# Clinical and histopathologic features of myeloid neoplasms with concurrent *JAK2* V617F and *KIT* D816V mutations

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**ABSTRACT**

**We report on 45 patients with myeloid neoplasms and concurrent *JAK2* V617F and *KIT* D816V (*JAK2*$^{pos.}$/*KIT*$^{pos.}$) mutations, which are individually identified in >60% of patients with classical myeloproliferative neoplasms (MPN) and >90% of patients with systemic mastocytosis (SM), respectively. In SM, the concurrent presence of a clonal non-mast cell neoplasm (SM with associated haematologic neoplasm, SM-AHN) usually constitutes a distinct subtype associated with poor survival. All 45 patients presented with a heterogenous combination of clinical/ morphological features typical of the individual disorders (e.g. leuko-/erythro-/thrombocytosis and elevated LDH for MPN; elevated serum tryptase and alkaline phosphatase for SM). Overlapping features identified in 70% of patients included splenomegaly, cytopenia(s), bone marrow fibrosis and additional somatic mutations. Molecular dissection revealed discordant development of variant allele frequency for both mutations and absence of concurrently positive single-cell derived colonies indicating disease evolution in two independent clones rather than monoclonal disease in > 60% of patients examined. Overall survival of *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients without additional somatic high-risk mutations (HRM, e.g. in *SRSF2*, *ASXL1* or *RUNX1*), at 5 years was 77%, indicating that the mutual impact of *JAK2* V617F and *KIT* D816V on prognosis is fundamentally different from the adverse impact of additional HRM in the individual disorders.**

## INTRODUCTION

*JAK2* V617F is the commonest mutation in *BCR-ABL1*-negative myeloproliferative neoplasms (MPN). It is present in > 95% of patients with polycythemia vera (PV) and in approximately 50-60% of patients with essential thrombocythemia (ET) and primary myelofibrosis (MF) (1). The constitutive activation of the JAK2 tyrosine kinase results in a sustained cell proliferation of the myeloid lineage and increased cytokine production, resulting in remodeling of the bone marrow (BM), resulting in fibrosis, splenomegaly and constitutional symptoms. More advanced MPNs, such as MF, are characterized by progressive BM failure and an increased risk of leukemic transformation (2-4). In addition, MF frequently exhibits additional, prognostically adverse somatic mutations, e.g. in *ASXL1*, *SRSF2*, *EZH2* or *IDH1/2*. These mutations have therefore been incorporated into prognostic scoring systems in MPN (5-7). A high *JAK2* V617F variant allele frequency (VAF) and/or additional somatic mutations confer an adverse impact on prognosis through an increase in thromboembolic complications, a more rapid progression to higher-grade fibrosis and leukemic transformation (7-14).

Mutations in the tyrosine kinase receptor gene *KIT*, most frequently *KIT* D816V, are identified in >90% of patients with systemic mastocytosis (SM) and represent a pivotal diagnostic criterion of SM beside the presence of diagnostic mast cell (MC) aggregates with expression of CD25 and/or atypical morphology and an elevated serum tryptase. SM is broadly subcategorised into indolent SM (ISM) and advanced SM (AdvSM) consisting of aggressive SM (ASM), mast cell leukemia (MCL) and SM with an associated haematologic neoplasm (SM-AHN). In AdvSM, 60-80% of patients have signs of multilineage involvement which is the basis for morphological diagnosis of an associated haematological neoplasm (AHN), most frequently featuring chronic myelomonocytic leukemia (SM-CMML), MPN or myeloproliferative/myelodysplastic neoplasm unclassified (SM-MDS/MPN-U) (15-18). A *KIT* D816V VAF >1-2% in peripheral blood (PB) suggests multilineage involvement/AHN and AdvSM is frequently associated with a *KIT* D816V VAF between 20 to 50% (19). The clinical features of SM range from mediator-induced symptoms (e.g. flushing, diarrhea, anaphylaxis) to organ damage due to neoplastic MC infiltration in AdvSM (e.g. cytopenias; liver dysfunction with portal hypertension and/or ascites; malabsorption with hypoalbuminemia and weight loss) (17, 18, 20). Patients with

AdvSM exhibit reduced median overall survival (OS) as compared to ISM or smoldering SM (SSM) patients. Recently established prognostic scoring systems including clinical and genetic parameters allow improved prognostication in SM (21-24). Very similar to *JAK2* V617F$^{pos.}$ MPN, presence and number of mutations in genes such as *SRSF2, ASXL1, RUNX1* (*S*/*A*/*R*) or *EZH2*, are present in at least 60-70% AdvSM patients and confer an adverse impact on OS and treatment response (25-27).

Pathogenesis, clinical phenotype and treatment options with specific tyrosine kinase inhibitors (TKI) such as ruxolitinib (28-30) towards *JAK2*-driven and midostaurin (19, 31, 32) towards *KIT*-driven myeloid neoplasms (MLN) are well characterised. However, little is known about the impact of tandem presence of *JAK2* V617F and *KIT* D816V (*JAK2*$^{pos.}$/*KIT*$^{pos.}$) in patients with MLN.

## PATIENTS AND METHODS

**Patients.** Clinical, morphological and genetic characteristics of 45 *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients were collected within the "German Registry on Disorders of Eosinophils and Mast Cells", Mannheim, Germany, the Guy's and St. Thomas Hospital, London U.K., and the Stanford Cancer Institute / Stanford University School of Medicine, Stanford, USA. Eighty-three patients with AdvSM from the German Registry on Disorders of Eosinophils and Mast cells and presence of a high-risk mutation in the *S*/*A*/*R* gene panel (*KIT*$^{pos.}$/*S*/*A*/*R*$^{pos.}$) were included as control group in the survival analysis (update from Jawhar et al. 2019 (21), Supplementary Table 1). The study design adhered to the tenets of the Declaration of Helsinki and was approved by institutional review boards. All patients gave written informed consent.

**Histopathology of BM biopsies.** BM biopsies, morphology and immunohistochemistry were performed according to standard procedures as previously reported (16, 33-35).

**Mutation analysis.** Detection and quantification of the variant allele frequency (VAF) of *JAK2* V617F and *KIT* D816V were assessed by digital PCR (*KIT* D816V, limit of detection (LOD) = 0.015%), or

next generation sequencing (NGS) analysis (*JAK2* V617F, LOD = 1%). The *KIT* D816V expressed allele burden (EAB) was assessed as previously described (36).

NGS (454 FLX amplicon chemistry, Roche, Penzberg, Germany) was performed using a standard myeloid gene panel covering 18 recurrently mutated genes in MLN (including the prognostically relevant *S*/*A*/*R* mutations) as previously published (27). Gene mutations were annotated compared to the reference sequence of the Ensembl Transcript ID (Ensembl release 85: July 2016).

**Cytogenetic analysis.** At least 20 Giemsa-banded metaphases were prepared from cultured BM aspirate specimens and cultivated for 24 or 48 hours as previously described (37) and analyzed by G-/R-banding techniques. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN 2016) (38).

**CFU-GM-colony assays.** BM or PB mononuclear cells (MNC) and/or sorted $CD34^+$cells were processed for a colony-forming unit granulocyte–macrophage (CFU-GM) assay and genotyped as previously published (39). For isolation of MNC from BM or PB samples we used Ficoll® Paque reagent (Sigma-Aldrich, Gelenau, Germany). Further isolation of CD34+ cells was performed by Magnetic Activated Cell Sorting (MACS) with CD34 Micro Beads (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions.

**Statistical analysis.** Statistical analyses were performed using GraphPad prism 8 software, San Diego, California). Survival probabilities were calculated by the Kaplan-Meier Method, correlation between variables was investigated using Mann-Whitney-U-test (t-approximation). A test result with a p-value less than 0.05 was considered statistically significant.

## RESULTS

**Disease characteristics.** Median age was 65 years (range 38-85) with a significant male predominance (M:F 1.8:1). Typical MPN features included leukocytosis (median leukocyte count 10.0 x $10^9$/l, range 2.7-18.3) and/or thrombocytosis (median platelet count 334 x $10^9$/l, range 22-993) in 28/45 (62%) patients, elevated haematocrit and/or haemoglobin in 15/45 (33%) patients, an elevated LDH (median 255 U/l, range 105-437) in 13/30 (43%) patients and a low serum erythropoetin (EPO, normal value 3.7-29.5 IU/l) level in 10/18 (56%) patients. Features only characteristic for SM included an elevated serum tryptase (median 66 µg/l, range 3-712) in 35/43 (81%) and an elevated alkaline phosphatase (median 88 U/l, range 35-640) in 19/44 (43%) patients. At least one characteristic SM-related symptom (gastrointestinal infiltration (GI)/symptoms [16/44, 36%], flushing [12/44, 27%], anaphylaxis [4/45, 9%] or skin infiltration with/without pruritus [12/44, 27%], ascites [9/45, 20%]) was present in 31/45 (69%) patients. Overlapping features for both MPN and SM, e.g. cytopenia(s) (indicating advanced phase of both SM and MF, i.e. haemoglobin <10 g/dl, platelets <100 x$10^9$/l, absolute neutrophil count <1 x$10^9$/l), were identified in 8/45 (18%) patients, splenomegaly in 32/43 (74%) patients and BM fibrosis of any grade in 28/41 (68%, high grade in 12/41 [29%] patients) (Figure 1). Detailed information on clinical and laboratory features of all 45 patients is listed in Table 1.

***KIT*$^{pos.}$/*S*/*A*/*R*$^{pos.}$ control group**. *KIT*$^{pos.}$/*S*/*A*/*R*$^{pos.}$ patients (n=83, Supplementary Table 1) had a higher BM MC infiltration, higher levels of serum tryptase and alkaline phosphatase, lower haemoglobin and platelet counts, higher absolute numbers of eosinophils and monocytes and a higher *KIT* D816V VAF. The majority (77%) of patients were high-risk according to the mutation-adjusted risk score (MARS (21)).

**Histopathology of BM biopsies**. Diagnostic MC infiltrates in BM were identified in all specimens (median 15%, range 3-70). If performed, tryptase, CD117 and CD25 stains were positive in 42/42 (100%), 41/42 (98%) and 40/42 (95%) patients, respectively. Megakaryocytes were increased in numbers in 27/42 (64%), pleomorphic in 22/42 (52%) and clustered in 6/27 (22%) of cases. BM eosinophilia was observed in 15/32 (46%) patients. Fibrosis was present in 28/41 (68%) patients, grade

1 in 16/41 (39%) and high grade (≥2) in 12/41 (29%) patients. Figure 2 depicts histological findings in 3 patients (Figure 2a-c, pat #5; d-f, pat #22; g-i, pat #42) with aspects indicative of SM (atypical CD117 and or tryptase positive MC infiltrates, Giemsa stain, Figure 2c, f, i), of MPN-only (increased, atypical pleomorphic megakaryopoiesis, Figure 2a, g, d) or findings simultaneously found in both entities (fibrotic remodeling around MC infiltrates, Figure 2b, e, h).

In 31 patients, a reference pathology review was performed: Reevaluation revealed initial incomplete diagnosis in 8/31 patients (26%) with missing of MPN in 3/8 patients (despite fibrosis >1 in 2/3 patients and knowledge of the molecular profile) and missing of SM in 5/8 patients (*KIT* D816V VAF in all 5 patients <5%). Median time of delay until correct diagnosis in these 8 patients was 6.6 years (range 5.8-9.3). In the remaining 37 patients, histopathologic diagnoses included SM-MPN (n=25; [MF, n=11, ET, n=6, PV, n=6, MPN-unclassified, n=2]), SM-MDS/MPN (n=8), SM-CMML (n=2), SM with chronic eosinophilic leukemia (SM-CEL, n=1) and MCL-PV (n=1).

**Mutation analysis.** In PB, the median *KIT* D816V VAF (n=33) of 4% (range 0-50) was significantly lower than the median *JAK2* V617F VAF (n=37) of 15% (range 1-80). NGS in 32/45 (71%) patients revealed 31 additional somatic mutations in 14 patients with 7 patients harboring more than one additional mutation: *TET2*, n=16; *SRSF2*, n=6; *RUNX1*, n=5; *SF3B1*, n=1; *CBL*, n=1; *ASXL1*, n=1, *NRAS*, n=1.

**Cytogenetic analysis.** Cytogenetic analysis were normal in 27/30 (90%) of patients analysed. A deletion del(5)(q14q34), a translocation t(X;3) and a trisomy 19 were present in one patient each (Table 1).

**Frequency of both mutations in random samples**. Screening for *JAK2* V617F in 187 randomly selected patients with *KIT*$^{pos.}$ AdvSM revealed 27/187 (14%) positive cases. At least 2/27 (7%) patients did not present with signs of myeloproliferation in PB or BM. Screening for *KIT* D816V in 50 randomly selected *JAK2*$^{pos.}$ MPN patients revealed 1/50 (2%) positive case. This patient (patient #44) was diagnosed with PV (elevated haemoglobin/haematocrit and need for phlebotomies), was asymptomatic

regarding SM associated symptoms, had a serum tryptase level of 17 µg/l, and the BM smear was indicative of a MPN. The BM biopsy was not made available for reference evaluation.

**CFU-GM-colony assays.** In 10 patients, cultured CFU-GM colonies (n=512, median per patient 30, range 5-30) were analysed for the presence or absence of *JAK2* V617F and *KIT* D816V mutations. Overall, 84/512 (16%) CFU-GM colonies were positive for at least one mutation. In 8/10 patients, >20 colonies were picked and tested for *JAK2* V617F and *KIT* D816V. No differences were found between colonies grown from MNC and colonies grown from sorted CD34+ cells. The vast majority of colonies (80/84, 95%) were positive for either *JAK2* V617F (69/80) or for *KIT* D816V (11/80, n=8 patients, Figure 3). The concurrent presence of both mutations was only observed in 4/84 (5%) CFU-GM colonies and in 2/11 (18%) patients analysed. In patient #4, CFU-GM colonies were also analysed for the presence of *TET2* (22/22 positive). *JAK2*$^{pos.}$/*TET*$^{pos.}$ colonies were significantly more frequent than *JAK2*$^{pos.}$/*KIT*$^{pos.}$ colonies.

**Treatment and sequential measurements of VAF**. Treatment information was available from 28 patients. Nine patients (32%) did not have any cytoreductive treatment. Midostaurin, ruxolitinib or hydroxycarbamide were used a single cytoreductive treatment in 2 (7%), 1 (4%) and 5 (18%) patients, respectively. In 11 (39%) patients, more than one (median 2, range 2-4) cytoreductive therapy was initiated during follow up, including hydroxycarbamide (n=10, 36%), midostaurin (n=7, 25%), ruxolitinib (n=5, 18%), cladribine (n=3, 11%) and interferon alpha (n=3, 11%). Sequential measurements of VAF were available for *JAK2* V617F and *KIT* D816V in 7 and 13 patients, respectively. Irrespective of treatment, *KIT* D816V VAF decreased or was stable in 11/13 (85%) patients, whereas *JAK2* V617F VAF increased in 7/7 (100%) patients (median follow up 60 months, range 24-78). Figure 4 depicts four patients with available VAF of both mutations.

**Prognosis.** The 5-year OS of 25 informative *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients without SM high-risk mutations (*S*/*A*/*R*) was 77%. Six *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients were positive for high-risk mutations. Figure 5 shows the survival of *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients with/without (6 vs 26 patients) high-risk mutations vs. the survival

of a control group of 83 SM-AHN patients with high-risk mutations (5-year OS 32%). The clinical, morphological and genetic characteristics of the vast majority of patients from this control cohort were recently reported (21), 64/83 (77%) patients were high-risk, 17 (21%) intermediate-risk and 2 (2%) low-risk according to MARS. At the time of analysis, 11/45 patients had died. When data were available (7/11), patients either had a high *JAK2* V617F >30%, a high *KIT* D816V VAF >30% (in one patient VAF of both mutations was >30%) or additional somatic mutations, predominantly in *SRSF2* or *RUNX1* (resulting in MARS intermediate or high-risk score in 57% of patients analysed).

## DISCUSSION

Patients with MLN and tandem phenotypic driver mutations, for example *BCR-ABL1* and *JAK2* V617F, are rare and have only been reported as single case reports or smaller series. In a multicentre effort, a cohort of 45 patients with a *JAK2*$^{pos.}$/*KIT*$^{pos.}$ MLN was analysed. At initial presentation, patients exhibited a heterogenous combination of clinical/morphological features typical of the individual disorders while several other features, e.g. cytopenias, splenomegaly, BM fibrosis and additional HRM, were overlapping and not clearly attributable to MPN or SM. MPN only features included -cytosis or an elevated LDH. Classical SM characteristics comprised BM MC infiltrates, an elevated serum tryptase level and an elevated alkaline phosphatase as typical features of AdvSM (22, 24). Of note, an elevated LDH is rather unusual for an AdvSM while an elevated AP is rather unusual for an MPN.

Although both driver mutations usually confer a distinct clinical phenotype and the molecular profile was known in many patients, the clinical and morphological phenotypes were not as conclusive as expected, resulting in an incomplete diagnosis (i.e. diagnosis of only MPN or only SM) in approximately 25% of patients. Interestingly, the SM component was overlooked in over 60% of these patients with an incomplete diagnosis, although the median tryptase level of approximately 100 µg/l in these individuals did not differ from the patients with correct initial diagnosis. However, the BM MC infiltration was low and the *KIT* D816V VAF in PB was also low in virtually all misdiagnosed patients highlighting the importance of serum tryptase as a fast and reliable marker for diagnosis of concurrent SM. Tryptase

measurement should therefore be performed in all patients with myeloid neoplasms. In a randomly selected cohort of *KIT*$^{pos.}$ AdvSM patients, concurrently present *JAK2* V617F was identified in 14% of patients. Beside prognostic mutations, NGS should therefore also include *JAK2* V617F, at least in all those AdvSM patients who present with signs of myeloproliferation, which are otherwise unusual for typical *KIT* D816V$^{pos.}$ SM.

A discordant PB VAF >20% (i.e. *JAK2*$^{high}$/*KIT*$^{low}$ or *JAK2*$^{low}$/*KIT*$^{high}$) at diagnosis, an increase in *JAK2* V617F VAF with decrease of *KIT* D816V VAF irrespective of the treatment chosen and the absence of concurrently positive CFU-GM-derived colonies indicated that the mutations likely arose in independent clones in at least 60% of informative patients. However, our colony analysis does not allow evaluation of mutation chronology. While approximately 60% of patients with AdvSM are positive for prognostically adverse mutations in the *S*/*A*/*R* gene panel, these mutations were only identified in approximately 20% of the *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients with a known mutational profile. The 5-year survival of *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients without high-risk mutations was 77%. All deceased patients with available data (n=7) either had a high *JAK2* V617F or *KIT* D816V VAF (>30%) or were positive for HRM. Therefore, *JAK2* V617F and *KIT* D816V appear to have less impact on prognosis compared to the adverse outcomes associated with additional somatic mutations, such as *SRSF2*, *ASXL1*, *EZH2*, *IDH1/2* or *RUNX1* in either MPN or SM. Laboratory parameters, MC infiltration and VAF were significantly different in a direct comparison between the *JAK2*$^{pos.}$/*KIT*$^{pos.}$ patients from this analysis and a control group of 83 *S/A/R*$^{pos.}$/*JAK*$^{pos.}$/*KIT*$^{pos}$ patients leading to a better prognosis in the study cohort. In multimutated SM, presence of a *JAK2* V617F mutation (*S/A/R*$^{pos.}$/*JAK*$^{pos.}$/*KIT*$^{pos}$) might therefore even outperform the negative impact of *S/A/R* mutations in terms of OS. However, the sample size is still small and larger studies are warranted to confirm this trend. Pathogenesis, phenotype and prognosis of *JAK2*$^{pos.}$/*KIT*$^{pos.}$ disease therefore implies that it should either not be diagnosed as SM-AHN (although this is the formal and correct term in this context) or should at least be seen as a distinct SM-AHN subentity representing a bi-clonal disorder with two distinct and separately developing subclones.

Where applicable, treatment decisions were based on the dominating clinical features and its potential relation to the individual entities following the guideline of our consensus group (17, 20, 40). In difficult situations, e.g. cytopenia or splenomegaly, mutation/disease burden (e.g. >30% vs <30% VAF) and the presence/absence of somatic mutations were taken into account. In the entire cohort, the median VAFs of *JAK2* V617F and *KIT* D816V were <10% indicating a more benign/indolent disease phenotype in the majority of patients. Data on treatment with midostaurin or ruxolitinib showed significant decrease in *KIT* D816V VAF in several patients while *JAK2* V617F remained unaffected or even increased in virtually all patients. We consider two possibilities to explain this phenomenon, i) midostaurin has a stronger activity towards *KIT* D816V than ruxolitinib towards *JAK2* V617F or ii) none of the drugs has decisive activity towards its specific mutation and the decrease of *KIT* D816V is predominantly the consequence of the evolution of *JAK2* V617F. In contrast, ruxolitinib was only used in 2 patients making an evaluation of disease burden- and VAF-development for the *JAK2*$^{pos.}$ compartment rather speculative. Introduction of interferon alpha as a potential treatment option with effective VAF decrease has been proven at least for *JAK2*$^{pos.}$ MPN and should also been taken into consideration in eligible patients.

In conclusion, morphological and genetic analyses allow the dissection of MLN into monoclonal and multiclonal disorders. At least patients with advanced disease should be routinely checked for type, number and VAF of additional phenotypic and prognostic mutations. Our analysis also shows that knowledge of the mutational profile is helpful and mandatory for the involved pathologist and should not be withheld. In *JAK2*$^{pos.}$ MPN and *KIT*$^{pos.}$ SM, epigenetic modifiers or spliceosome mutations usually occur in the same clone with the driver mutation(s) and confer an adverse impact on phenotype, response to treatment and prognosis. In contrast, the mutual impact of *JAK2* V617F and *KIT* D816V is at least in part distinctively different because MPN and SM may represent two independent (sub-)clones in a significant proportion of patients. Treatment with JAK-inhibitors, or KIT-inhibitors, should be chosen according to quantitative assessment of individual disease characteristics.

**Figure legend:**

**Figure 1: Clinical and molecular characteristics of *JAK2* V617F$^{pos.}$and *KIT* D816V$^{pos.}$ myeloid neoplasms.** Features typical for myeloproliferative neoplasms (MPN) are highlighted in light grey, mastocytosis (SM) related findings are highlighted in white, overlapping features are marked in dark grey. Data on mutation status is marked in black. LDH, lactate dehydrogenase; Epo, erythropoietin; BM, bone marrow; AP, alkaline phosphatase; MC, mast cell; GI, gastrointestinal; VAF, variant allele frequency.

**Figure 2: Histological analysis in *JAK2* V617F$^{pos}$ and *KIT* D816V$^{pos}$ patients.** a,g – atypical megakaryocytes; d – increased number of pleomorphic megakaryocytes (including juvenile forms), CD 42 stain; b,e,h – fibrosis with concomitant increase of spindle shaped mast cells (Gomori reaction); c - CD117 staining; f – Giemsa staining; i – tryptase staining.

**Figure 3: Colony-forming unit granulocyte–macrophage (CFU-GM) assay derived single cell colony analysis for additional mutations.** In 10 patients analysed, altogether 84 colonies were tested positive for *JAK2* V617F (n=69), *KIT* D816V (n=11) and *JAK2* V617F/*KIT* D816V (n=4). Colonies tested for *JAK2* V617F are highlighted in light grey, colonies tested for *KIT* D816V are highlighted in dark grey. Positive colonies are labeled with "+".

**Figure 4: Variant allele frequency (VAF) for *JAK2* V617F and *KIT* D816V during treatmen**t. Analysis of serial *JAK2* V617F and *KIT* D816V VAF measurements. *JAK2* V617F VAF is marked in red, *KIT* D816V VAF in blue. Timepoint of colony assay is highlighted with a green star. Treatment sequences: ruxolitinib start/stop dates are marked in light red, hydroxurea start/stop dates in dark red and midostaurin treatment in blue.

**Figure 5: Overall survival according to *JAK2* V617F, *KIT* D816V and high risk mutational status (*SRSF2*, *ASXL1, RUNX1*; *S/A/R*).** *S/A/R*$^{neg.}$/*JAK*$^{pos.}$/*KIT*$^{pos.}$ patients (n=26) are depicted with a dashed line, *S/A/R*$^{pos.}$/*JAK*$^{pos.}$/*KIT*$^{pos}$ patients (n=6) are depicted with a continuous line and

*S/A/R*$^{pos.}$/*JAK*$^{neg.}$/*KIT*$^{pos}$ patients are depicted with a spotted line. The 83 *S/A/R*$^{pos.}$/*JAK*$^{neg.}$/*KIT*$^{pos}$ patients were derived from the German Registry on Disorders of Eosinophils and Mast Cells.

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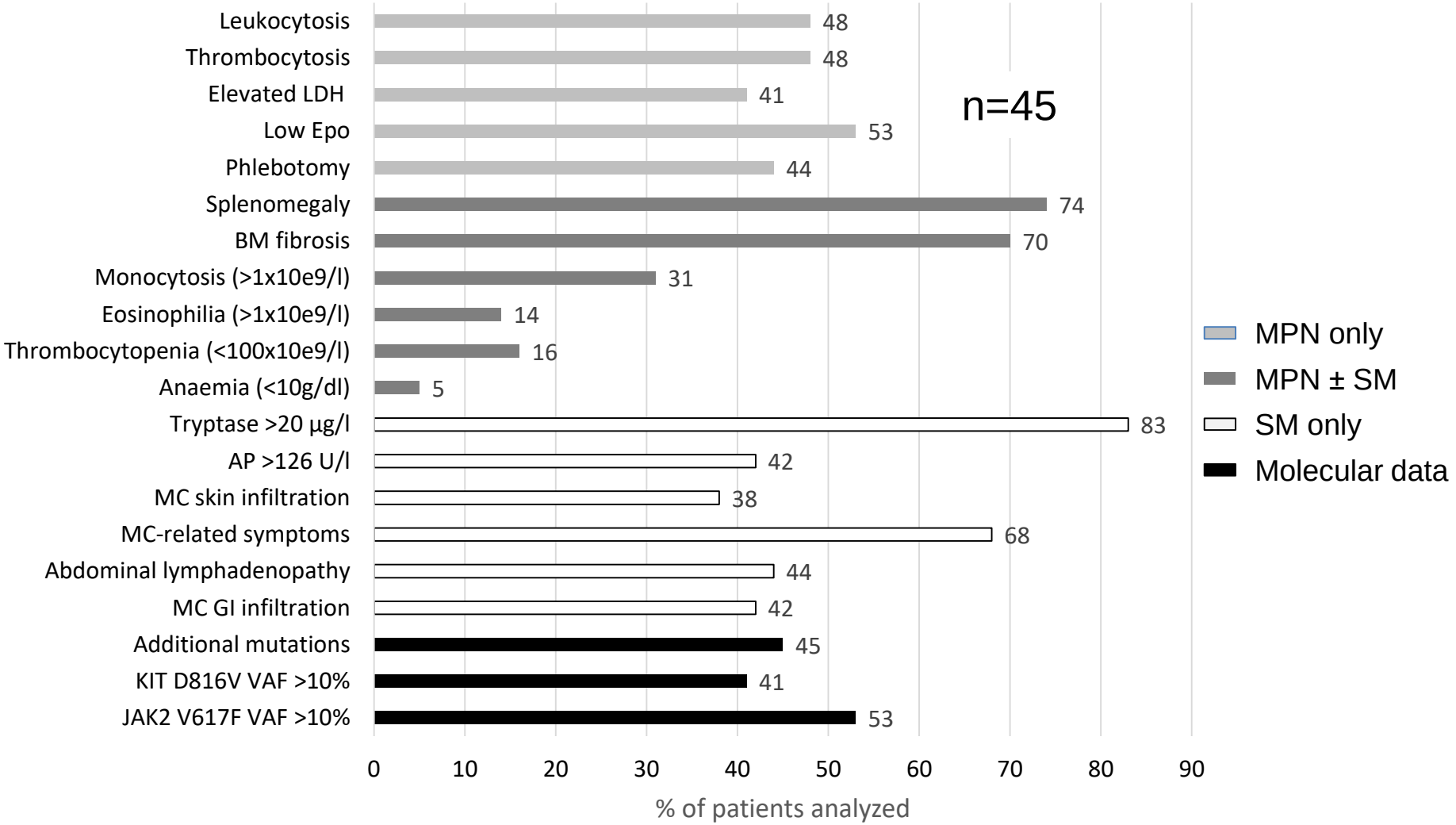
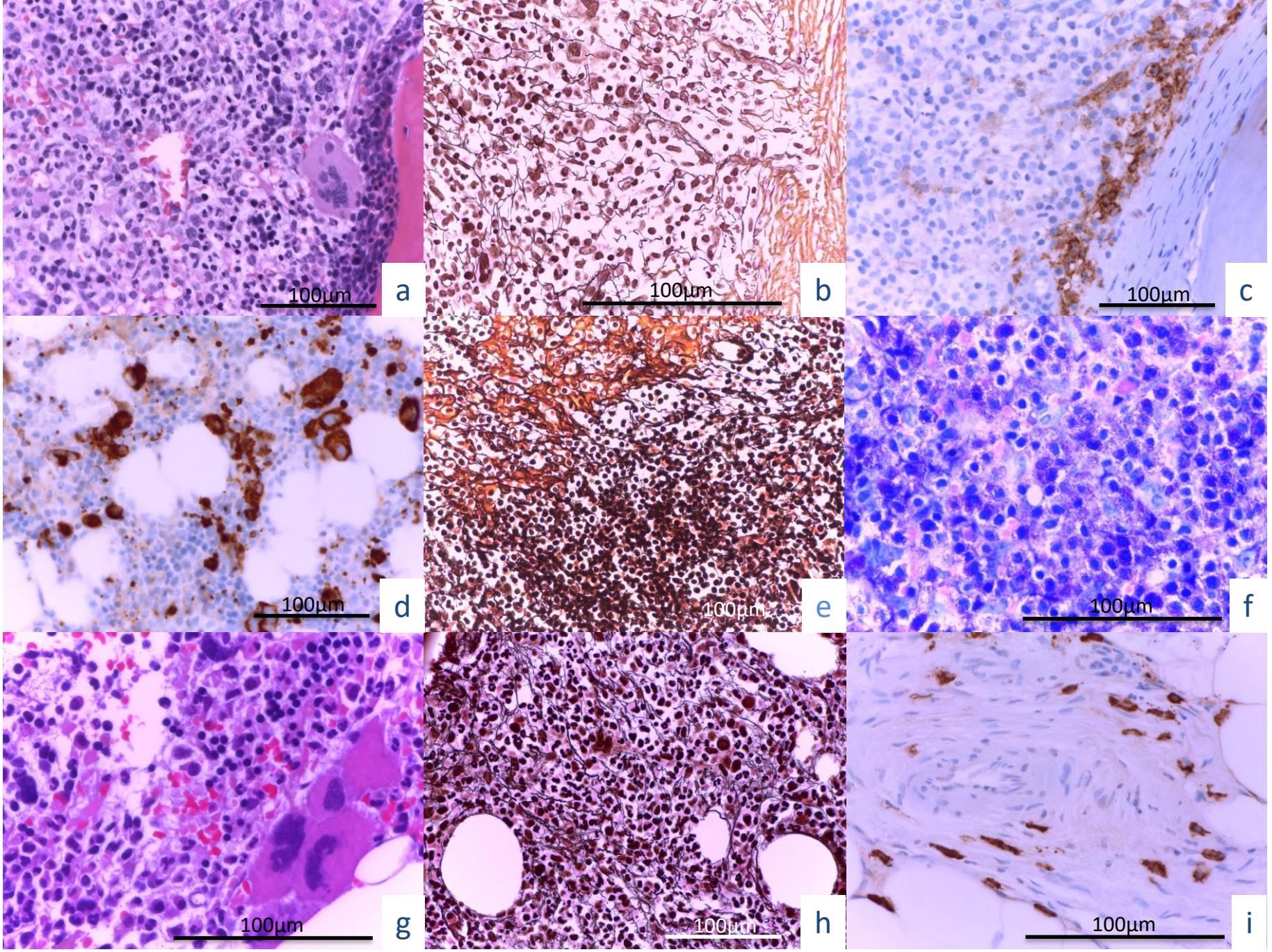

Leukocytosis 48
Thrombocytosis 48
Elevated LDH 41
Low Epo 53
Phlebotomy 44
Splenomegaly 74
BM fibrosis 70
Monocytosis (>1x10e9/l) 31
Eosinophilia (>1x10e9/l) 14
Thrombocytopenia (<100x10e9/l) 16
Anaemia (<10g/dl) 5
Tryptase >20 µg/l 83
AP >126 U/l 42
MC skin infiltration 38
MC-related symptoms 68
Abdominal lymphadenopathy 44
MC GI infiltration 42
Additional mutations 45
KIT D816V VAF >10% 41
JAK2 V617F VAF >10% 53
n=45
0 10 20 30 40 50 60 70 80 90
% of patients analyzed
MPN only
MPN ± SM
SM only
Molecular data


100µm
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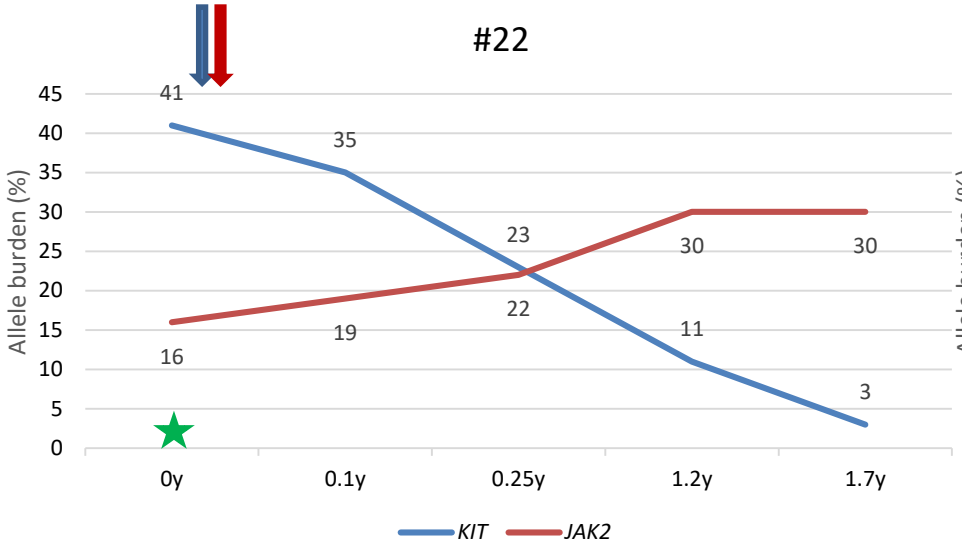
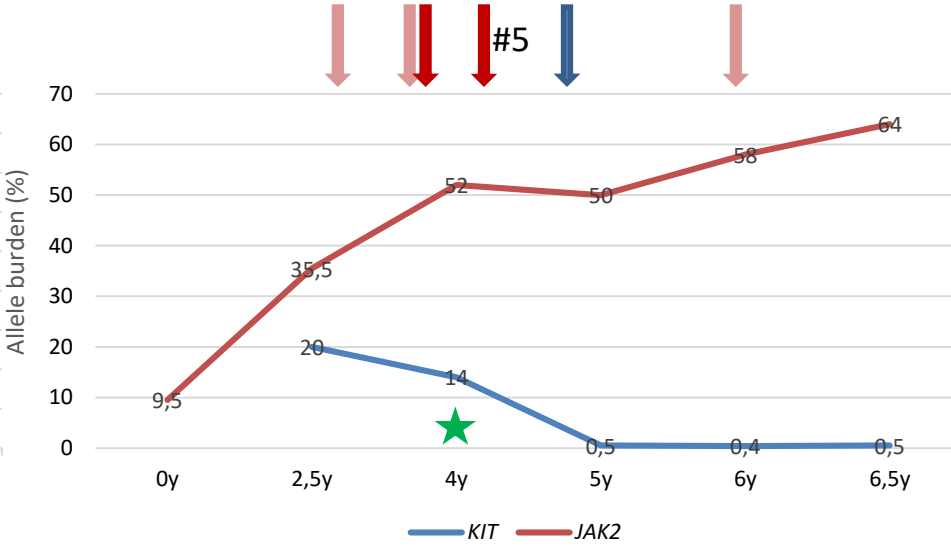
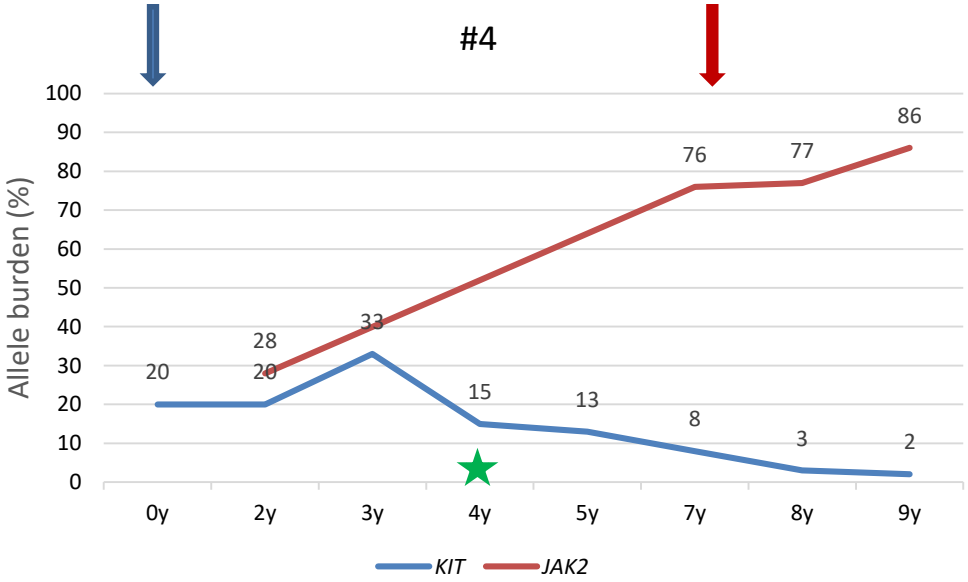
| UPN | Muta-tion | * | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | *JAK2* | 30 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | | | | | | | | | |
| | *KIT* | 5 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | + | + | + | + | | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 17 | *JAK2* | 30 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | | | | | | | | | | | | | | | |
| | *KIT* | 30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | *JAK2* | 19 | + | + | + | + | + | + | + | + | + | | | | | | | | | | | N/A | N/A | N/A | | | | | | | | |
| | *KIT* | 22 | | | | | | | | | | + | + | + | + | | | | | | | | | | | | | | | | | |
| 4 | *JAK2* | 30 | + | + | + | + | + | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | *JAK2* | 30 | + | + | + | + | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 30 | | | | | | | | | + | | | | | | | | | | | | | | | | | | | | | |
| 22 | *JAK2* | 20 | + | + | + | + | + | + | + | | | | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 20 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 19 | *JAK2* | 26 | + | + | | | | | | | | | | | | | | | | | | | | | | | | | N/A | N/A | N/A | N/A |
| | *KIT* | 30 | | | + | + | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | *JAK2* | 30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 30 | + | + | + | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | *JAK2* | 30 | + | + | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | *JAK2* | 20 | + | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | *KIT* | 20 | | + | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

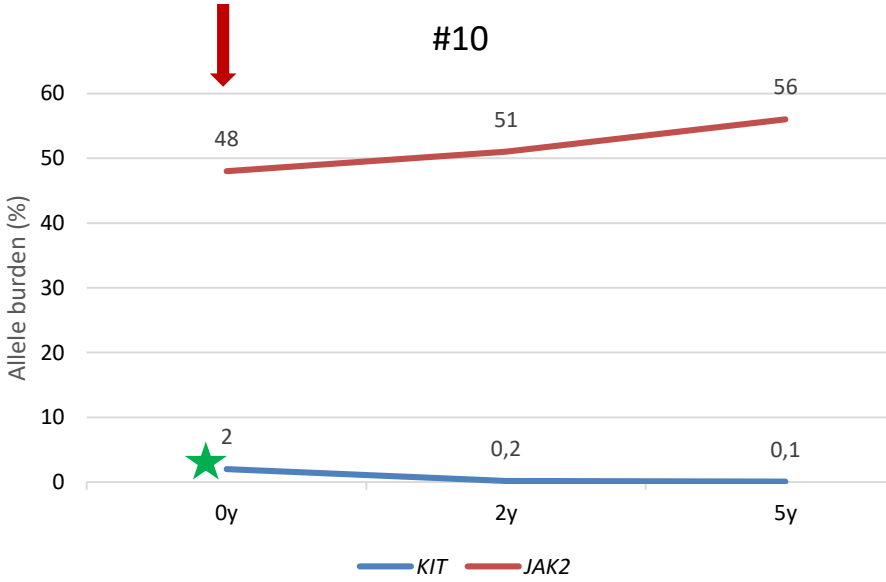
* No. of investigated CFU-GM colonies, + positive CFU-GM colonies, N/A not applicable




Ruxolitinib start/stop
Hydroxycarbamide start/stop
Midostaurin start/stop
Timepoint of colony assay



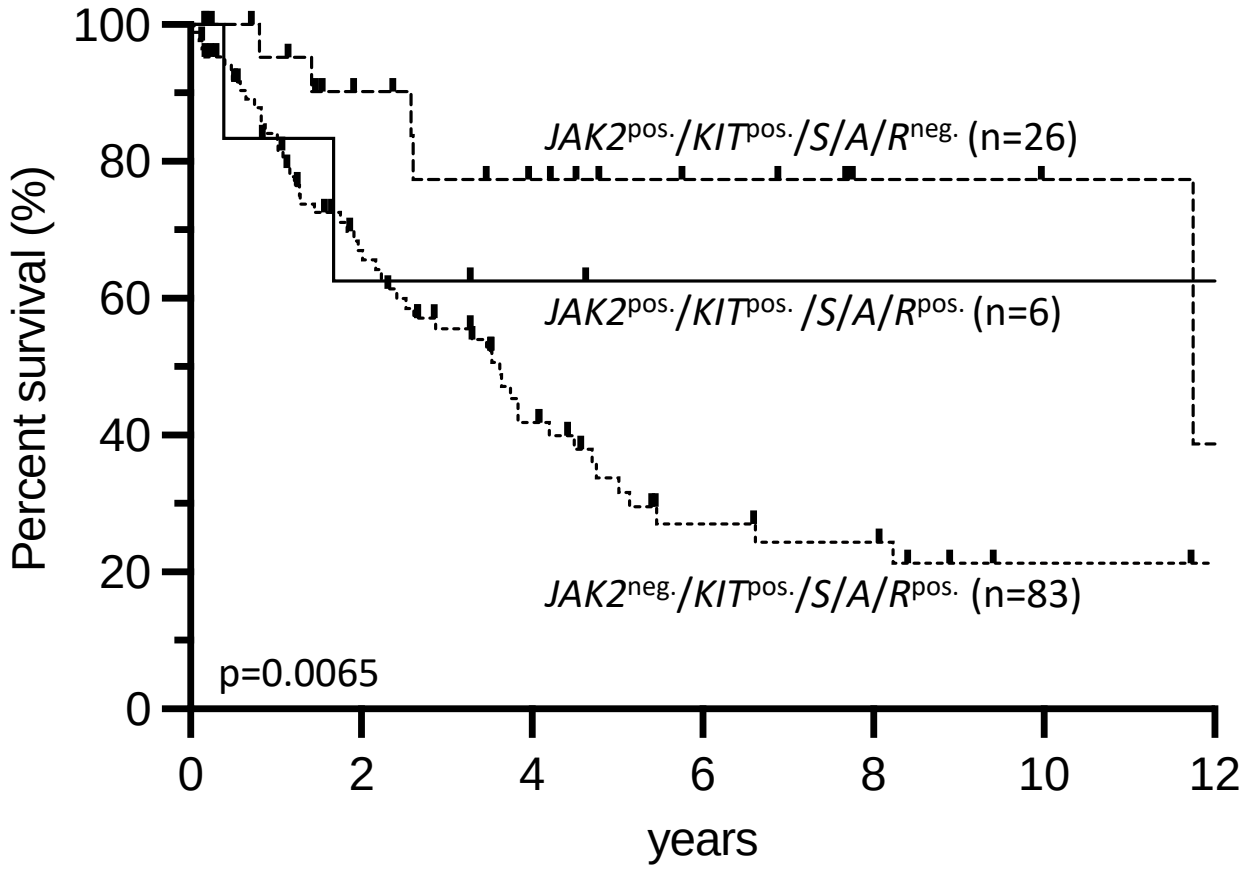

100
80
60
40
20
0
Percent survival (%)
0
2
4
6
8
10
12
years
JAK2pos./KITpos./S/A/Rneg. (n=26)
JAK2pos./KITpos./S/A/Rpos. (n=6)
JAK2neg./KITpos./S/A/Rpos. (n=83)
p=0.0065

**Table 1:** Clinical characteristics of 45 *JAK2* V617F$^{pos.}$/*KIT* D816V$^{pos.}$ patients

| Patient # | Age at diagnosis | Sex | Diagnosis | Karyotype | BM mast cell infiltration (%) | Serum Tryptase (ug/l) | *JAK2* VAF in PB | *KIT* VAF in PB | Other Mutations | Leuko-cytes (x10$^9$/l) | Haemo-globin (g/dl) | Platelets (x10$^9$l) | Monocytosis/ Eosinophilia | LDH | AP U/l (norm < 126) | Spleen size° |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38 | M | SM-PV | 46,XY | nd | 16 | 25 | 0 | none | 15.0 | 16.0 | 800 | -/- | 163 | 96 | 17 |
| 2 | 44 | F | ISM | 46,XX | 10 | 100 | 2.5 | 3 | none | 13.2 | 14.1 | 485 | -/- | 186 | 68 | 12 |
| 3 | 52 | M | SM-ET | 46,XY | 10 | 137 | 24 | 0 | nk | 10.0 | 16.3 | 745 | +/- | 307 | 78 | 9 |
| 4 | 61 | F | SM-CMML | 46,XX | 50 | 105 | 29 | 27 | *TET2* (3x) | 7.1 | 12.2 | 83 | +/- | 111 | 82 | 18 |
| 5 | 56 | F | SM-MF | 46,XX | 25 | 186 | 35 | 20 | none | 17.8 | 13.3 | 481 | +/- | 199 | 337 | 14 |
| 6 | 57 | F | SM-ET | Nk | 15 | 34 | 35 | 0.5 | *TET2* | 11.5 | 12.5 | 795 | -/- | 244 | 52 | 9 |
| 7 | 61 | M | MCL-MDS/MPN | 46,XY | 35 | 371 | 2 | 45 | *SRSF2, RUNX1, TET2* | 9.8 | 10.8 | 87 | +/+ | 105 | 511 | 17 |
| 8 | 58 | F | SM-MF | 46,t(X;3)* | 5 | nk | 78 | 50 | *SF3B1* | 10.8 | 11.3 | 630 | -/- | 145 | 185 | 17 |
| 9 | 62 | M | SM-PV | 46,XY | 5 | 148 | nk | nk | none | 13.0 | 20.5 | 464 | -/- | 289 | 133 | 16 |
| 10 | 65 | M | SM-MF | 46,XY | 15 | 17 | 48 | 0.2 | none | 11.1 | 14.6 | 993 | -/- | 233 | 64 | 16 |
| 11 | 71 | F | SSM | 46,XX | 30 | 101 | 48 | 12 | none | 6.6 | 14.3 | 251 | -/- | 165 | 179 | 14 |
| 12 | 64 | M | ISM | nk | 20 | 63 | 4 | 0 | none | 4.68 | 15.0 | 244 | -/- | 129 | 69 | 11 |
| 13 | 66 | M | SM-CEL | 47,XY,+19 | 10 | 146 | 6 | 12 | none | 7.51 | 11.2 | 323 | -/+ | 266 | 134 | 15 |
| 14 | 68 | M | SM-MF | nk | 50 | 53 | nk | Nk | nk | 7.0 | 14.0 | 250 | nk | nk | 133 | 19 |
| 15 | 69 | M | SM-CMML | 46,XY | 5 | 197 | 54 | 12 | *RUNX1, SRSF2, TET2* | 15.0 | 10.7 | 297 | +/+ | 437 | 186 | ** |
| 16 | 70 | M | SM-MDS/MPN | 46,XY,del (5)(q14q34) | 5 | 13 | 11 | 7 | none | 13.0 | 14.2 | 549 | -/- | 211 | 64 | 12 |
| 17 | 72 | F | SM-MDS/MPN | 46,XX | 10 | 42 | 52 | 7 | none | 5.02 | 10.4 | 69 | -/- | 226 | 194 | 22 |
| 18 | 72 | M | SM-AHN | nk | 30 | 66 | nk | nk | none | 3.0 | 8.8 | 176 | -/- | 179 | 61 | 20 |
| 19 | 72 | M | SM-MDS/MPN | 46,XY | 35 | 377 | 1.5 | 18 | *SRSF2* | 6.64 | 12.7 | 120 | -/+ | 180 | 575 | 17 |
| 20 | 78 | M | SM MDS | 46,XY | 20 | 195 | 1.1 | 4 | *RUNX1, SRSF2, TET2* (2x) | 3.6 | 10.2 | 370 | -/- | 300 | 383 | 15 |
| 21 | 48 | F | SM-MF | 46,XX | 5 | 28 | 37 | 0.03 | nk | 12.9 | 16.0 | 445 | -/- | 190 | 108 | nk |
| 22 | 76 | M | SM-MDS/MPN | 46,-Y | 50 | 101 | 20 | 39 | none | 12.8 | 14.5 | 958 | +/- | 331 | 640 | 16 |
| 23 | 60 | M | SM-ET | 46,XY | 15 | 32 | 7 | nk | nk | 10.6 | 15.1 | 632 | -/- | 179 | 84 | 14 |
| 24 | 52 | F | SM-ET | nk | 10 | 45 | 5 | 1.2 | none | 13.11 | 14.0 | 736 | -/- | 171 | 61 | 9 |
| 25 | 46 | M | SM-MF | nk | 5 | 12 | 16 | 0.3 | none | 8.26 | 14.5 | 649 | -/- | 226 | 61 | 12 |
| 26 | 71 | F | SM-ET | nk | 5 | 90 | 11 | 0.5 | nk | 11.9 | 13.3 | 650 | -/- | 286 | 57 | nk |
| 27 | 85 | M | SM-MDS/MPN | 46,XY | 40 | 178 | 5 | 39 | *RUNX1, SRSF2, TET2* | 2.7 | 10.5 | 81 | -/- | 251 | 154 | 14 |
| 28 | 55 | F | SM-PV | 46,XX | 15 | 68 | 7 | 0.5 | nk | 8.6 | 13.9 | 527 | -/- | nk | 105 | 9 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 78 | M | SM-MDS/MPNu | 46,XY | 20 | 593 | 50 | nd | *RUNX1, SRSF2, ASXL1* | 16.5 | 12.9 | 121 | -/+ | nk | 229 | 15 |
| 30 | 57 | M | MCL-PV | nk | 70 | 152 | nd | 28 | nk | 5.43 | 11.3 | 232 | -/- | nk | 263 | 12 |
| 31 | 75 | M | SM-ET | nk | nd | 22 | nd | nd | nk | 2.8 | 12 | 334 | -/- | nk | 75 | 10 |
| 32 | 53 | M | SM-PV | nk | nd | 10 | 56 | nd | *TET2* | 14.3 | 14.3 | 65 | +/- | nk | 67 | 9 |
| 33 | 73 | M | SM-ET | 46,XY | 3 | 118 | nd | 2 | nk | 2.9 | 9.9 | 22 | -/- | nk | 192 | 21 |
| 34 | 70 | F | SM-PV | nk | 5 | 9 | 72 | 11 | *TET2* | 12.5 | 11.3 | 75 | -/- | nk | 236 | 25 |
| 35 | 52 | M | SM-ET | 46,XY | 7 | 97 | 9 | 2 | nk | 7.1 | 14.2 | 590 | -/- | nk | 63 | 10 |
| 36 | 63 | F | SM-MPNu | 46,XX | 40 | 435 | 7 | 25 | *CBL,NRAS, TET2* | 8.3 | 12.7 | 319 | +/- | nk | 171 | nk |
| 37 | 76 | F | SM-MF | 46,XX | 40 | 712 | 8 | nd | nk | 18.3 | 10.6 | 125 | +/+ | nk | 88 | nk |
| 38 | 64 | F | SM-PV | 46,XX | 10 | 153 | 9 | nd | *TET2* | 6.1 | 13.5 | 320 | -/- | nk | 114 | nk |
| 39 | 64 | M | SM-PV | 46,XY | 5 | 21,5 | 1 | nd | none | 9.8 | 18.1 | 305 | +/- | nk | 71 | nk |
| 40 | 75 | M | ASM-ET | nk | 50 | 167 | nk | nd | nk | 11.4 | 12.1 | 731 | +/- | nk | 76 | nk |
| 41 | 45 | M | SM-MPN | 46,XY | nk | 31 | 5 | nd | none | 9.0 | 16.7 | 466 | -/- | nk | 66 | nk |
| 42 | 80 | M | SM-MDS/MPN | 46,XY | 3 | 14 | nk | 2 | *TET2* | 6.43 | 15.6 | 641 | -/- | 404 | 35 | 9 |
| 43 | 64 | M | SM-MF | nk | 15 | 56 | 28 | 7 | *TET2* | 12.7 | 16.4 | 1290 | -/- | 255 | nk | 14 |
| 44 | 76 | M | MF | nk | nk | nk | 80 | 4 | nk | 17.13 | 17.2 | 470 | -/- | 289 | 102 | 17 |
| 45 | 65 | F | SM-PMF | nk | 8 | 3 | 15 | 0 | none | 10.12 | 14,4 | 792 | -/- | 284 | 128 | 9 |
| Median (Range) | 64 (38-85) | | | | 15 (1-70) | 66 (3-712) | 4 (0-50) | 15 (1-80) | | 10 (2.7-18.3) | 14.2 (8.8-20.5) | 334 (22-993) | | 226 (105-437) | 88 (35-640) | 15 (9-25) |

*constitutional aberration; **splenectomy due to massive enlargement; °spleen size in ultrasound; nk, not known; nd, not done; SM; Systemic mastocytosis, PV, polycythaemia vera; ISM, indolent systemic mastocytosis, ET, essential thrombocythemia; MF, myelofibrosis; CMML, chronic myelomoncytic leukemia; MDS/MPN, myelodysplastic/myeloproliferative neoplasm; CEL, chronic eosinophilic leukemia; SSM, smoldering SM; MCL, mast cell leukemia; MPNu, myeloproliferative neoplasm unclassified; ASM, aggressive SM.

**Supplementary Table 2**

| | *KIT D816V* | | |
|---|---|---|---|
| | **+ *JAK2* V617F (n=45)** | **+ *SRSF2*, *ASXL1* or *RUNX1* (n=83)** | **P- value** |
| **Age, median (range)** | 64 (38-85) | 68 (23-86) | n.s. |
| **Sex,** | | | n.s. |
| **male** | 29 | 62 | |
| **female** | 16 | 21 | |
| **Diagnosis:** | | | |
| **SM-AML** | - | 5 | |
| **SM-CMML** | 2 | 37 | |
| **SM-HES/CEL** | 1 | 7 | |
| **SM-MDS/MPNu** | 7 | 19 | |
| **SM-MDS** | 1 | 8 | |
| **SM-MPN** | 18 | 3 | |
| **SM AHN (unclassified)** | 1 | - | |
| **MCL-AHN** | 2 | - | |
| **no SM** | 10 | - | |
| **no AHN** | 3 | 4 | |
| **Bone marrow infiltration in %** | 15 (1-70) | 30 (4-95) | 0.0017 |
| **Aberrant karyotype, n (%)** | 4/30 (13%) | 13/66 (20%) | n.s |
| **Serum tryptase, median (range)** | 66 (3-712) | 188 (10-1,675) | <0.0001 |
| **Leukocytes (x10$^9$/l), median (range)** | 10 (2.7-18.3) | 10.7 (1-130) | n.s. |
| **Haemoglobin (g/dl), median (range)** | 14.2 (8.8-20.5) | 10.1 (6.1-14.8) | <0.0001 |
| **Platelets (x10$^9$/l), median (range)** | 334 (22-993) | 98 (11-572) | <0.0001 |
| **Monocytes (x10$^9$/l), median (range)** | 0.62 (0-3.07) | 1.16 (0-17.95) | 0.0037 |
| **Eosinophils (x10$^9$/l), median (range)** | 0.26 (0-5.86) | 0.47 (0-100) | n.s. |
| **LDH, median (range)** | 226 (105-437) | 191 (81-575) | n.s. |
| **Alkaline phosphatase, median (range)** | 88 (35-640) | 222 (47-1,206) | <0.0001 |
| **Spleen size in cm, median (range)** | 15 (9-25) | 17 (9-25) | 0.021 |
| ***KIT* D816V allele burden in %, median (range)** | 15 (1-80) | 36 (0-74) | <0.0001 |
| **MARS Score, n (%)** | | | |
| **Low (0-1P)** | 24 (73) | 2 (2.4) | |
| **Intermediate (2P)** | 3 (9) | 17 (20.5) | <0.0001 |
| **High (3-5P)** | 6 (18) | 64 (77.1) | |
| **Median overall survival, years** | Not reached | 3.6 | 0.0065 |