

**Response and progression on midostaurin
in advanced systemic mastocytosis:
impact of *KIT* D816V and other molecular markers**

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Key Point: The complexity and dynamics of mutations significantly impact on response, progression, and prognosis in midostaurin treated advanced SM patients

ABSTRACT

In advanced systemic mastocytosis (advSM), disease evolution is often triggered by activating *KIT* mutations (D816V in >80% of cases) and by additional mutations, e.g. in *SRSF2*, *ASXL1* and/or *RUNX1* (S/A/R^{pos}, >60% of cases). In a recently reported phase-II-study, midostaurin, a multikinase/*KIT* inhibitor, demonstrated an overall response rate (ORR) of 60% in advSM but biomarkers predictive of response are lacking. We evaluated the impact of molecular markers (*KIT* D816V, S/A/R^{pos}) at baseline and during follow-up in 38 midostaurin-treated advSM patients. The median overall survival (OS) was 30 months (95% CI, 6-54) from start of midostaurin. ORR and OS were significantly different between S/A/R^{neg} (n=12) vs. S/A/R^{pos} (n=23) patients (ORR: 75% vs. 39%, P=0.04; OS: P=0.01, HR 4.5 [1.3-16.2]). Depending on the relative reduction of the *KIT* D816V expressed allele burden (EAB) at month 6, patients were classified as *KIT*-responders (≥25%, n=17) or *KIT*-non-responders (<25%, n=11). In univariate analyses of clinical and molecular response parameters at month 6, reduction of *KIT* D816V EAB ≥25%, serum tryptase ≥50% and alkaline phosphatase ≥50% were significantly associated with improved OS. In multivariate analysis, only *KIT* D816V EAB reduction ≥25% remained an independent on-treatment marker for improved OS (P=0.004, HR 6.8 [1.8-25.3]). Serial NGS analysis of 28 genes in 16 patients revealed acquisition of additional mutations or increasing variant allele frequency in *K/NRAS*, *RUNX1*, *IDH2* or *NPM1* associated with progression in 7 patients. In midostaurin-treated advSM patients, the complexity and dynamics of mutational profiles significantly impact on response, progression, and, prognosis.

INTRODUCTION

Systemic mastocytosis (SM) is a rare myeloid neoplasm characterized by clonal expansion of mast cells (MC) in various organ systems. Advanced SM (advSM), which comprises aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN), and mast cell leukemia (MCL), has a poor prognosis.¹⁻³ In patients with SM-AHN, the SM component can resemble indolent SM (ISM), ASM or MCL.²⁻⁴

SM is characterized by somatically acquired, activating mutations in the gene encoding the receptor tyrosine kinase *KIT*, most commonly *KIT* D816V, which is seen >80% of all SM patients. Recent data, however, have highlighted that the molecular pathogenesis of advSM is complex with one or more additional mutations, e.g. in *ASXL1*, *CBL*, *JAK2*, *RUNX1*, *SRSF2* or *TET2*, present in >60% of advSM patients.⁵⁻⁷ These additional mutations are usually acquired prior to *KIT* D816V, thereby indicating a multi-mutated stem cell disease.⁸ The *KIT* D816V expressed allele burden (EAB) and the presence and number of additional molecular aberrations, notably in *SRSF2*, *ASXL*, or *RUNX1* (S/A/R^{pos}), have a strong adverse impact on disease phenotype and prognosis.^{6,9,10} A new risk classification was recently proposed including clinical, e.g. splenomegaly, elevated alkaline phosphatase (AP), and S/A/R^{pos}.¹¹

A recently reported phase-II-study exploring the efficacy and safety of midostaurin, a multi-targeted *KIT* inhibitor, in patients with advSM demonstrated an overall response rate (ORR) of 60%. Notably, achievement of responses was significantly associated with an improved outcome.¹² However, the impact of the clonal architecture on responsiveness to midostaurin and the mechanisms of progression are unknown. In the current study, we therefore sought to evaluate the impact of the presence and dynamics of molecular markers at baseline and during follow-up on response, progression, and prognosis in midostaurin-treated advSM patients.

MATERIAL/METHODS

Patients, diagnosis and response criteria

The diagnosis of SM was established according to the WHO classification.^{2,3,13,14} All BM biopsies were evaluated by reference pathologists of the ECNM (H.-P.H. and K.S.). The diagnosis of ASM was based on the presence of one or more C-findings. Only measurable C-findings were eligible for this study: transfusion-independent and -dependent cytopenia(s); liver function abnormalities (increased alanine aminotransferase, aspartate aminotransferase, and/or total bilirubin); hypoalbuminemia; medically documented weight loss $\geq 10\%$ in the 6 months prior to the study. Response was evaluated according to Valent criteria.¹⁵ The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Medical Faculty of Mannheim, Heidelberg University, as part of the 'German Registry on Disorders of Eosinophils and Mast Cells'. All patients gave written informed consent.

Quantitative assessment of *KIT* D816V

Quantitative assessments of *KIT* D816V EAB were performed using allele-specific quantitative real-time reverse-transcriptase polymerase chain reaction (RQ-PCR) analysis on RNA/cDNA as previously described. It was also shown that quantitative measurements on RNA were equivalent to DNA.⁹ Assessments were performed at baseline and at least every three months during and after stopping treatment. Baseline was defined as last measurement prior to first dose of treatment.

Targeted Next-Generation Sequencing (NGS) analysis

Next-Generation Deep Amplicon Sequencing (NGS) by 454 FLX amplicon chemistry (Roche, Penzberg, Germany) was performed at baseline in all patients to investigate 18 candidate genes. The customized sequencing panel targeted the hotspot or complete coding regions of the following 18 genes: *ASXL1*, *CBL*, *ETV6*, *EZH2*, *IDH1*, *IDH2*, *JAK2*, *KRAS*, *NPM1*, *NRAS*, *RUNX1*, *SETBP1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *ZRSR2*.⁵ Sequential NGS, with consistent detection sensitivity of variant allele

frequency (VAF) down to 3%, was performed using the 18 gene panel above or a wider 28 gene panel (as previously described)¹⁶ in 16 patients with signs of progressive disease.^{17,18} The sequential NGS approach is based on library preparation by the Access Array Technology (Fluidigm, San Francisco, CA) and sequencing on the MiSeq Instrument (Illumina, San Diego, CA). Gene mutations were annotated using the reference sequence of the Ensembl Transcript ID (Ensembl release 85: July 2016).

Statistical analyses

Statistical analyses considered clinical, laboratory, and molecular parameters obtained at the time of diagnosis, start of treatment, and at multiple time points during treatment. OS was defined as time from start of treatment to date of death or last contact. Differences in the distribution of continuous variables between categories were analyzed by the Mann-Whitney test (for comparison of two groups). For categorical variables, Fishers's exact test was used. For *KIT* D816V EAB reduction, receiver operating characteristic (ROC) curve with a time-dependent survival probability were used to identify optimal cut-off points. OS probabilities were estimated using the Kaplan-Meier method and compared by the log-rank test for univariate analysis. For the estimation of hazard ratios (HRs) and multivariate analysis, the Cox proportional hazard regression model was used. *P*-values <0.05 (two-sided) were considered significant. There was no adjustment for multiple testing as all analyses were explorative. SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) and SAS version 9.2 (SAS Institute, Cary, NC, USA) were used for statistical analysis.

RESULTS

Clinical characteristics at baseline

Between 2009 and 2015, 38 advSM patients (median age 67 years, range 48-76, 69% male) were treated with midostaurin at our institution (international phase-II-study on the efficacy and safety of

midostaurin in advSM, ClinicalTrials.gov number, NCT00782067, n=20; compassionate use program, CUP, funded by Novartis Pharma, n=18). There were no differences in eligibility criteria and treatment design between the phase-II-study and the CUP. All patients started at 100 mg bid. Fifteen of 35 (43%) patients had at least 1 prior therapy (median 1, range 1-4).

Diagnoses at baseline were as follows: SM-AHN, n=22; ASM, n=5; MCL, n=4; MCL-AHN, n=7.^{1,3,13,14} The AHN comprised chronic myelomonocytic leukemia (CMML, n=15), myelodysplastic syndrome/myeloproliferative neoplasm unclassified (MDS/MPN-U, n=11) and chronic eosinophilic leukemia (CEL, n=3). *KIT* mutation status was as follows: *KIT* D816V (n=35, 92%), *KIT* D816Y (n=1), *KIT* D816 negative (n=2). Three of 38 (8%) patients stopped midostaurin within the first 4 months because of intolerance (nausea, vomiting) and were excluded from further analyses. Clinical parameters at diagnosis of the 35 remaining patients are shown in Table 1.

Response rates, progression, and survival on midostaurin

The median time from diagnosis of advSM to start of midostaurin was 12 months (range 1-60) and the median treatment time was 13 months (range 1-88). Responses according to Valent criteria at month 6 (n=29) or at the end of treatment due to progression/death prior to month 6 (n=6) were as follows: ORR, 51% (18/35 patients) with major response (MR), n=12 (34%) and partial response (PR), n=6 (17%); stable disease, n=8 (23%) and progressive disease (PD), n=9 (26%). ORRs were not statistically different between patients with or without MCL, AHN or prior therapies (Figure 1A).

During follow-up, secondary MCL (sMCL), sMCL with secondary AML (sMCL-sAML) and SM-sAML were diagnosed in 4, 1, and 1 patient(s), respectively. In 5/6 (83%) cases, the disease evolved from SM-AHN within a median time of 14 months (range 6-58) from start of midostaurin. The median OS was 30 months (95% confidence interval [CI], 6-54) from start of midostaurin and 45 months (95% CI,

34-56) from diagnosis of advSM. Twenty of 35 (57%) patients died, 6 of them within the first 6 months because of early PD.

Impact of mutations in the S/A/R gene panel on response, progression, and survival

At baseline, significant differences between S/A/R^{POS} (n=23) and S/A/R^{NEG} (n=12) patients were observed for hemoglobin <10 g/dL and elevated AP >150 U/L (Table 1). Five of 6 (83%) patients who stopped treatment prior to month 6 due to progression/death were S/A/R^{POS} and the remaining S/A/R^{NEG} patient had MCL. Number of patients on midostaurin at the time of last contact (17% vs. 58%, P=0.02), ORR (39% vs. 75%, P=0.04, Figure 1A) and OS (median not reached vs. 27 months, 95% CI [16-38], P=0.01; HR 4.0, 95% CI [1.3-16.2], Figure 1B and 3A) were all significantly in favor of S/A/R^{NEG} patients. Long-term survival >5 years (n=5) was only observed in S/A/R^{NEG} patients. Of note, 5/6 (83%) patients with progression to sMCL or sAML were S/A/R^{POS}.

Within an unselected historical control group ('German Registry on Disorders of Eosinophils and Mast Cells') of advSM patients without midostaurin treatment (n=50), the molecular profile was available in 30 patients; 17/30 (57%) patients (SM-AHN, n=15; MCL-AHN, n=1; ASM, n=1) were S/A/R^{POS}. The median OS calculated from diagnosis of advSM in those 17 patients without midostaurin treatment was 14 months compared to 40 months in 23 S/A/R^{POS} patients (SM-AHN, n=16; MCL-AHN, n=6; MCL, n=1) with midostaurin treatment (P=0.004, HR 3.0 [1.4-6.5]). Progression to sAML was not different between the two cohorts (4/23 [17%] vs. 3/17 [18%]).

KIT D816V EAB changes during treatment with midostaurin

For the 28 KIT D816V positive patients with at least 6 months on midostaurin, the KIT D816V EAB in PB was assessed at baseline and at least every 3 months (Figure 2A). The best median response in all patients was -29% (range -100 to 71). Nineteen (68%) and 13 patients (46%) had a relative KIT D816V EAB reduction of ≥25% or ≥50%, respectively, which were independent of baseline EAB (Figure 2B).

As a result of ROC-analysis, patients were classified according to the *KIT* D816V EAB reduction at month 6 as *KIT*-responders ($\geq 25\%$ reduction, $n=17$) or *KIT*-non-responders ($< 25\%$ reduction, $n=11$) (Figures 2C to 2D). *KIT*-responders were found to have significantly higher ORR (13/17 vs. 2/11 patients, $P=0.006$), a median longer time on midostaurin (25 months vs. 9 months, $P=0.01$) and improved OS (median not reached vs. 27 months, 95% CI [7-51], $P=0.0004$) (Table 2, Figures 1C and 3B). All 11 *KIT*-non-responders were found to have multi-mutated advSM (*S/A/R*^{pos}, $n=9$, 82%; *TET2/EZH2*, $n=1$; *TET2*, $n=1$). The mutation profile of the *KIT* EAB responders ($n=17$) were as follows: *S/A/R*^{pos} ($n=9$, 53%), *TET2/CBL/JAK2* ($n=1$), *TET2/JAK2* ($n=1$), *TET2* ($n=1$), no mutations ($n=4$). Of note, all patients ($n=4/17$, 24%) who lost their molecular response (*KIT* D816V EAB reduction $< 25\%$) sustainably (during follow up) were also *S/A/R*^{pos}.

Univariate and multivariate analyses of on-treatment variables

Univariate analyses of key clinical (measureable) parameters (MC infiltration in BM, serum tryptase level, AP, cytopenia, albumin) and molecular markers (*KIT* D816V EAB) and their response at month 6, identified reduction of serum tryptase ($P=0.03$, HR 4.8 [1.1-22.1]), reduction of AP ($P=0.04$, HR 3.2 [1.0-10.1]), reduction of *KIT* D816V EAB ($P=0.0004$, HR 7.6 [2.0-28.3]) and any response (MR and PR) according to Valent criteria ($P=0.01$, HR 3.6 [1.2-10.5]) as favorable prognostic variables regarding OS. In multivariate analysis, only *KIT* D816V EAB reduction $< 25\%$ remained an independent risk factor for poor OS ($P=0.004$, HR 6.8 [1.8-25.3]) (Figures 1C and 3B).

Serial NGS analyses during treatment with midostaurin

Serial NGS analyses of PB samples in a cohort of multi-mutated patients ($n=16$) were performed at baseline and up to three times per patient (median 2, range [1-3]) during midostaurin treatment at a median interval of 14 months (range 6-48). In total, 70 serial NGS (including baseline NGS) analyses were performed. The dynamics of VAF of recurrently mutated genes are shown in Figure 4.

Among *KIT*-responders, a significant increase in the VAF of additional mutations was identified in patients #06 (*KRAS*, 11% to 39%) and #12 (*RUNX1*, 42% to 86%). In patient #12, progression of ASM-MDS/MPN to ASM-AML was observed although *KIT* D816V had virtually disappeared (VAF, 1%). Both patients died, 22 months (patient #09) and 15 months (patient #12) after start of midostaurin, respectively. In the remaining 6 patients, the appearance of new mutations was observed in 2 patients (#10 and #11, *JAK2* V617F [VAF, 3% and 52%, respectively] in both cases) and the virtual disappearance (VAF \leq 1%) of mutations in 4 patients (*KIT* D816V in patients #16 and #12; *CBL* in patient #07; *TET2* in patient #11, baseline VAF [7%, 48%, 4%, 8%, respectively]). In patient #11 (acquisition of *JAK2* V617F, VAF 52%), progression of ASM to ASM-polycythemia vera was observed. All 6 patients are alive and on midostaurin at a median of 40 months (range 22-79).

Of the 8 *KIT*-non-responders, 7 (88%) patients died at a median of 21 months (range 6-54) after start of midostaurin. An increase in the *KIT* D816V EAB was observed in 7/8 patients (88%) and a significant increase of the VAF of additional mutations already present at start of midostaurin was observed in patients #13 (*IDH2*, 20% to 49%) and #02 (*KRAS*, 4% to 16%), respectively. The appearance of new mutations was seen in patients #15 (*NPM1*, VAF 3%) and #04 (*RUNX1*, VAF 19%), and was associated with progression to secondary AML (sAML) after 6 (patient #15) and 25 (patient #4) months on midostaurin, respectively. Both patients failed to achieve a response to intensive chemotherapy and died 1 and 12 months, respectively, after diagnosis of sAML. In patient #03, midostaurin was stopped after 8 months because of progression. After 6 cycles of cladribine, a significant reduction of AP and serum tryptase levels were observed, which was associated with a reduction of the *KIT* D816V EAB from >50% to 5%. Relapse occurred 6 months later and 5-azacytidine was initiated. The patient remained in SD with a significant reduction of the *KIT* D816V EAB after 11 cycles of 5-azacytidine. Acquisition of *KRAS* (VAF, 17%) and *SETBP1* (VAF, 11%) mutations was associated with rapid progression and death at 12 months after start of 5-azacytidine (Figure 4).

Overall, serial NGS analysis of 7 deceased patients revealed acquisition of additional mutations in *RUNX1* (n=2), *K/NRAS* (n=3), *IDH2* (n=1) or *NPM1* (n=1) and/or increasing VAF of additional mutations, while *KIT* D816V EAB was increasing (n=4), stable (n=1), or even low (n=2). In contrast, 2 patients acquired a *JAK2* V617F mutation but remained in durable PR on midostaurin (Figure 4).

DISCUSSION

In addition to the known beneficial effects of midostaurin on BM MC infiltration, serum tryptase and C-findings,^{12,21} we demonstrate here that midostaurin also reduces the *KIT* D816V allele burden. In patients who had been treated for at least 6 months, a significant reduction $\geq 25\%$ at month 6 was the strongest on-treatment predictor for improved survival in multivariate analysis and was even superior to Valent response criteria. Because all patients with *KIT* D816V positive advSM have at diagnosis a measurable allele burden in PB,⁹ regular measurements of the *KIT* D816V allele burden should be performed in the routine follow-up for residual disease on or after potentially effective treatment regimens, including midostaurin, chemotherapy and/or allogeneic stem cell transplantation (SCT).²²⁻²⁵

Disparate mechanisms may be responsible for progression on midostaurin. In addition to the known negative effects on phenotype and prognosis,^{6,11} we also show that response rates due to early or late progression and consequently survival were significantly inferior in *S/A/R*^{pos} patients suggesting persistence and/or outgrowth of an aggressive and multi-mutated *KIT* D816V positive clone. Almost all patients who stopped due to early progression, who lost the *KIT*-response or who progressed to sMCL or sAML had at least one mutation in the *S/A/R* gene panel. However, the median OS of midostaurin-treated *S/A/R*^{pos} patients is better than the median OS of *S/A/R*^{pos} patients without midostaurin treatment while the rate of progression to sAML during follow-up is not different.

There is a strong link between AHN and additional mutations, however, no significant differences were observed between advSM with or without AHN because advSM with AHN was also associated with other mutations than in S/A/R, e.g. *JAK2* V617F. It is therefore not the presence of AHN *per se* but the molecular background of AHN that impacts on response and prognosis. Of note, the majority of patients who progressed on midostaurin also failed to respond to subsequent chemotherapy, e.g. cladribine, and even allogeneic SCT in one patient. This data is reminiscent of the treatment of myelofibrosis (MF) with ruxolitinib for which the presence of mutations in *ASXL1* or *SRSF2* was also inversely correlated with spleen response, time to treatment discontinuation and shortened OS.²⁶ We found no evidence for additional *KIT* mutations that might be conferring resistance to midostaurin (data not shown).

A second mechanism leading to progression was associated with increasing VAF or new emergence of mutations in *RUNX1*, *K/NRAS* or *IDH2*, despite otherwise durable response of C-findings and significant reduction of the *KIT* D816V allele burden. In this regard it is noteworthy that the first published patient with advSM receiving midostaurin also relapsed with a *KIT* D816V negative sub-clone resembling AML.²⁷ The data are also reminiscent to other myeloid neoplasms and their clonal diversity, in which mutations in *RUNX1*, *N/KRAS* or *IDH2* were also identified as late events and drivers for disease progression.^{19,28-31} Of interest, no significant increase in clone size was observed for mutations in *TET2* or *SRSF2*, although they represent early mutations in the multistep pathogenesis of SM⁸, CMML³² or MDS¹⁹. These results suggest that the extensive clinical heterogeneity of advSM is most likely due to the presence and dynamic evolution of several molecularly disparate sub-clones, which have a variable impact on clinical characteristics and response to treatment.

Dysregulated KIT and JAK2 signaling are certainly pivotal for the pathogenesis of SM and MF. However, it is not yet fully understood how one mutation can cause distinct phenotypes, e.g. *KIT* D816V in ISM and advSM or *JAK2* V617F in classical MPN. In addition, it has also become apparent that patients do not experience complete hematologic remissions on targeted treatment with midostaurin or ruxolitinib.³³ But there is now accumulating evidence that complex mutation profiles are strongly associated with primary and secondary progression on targeted treatment of these neoplasms. Whether additional mutations cause progression directly or whether they are simply markers for other factors remains yet unknown. Based on promising *in vitro* studies,^{34,35} the combination of midostaurin with other effective anti-SM therapies such as chemotherapy with or without allogeneic SCT²²⁻²⁵ may become a reasonable approach to overcome this frequently occurring scenario.

In conclusion, we have found that the absence of mutations in the *S/A/R* gene panel at baseline and a reduction of the *KIT* D816V allele burden $\geq 25\%$ at month 6 are the most favorable predictors for improved survival in midostaurin-treated advSM patients. Serial sequencing of relevant mutations at multiple time points during treatment has led to a more detailed understanding of the clonal dynamics for response assessment. Progression may be caused by expansion of sub-clones exhibiting new mutations in critical target genes independent of *KIT* D816V. Prospective clinical trials are warranted which integrate NGS for clinical decision-making in advSM and use combinations of midostaurin with drugs, which are able to also suppress *KIT* D816V-negative subclones.

Authorship: MJ, JS, NN, SB, TH, AF, NCPC, MM, AR performed the laboratory work for the study. MJ, JS, SK, GM, MM and AR provided patient material and information. HPH and KS reviewed the bone marrow biopsies. MJ, JS, NN, TH, GM, AF, PV, WKH, NCPC, MM and AR wrote the paper.

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Table 1: Clinical, laboratory, treatment characteristics and outcome of 35 patients with advanced systemic mastocytosis, stratified by absence (S/A/R^{neg}) or presence (S/A/R^{pos}) of mutations in the *SRSF2/ASXL1/RUNX1* (S/A/R) gene panel.

Variables	All patients	S/A/R ^{neg}	S/A/R ^{pos}	P*
No. of patients (n)	35	12	23	
Age in years, median (range)	67 (48-76)	64 (48-76)	68 (55-75)	n.s.
Males, n (%)	24 (69)	5 (42)	19 (83)	0.02
Diagnosis				
ASM, n (%)	4 (6)	4 (33)	0	n.s.
MCL, n (%)	4 (11)	3 (25)	1 (4)	n.s.
SM-AHN	27 (83)	5 (42)	22 (96)	0.01
SM, n (%)	20 (57)	4 (80)	16 (73)	n.s.
MCL, n (%)	7 (26)	1 (20)	6 (27)	n.s.
Disease related parameters				
Hemoglobin, g/dL, median (range)	10 (6-15)	11 (7-15)	9 (6-14)	0.04
<10 g/dL, n (%)	21 (60)	4 (33)	17 (74)	0.03
Platelets, x 10 ⁹ /L, median (range)	97 (7-426)	113 (30-344)	94 (7-426)	n.s.
<100 x 10 ⁹ /L, n (%)	20 (57)	6 (50)	14 (61)	n.s.
Hemoglobin < 10 g/dL and/or platelets < 100 x 10 ⁹ /L, n (%)	30 (86)	8 (67)	22 (96)	0.04
MC infiltration in BM, %, median (range)	50 (15-95)	40 (15-90)	50 (20-95)	n.s.
Serum tryptase, µg/L, median (range)	246 (33-1690)	214 (65-1690)	296 (33-1200)	n.s.
>200 µg/L, n (%)	21 (60)	7 (58)	14 (61)	n.s.
<i>KIT</i> D816V ⁺ EAB in PB, %, median (range)	40 (4-64)	35 (4-63)	40 (7-64)	n.s.
>30 %, n (%)	24 (69)	8 (67)	16 (70)	n.s.
Albumin, g/L, median (range)	34 (23-45)	34 (23-43)	34 (26-45)	n.s.
<35 g/L, n (%)	21 (60)	7 (58)	14 (61)	n.s.
Alkaline phosphatase, U/L, median (range)	287 (61-1067)	179 (61-479)	355 (134-1067)	0.004
>150 U/L, n (%)	29 (83)	8 (67)	22 (96)	0.04
Spleen volume, mL, median (range), n=28	918 (333-2058)	800 (333-1796)	919 (338-2058)	n.s.
Marked splenomegaly (≥1200 mL)	10 (36)	2 (22)	8 (42)	n.s.
Treatment related parameters				
Time on midostaurin therapy, median (range)	13 (1-88)	13 (5-88)	11 (1-57)	n.s.
≥6 months on midostaurin, n (%)	29 (83)	11 (92)	18 (78)	n.s.
Number of patients on midostaurin at time of reporting	11 (31)	7 (58)	4 (17)	0.02
Outcome				
Median OS, months (95% CI)	30 (6-54)	NR	27 (16-38)	0.01**
Death, n (%)	20 (57)	3 (25)	17 (74)	

The *P*-values refer to the Mann–Whitney U test, Fisher’s exact test or the **log-rank tests comparing S/A/R^{neg} and S/A/R^{pos} patients. Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive SM; CI, confidence interval; BM, bone marrow; EAB, expressed allele burden; MC, mast cells; MCL, mast cell leukemia; n.s., not significant; OS, overall survival; PB, peripheral blood; SM, systemic mastocytosis.

Table 2: Clinical, laboratory, molecular and treatment characteristics and outcome of 28 patients with advanced systemic mastocytosis, stratified by *KIT* D816V expressed allele burden (EAB) reduction at month 6 in peripheral blood ($\geq 25\%$ vs. $< 25\%$).

Variables	All patients	Decrease of <i>KIT</i> D816V EAB $\geq 25\%$	Decrease of <i>KIT</i> D816V EAB $< 25\%$	<i>P</i> *
No. of patients (n)	28	17	11	
Age in years, median (range)	65 (48-76)	68 (48-76)	63 (55-75)	n.s.
Males, n (%)	19 (68)	10 (59)	9 (82)	n.s.
Diagnosis				
ASM, n (%)	3 (11)	3 (18)	0	
MCL, n (%)	2 (07)	2 (12)	0	
SM-AHN	23 (82)	12 (71)	11 (100)	n.s.
SM, n (%)	19 (83)	10 (83)	9 (82)	
MCL, n (%)	4 (17)	2 (17)	2 (18)	
Any response according Valent criteria	15 (54)	13 (76)	2 (18)	0.006
Molecular profile				
S/A/R ^{pos} , n (%)	18 (65)	9 (53)	9 (82)	n.s.
Other mutation (n (%))	6 (21)	4 (24)	2 (18)	
No mutation, n (%)	4 (14)	4 (24)	0	
Loss of <i>KIT</i> response***, n (%)		4 (24)	-	
S/A/R ^{pos} , n (%)		4 (24)	-	
Median time on midostaurin, months (range)	19 (6-88)	25 (6-88)	9 (7-35)	0.01
Number of patients on midostaurin at time of reporting, n (%)	10 (36)	10 (59)	0	0.002
Outcome				
Median OS, months (95% CI)	45 (17-73)	NR	27 (7-51)	0.0004**
Death, n (%)	14 (50)	4 (24)	10 (91)	

The *P*-values refer to the Mann–Whitney U test, Fisher’s exact test or the **log-rank tests comparing decrease of *KIT* D816V EAB $\geq 25\%$ vs. $< 25\%$.

***Lost of *KIT* D816V response during follow-up (after month 6). Abbreviations: AHN, associated hematologic neoplasm; ASM, aggressive SM; CI, confidence interval; EAB, expressed allele burden; MCL, mast cell leukemia; n.s., not significant; OS, overall survival; S/A/R^{pos}: mutation(s) in the *SRSF2/ASXL1/ RUNX1* gene panel; SM, systemic mastocytosis.

FIGURES

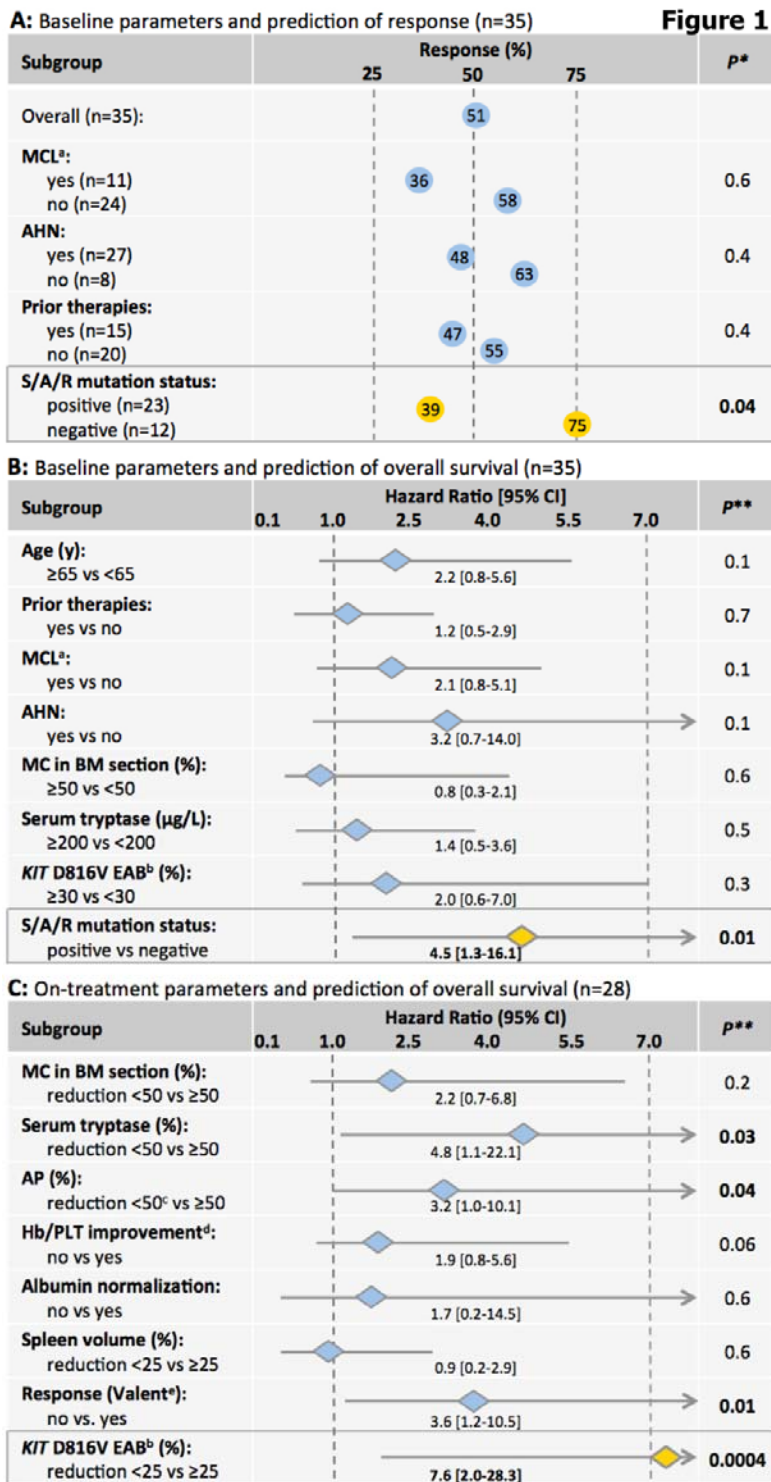


Figure 1: Response rates (A) and overall survival (B) in 35 midostaurin-treated advanced systemic mastocytosis (SM) patients according to various baseline parameters. Only the mutational status in the *SRSF2/ASXL1/RUNX1* (S/A/R) gene panel was a significant parameter for prediction of response

and overall survival. (C) On-treatment response parameters in 28 patients at month 6: significant reductions of serum tryptase, alkaline phosphatase (AP), *KIT* D816V expressed allele burden (EAB) and any response according to Valent criteria were identified as favorable prognostic parameters concerning OS. In multivariate analysis, only a *KIT* D816V EAB reduction <25% remained an independent risk factor for poor OS. Abbreviation: AHN, associated hematologic neoplasm; BM, bone marrow; CI, 95% confidence interval; Hb, hemoglobin; HR, hazard ratio; MCL, mast cell leukemia; PLT, platelets. *The P-values refer to Fishers's exact tests; **The P-values refer to the log-rank tests. ^aMCL patients with and without AHN, ^b*KIT* D816V in peripheral blood, ^cAP reduction ≥50% or normalization, ^dCheson criteria for transfusions, ^eresponse criteria according to Valent.¹⁵

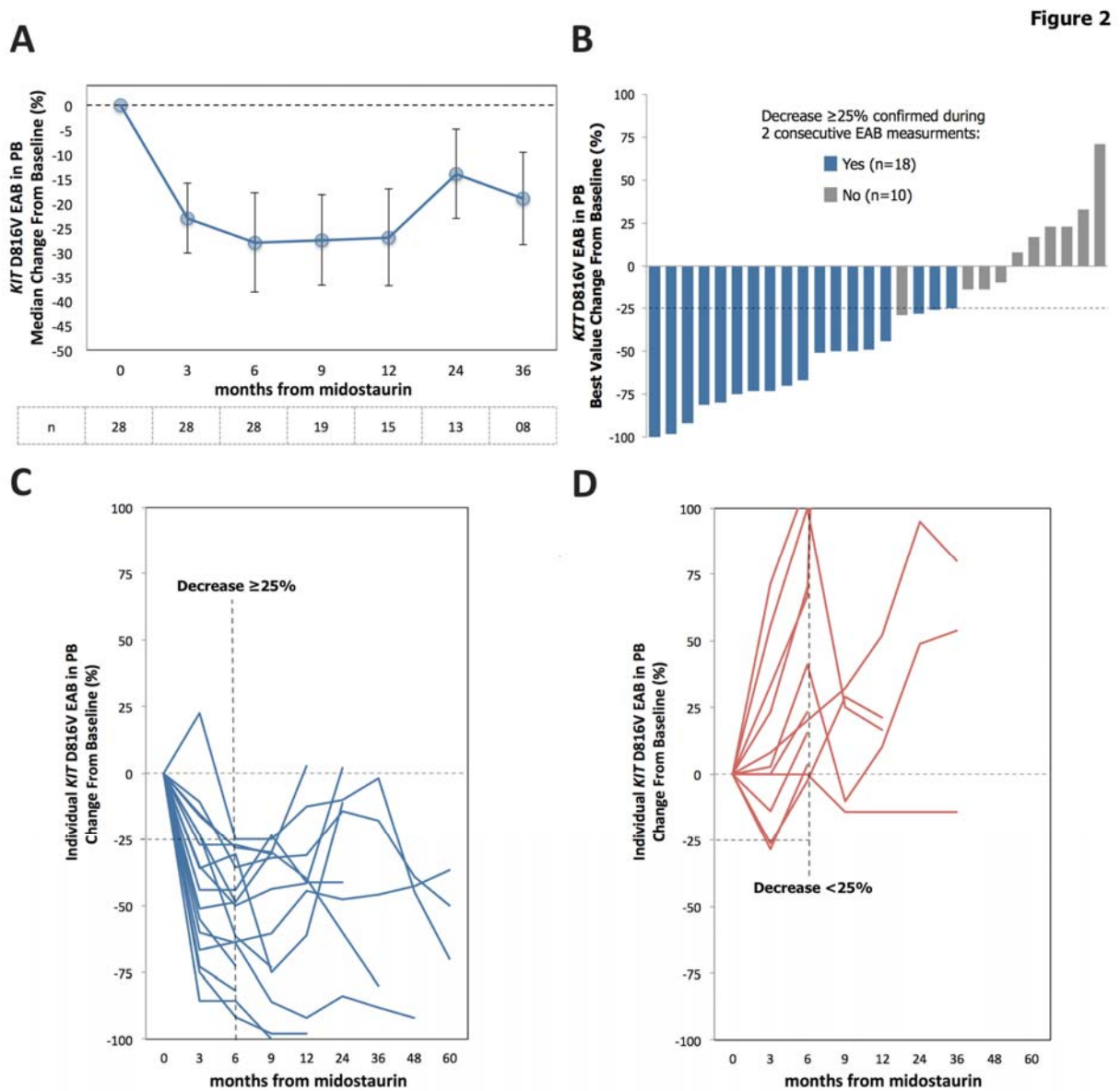


Figure 2: (A) Mean percent change (\pm SE) of the *KIT* D816V expressed allele burden (EAB) in peripheral blood (PB) from baseline in 28 advanced systemic mastocytosis patients treated with midostaurin, B) waterfall plot of the best percentage change of *KIT* D816V EAB in individual patients at any time, C) and D) EAB changes over time in individual patients with $\geq 25\%$ (C) or $< 25\%$ (D) reduction of *KIT* EAB at month 6. Abbreviations: SE, standard error.

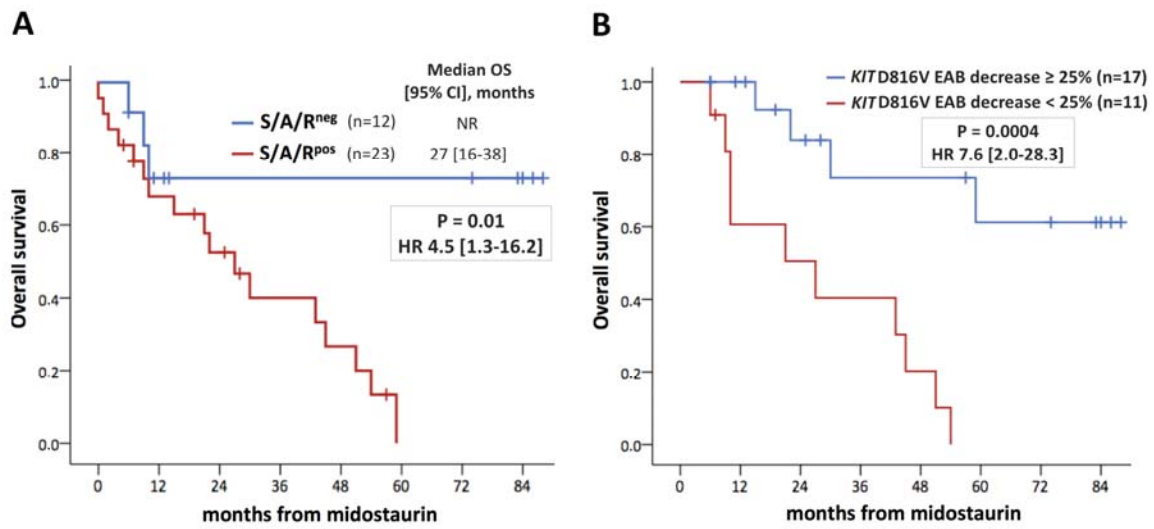


Figure 3: (A) Kaplan–Meier estimates of overall survival (OS) depending on mutational status in the *SRSF2/ASXL1/RUNX1* (*S/A/R*) gene panel ($S/A/R^{pos}$ vs. $S/A/R^{neg}$) at baseline. (B) OS depending on the expressed *KIT* D816V allele burden (EAB) reduction at month 6 in peripheral blood ($\geq 25\%$ vs. $< 25\%$). Abbreviation: HR, hazard ratio 95% confidence interval; NR, not reached.

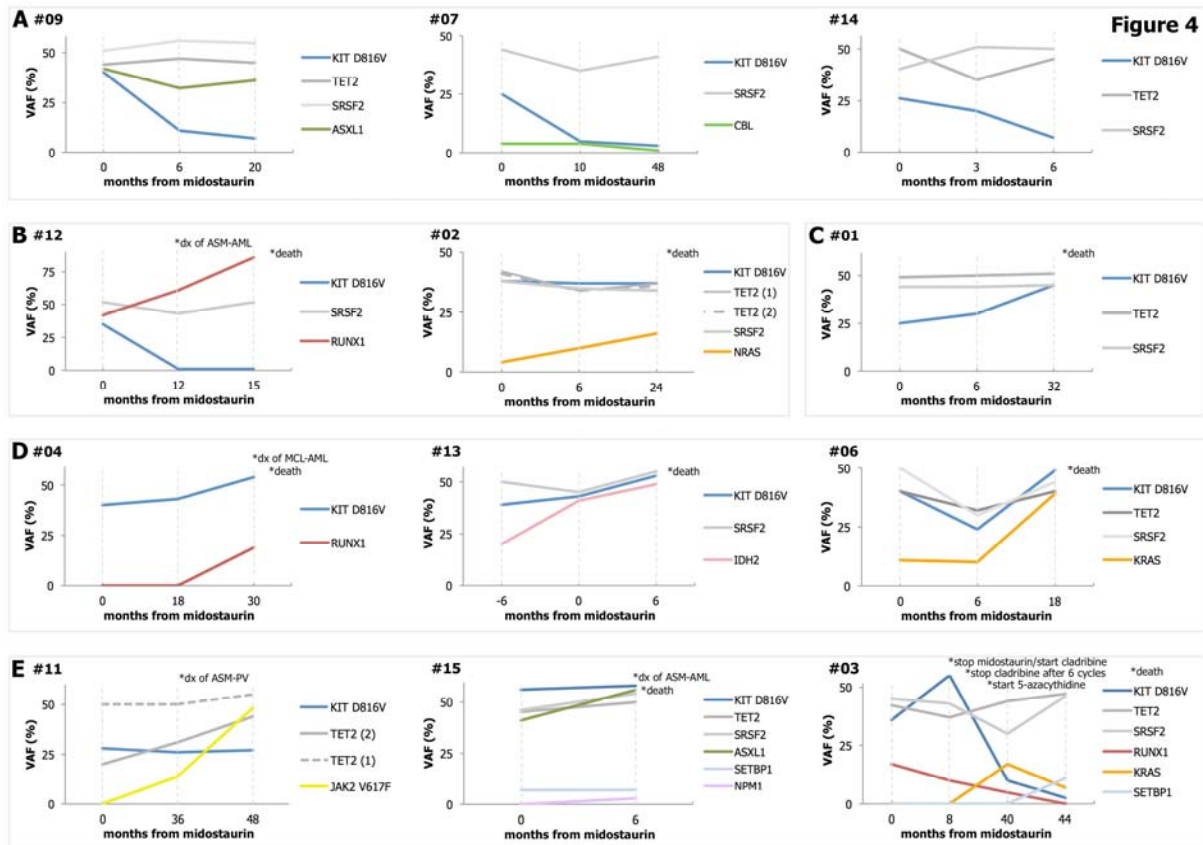


Figure 4: Schematic presentation of the variant allele frequency (VAF) changes of *KIT* D816V and additional mutations in 12 patients during treatment with midostaurin. Five clusters were identified (A-E): (A) Significant reduction of *KIT* D816V but without significant VAF changes of the additional mutations. All patients were maintained on midostaurin. (B) Significant reduction of *KIT* D816V but expansion of *RUNX1* (#12) or *NRAS* (#02) with subsequent disease progression and death (in #12 after progression into secondary acute myeloid leukemia). (C) Significant increase of *KIT* D816V but stable VAF of additional mutations with subsequent disease progression and death. (D) Significant increase of *KIT* D816V in combination with increase of VAF of additional mutations (*RUNX1*, #04; *IDH2*, #13; *KRAS*, #06) followed by disease progression and rapid death. Patient #04 progressed into secondary mast cell leukemia (MCL) and then secondary AML. (E) Acquisition of new mutations (*JAK2* V617F, #11; *NPM1*, #15; *KRAS/SETBP1*, #03) with disease progression into aggressive systemic mastocytosis and associated polycythemia vera (ASM-PV, #11) or ASM-AML (#15) followed by death (#15, #03). Patient #11 with transfusion-dependent anemia at baseline became transfusion-independent. *clinical end points, including type of secondary neoplasm observed at the time of

progression and death. Abbreviation: dx, diagnosis.