Mast Cell Leukemia

Kaplan-Meier estimates of overall survival for (A) the full cohort of patients with mast cell leukemia (MCL) (n = 92) and stratified by (B) MCL vs MCL with an associated hematologic neoplasm (MCL-AHN); (C) chronic vs acute MCL; (D) *de novo* vs. secondary MCL; (E) normal vs abnormal karyotype; and (F) any vs no peripheral blood mast cells. MC, mast cells.

Figure 3. *KIT* mutations in patients with mast cell leukemia

(A) Relative frequency distribution of *KIT* mutations in 85 patients with mast cell leukemia. (B) Kaplan-Meier estimates of overall survival stratified by *KIT* D816V mutation status; and (C) stratified by *KIT* D816V positivity vs alternative *KIT* mutation positivity vs. no detectable *KIT* mutations.

Figure 4. Mutational Profile of Patients with Mast Cell Leukemia

Mutational profiles for 36 patients with mast cell leukemia (MCL), stratified by diagnosis of MCL vs. MCL with an associated hematologic neoplasm (MCL-AHN) (2^{nd} row). Each column represents an individual patient.

Figure 5. Treatment Modalities in Patients with Mast Cell Leukemia

Treatment modalities administered to patients with mast cell leukemia in (A) at any time during the treatment course; and (B) stratified by therapeutic line. The numbers above each data point indicate the number of patients who received each treatment.

Figure 1

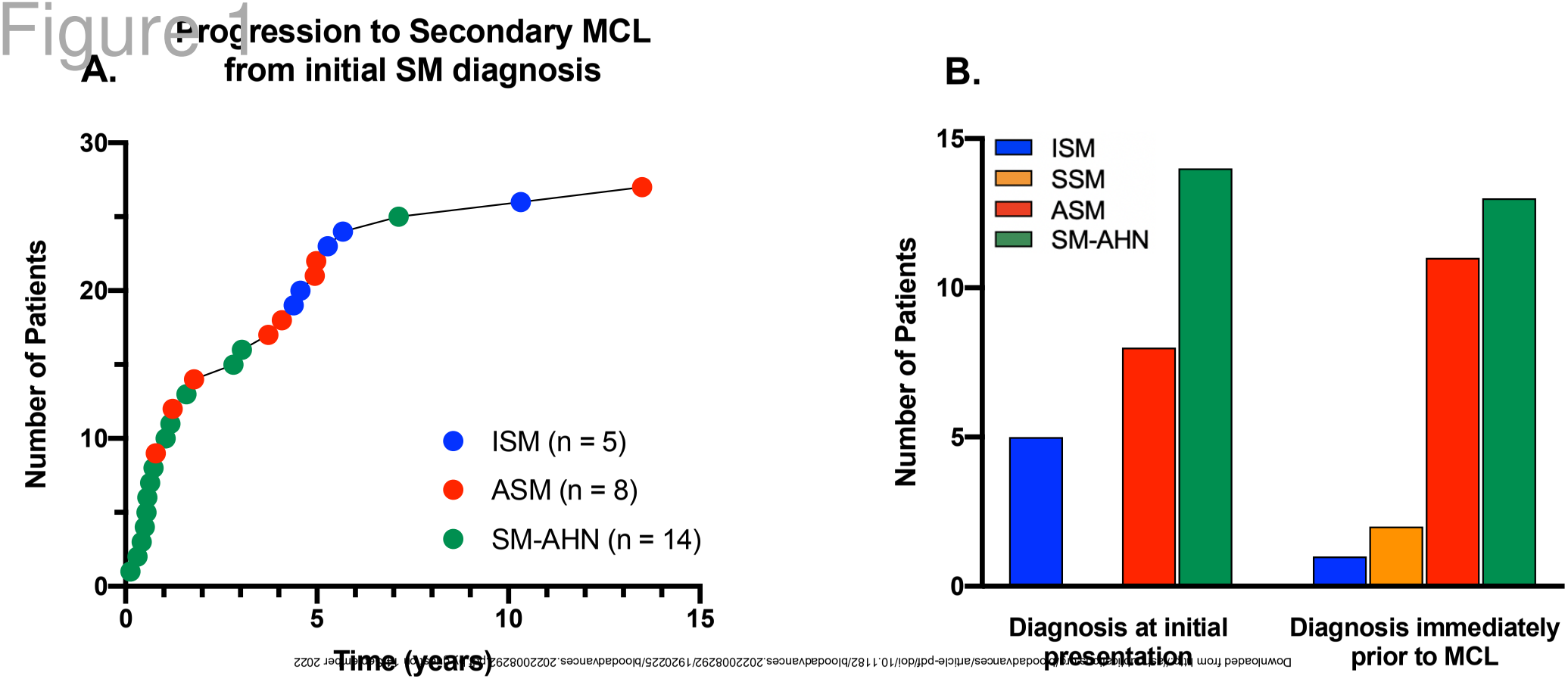
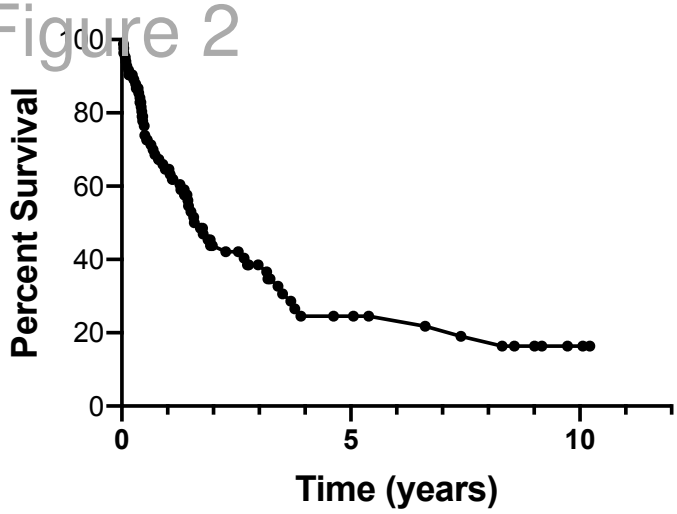
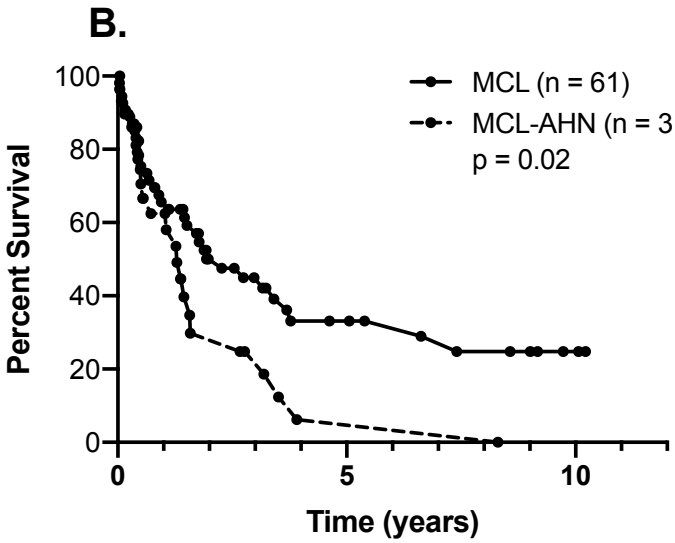
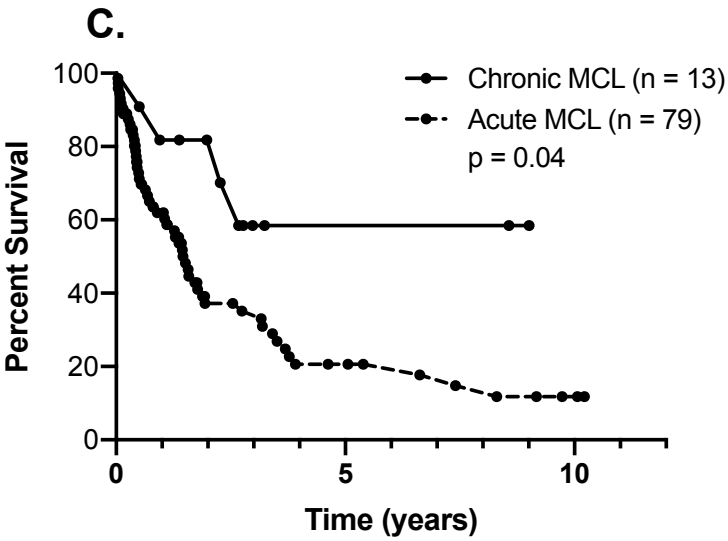
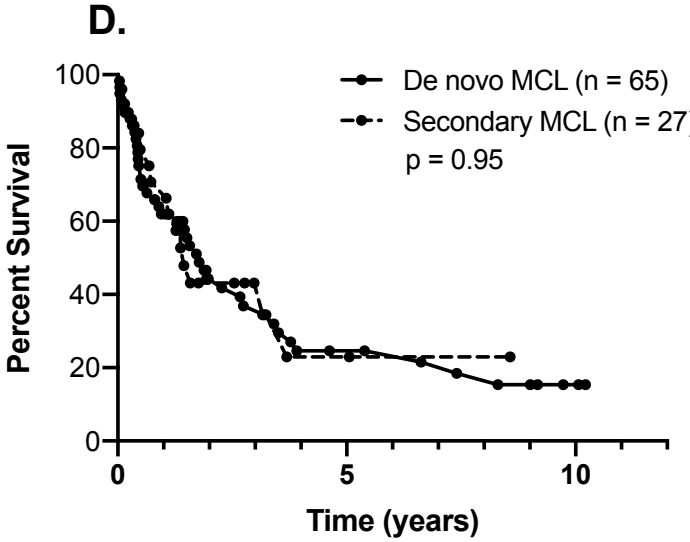


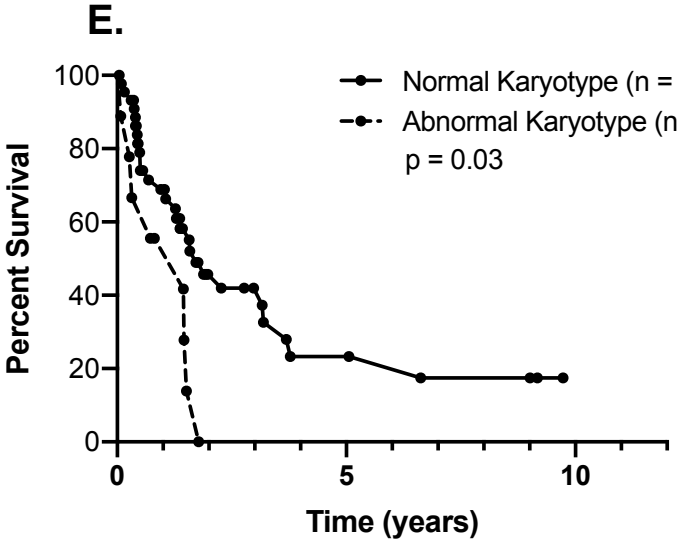
Figure 2











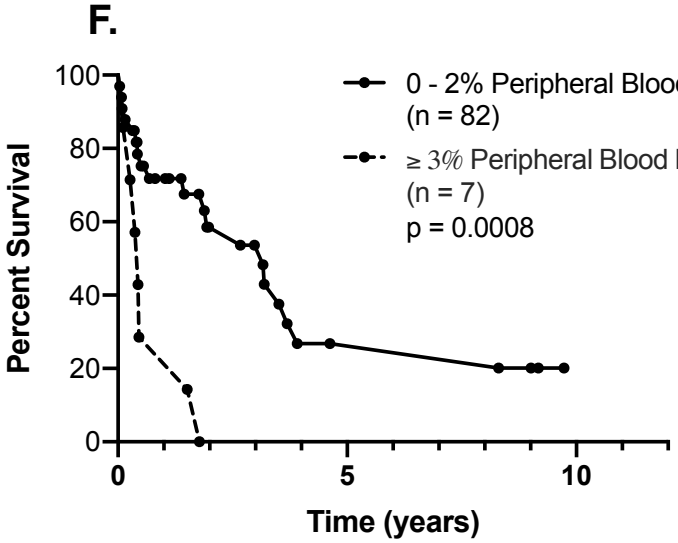
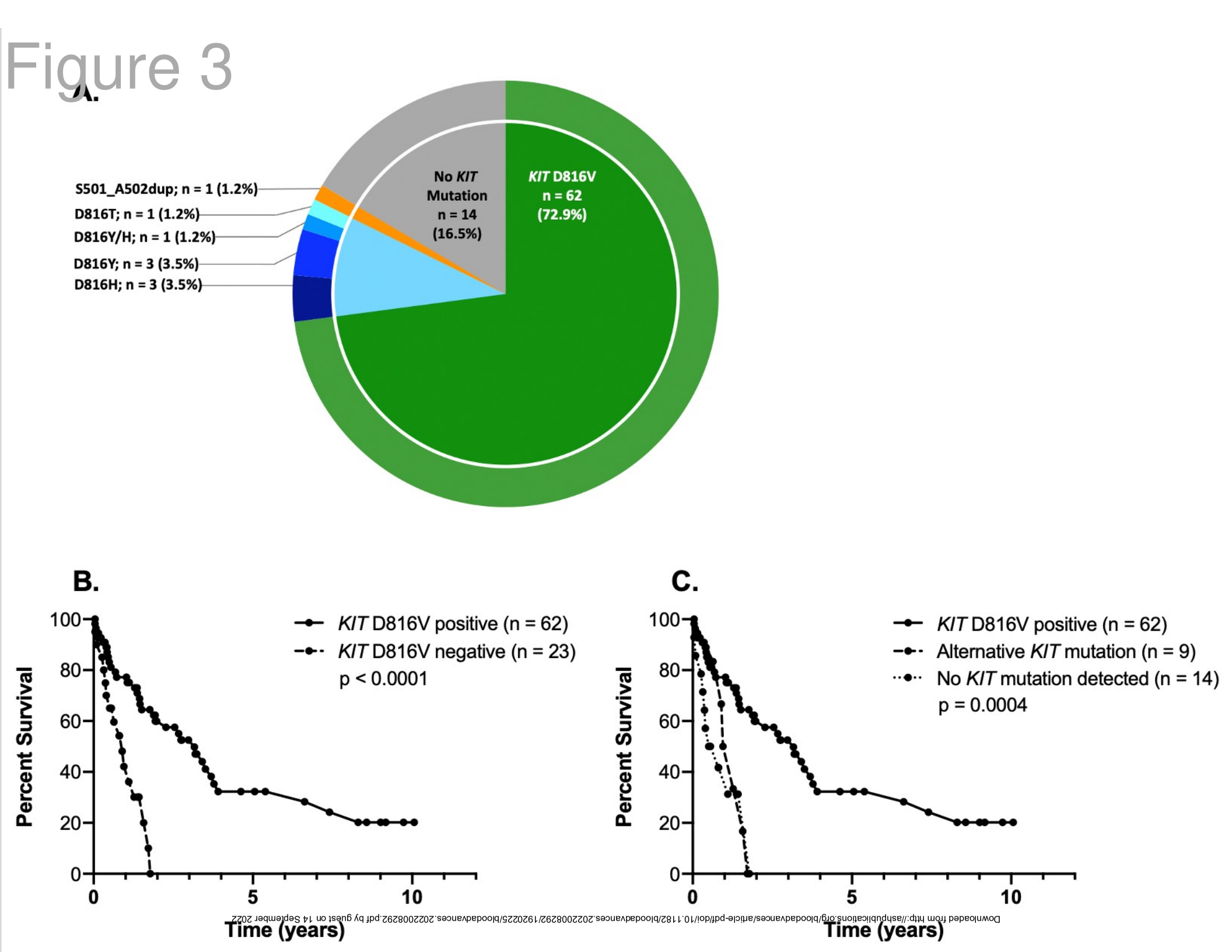


Figure 3



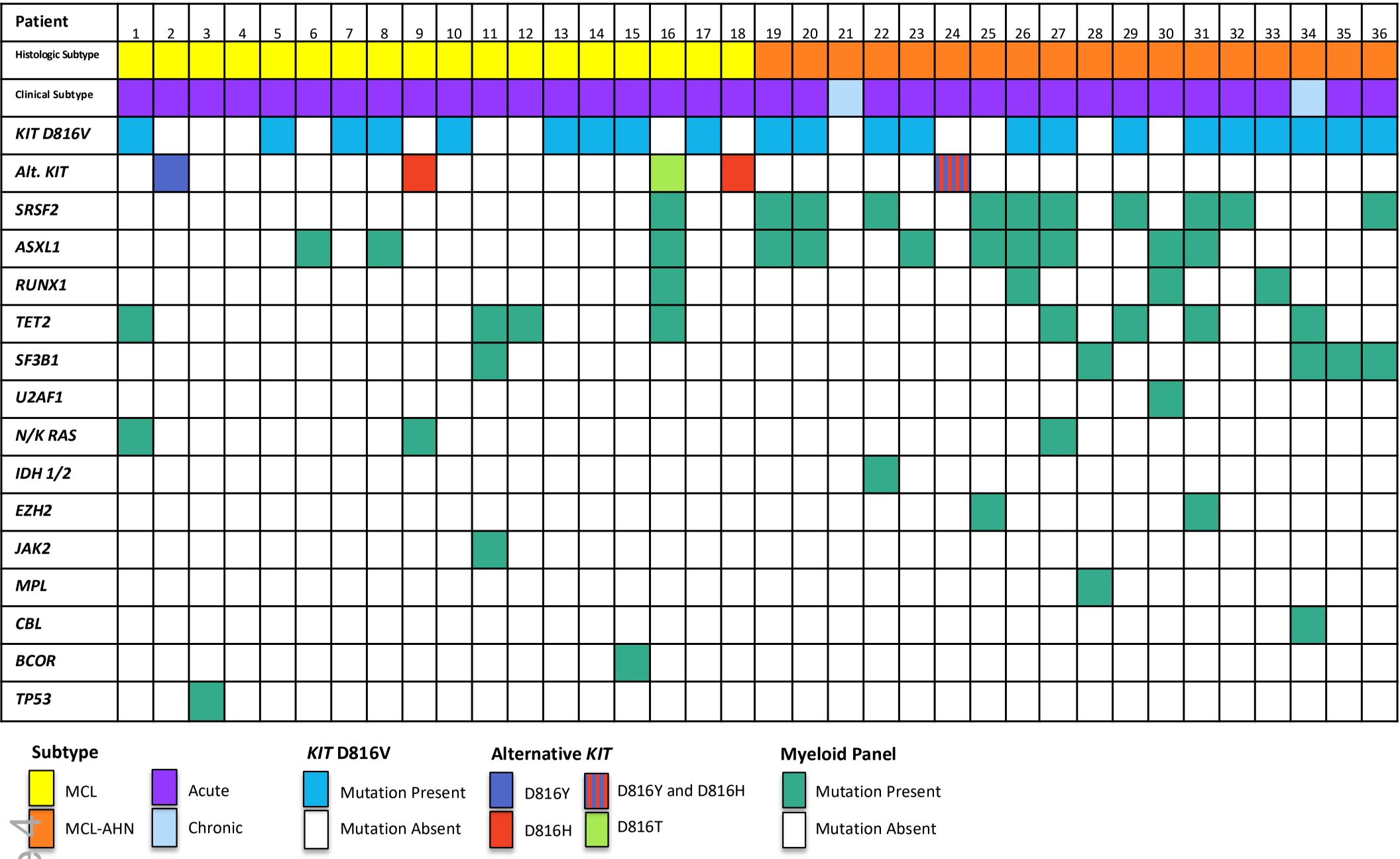


Figure 4

Figure 5