

BRIEF COMMUNICATION

The *KIT* D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease

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To cite this article: Hoermann G, Gleixner KV, Dinu GE, Kundi M, Greiner G, Wimazal F, Hadzijusufovic E, Mitterbauer G, Mannhalter C, Valent P, Sperr WR. The *KIT* D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. *Allergy* 2014; **69**: 810–813.

Keywords

allele burden; *KIT* D816V; mastocytosis; survival; treatment response.

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Accepted for publication 5 March 2014

DOI:10.1111/all.12409

Edited by: Hans-Uwe Simon

Abstract

KIT D816V is present in a majority of patients with systemic mastocytosis (SM). We determined the *KIT* D816V allele burden by quantitative real-time PCR in bone marrow and peripheral blood of 105 patients with mastocytosis. *KIT* D816V was detected in 92/105 patients (88%). Significant differences in the median allele burden were observed between disease subgroups: cutaneous mastocytosis (0.042%), indolent SM (0.285%), smoldering SM (5.991%), aggressive SM (9.346%), and SM with associated hematologic non-mast cell lineage disease (3.761%) ($P < 0.001$). The *KIT* D816V burden also correlated with serum tryptase ($R = 0.5$, $P < 0.005$) but not with mast cell infiltration in bone marrow or mediator symptoms. Moreover, the allele burden was of prognostic significance regarding survival ($P < 0.01$). Patients responding to cytoreductive therapy showed a significant decrease in *KIT* D816V ($P < 0.05$). To conclude, the *KIT* D816V burden correlates with the variant of mastocytosis, predicts survival, and is a valuable follow-up parameter in SM.

The great majority of patients with systemic mastocytosis (SM) harbor the somatic point mutation *KIT* D816V (1–4). Kristensen et al. (5) reported a highly sensitive quantitative real-time PCR (qPCR) for *KIT* D816V. Recently, the importance of allele burden measurements by qPCR techniques has been confirmed applying different methods (6). Moreover, differences in *KIT* D816V allele burden among

indolent and aggressive forms have been observed (6, 7). The aims of the present study were to evaluate the impact of *KIT* D816V allele burden on the clinical course and survival in SM.

Patients and methods

A total of 105 adult patients with mastocytosis, that is, cutaneous mastocytosis (CM, $N = 12$), mastocytosis in the skin (MIS, $N = 3$), indolent SM (ISM, $N = 67$), smoldering SM (SSM, $N = 5$), aggressive SM (ASM, $N = 7$), mast cell leukemia (MCL, $N = 1$), and SM with an associated hematologic non-MC lineage disease (SM-AHNMD, $N = 10$), were examined. Patients' characteristics are shown in Table S1. *KIT* D816V allele burden was quantified from genomic DNA of bone marrow (BM) aspirates and peripheral blood using allele-specific qPCR as described (5).

Abbreviations

AML, acute myeloid leukemia; ASM, aggressive systemic mastocytosis; BM, bone marrow; CM, cutaneous mastocytosis; CMML, chronic myelomonocytic leukemia; ISM, indolent systemic mastocytosis; MC, mast cell; MCL, mast cell leukemia; qPCR, quantitative real-time PCR; SM, systemic mastocytosis; SM-AHNMD, systemic mastocytosis with an associated hematologic non-mast cell lineage disease; SSM, smoldering systemic mastocytosis.

Results

At diagnosis, *KIT* D816V was detectable in 92/105 patients (88%) with a median *KIT* D816V allele burden of 0.315% (range 0.005–50.183%). In *KIT* D816V+ patients ($N = 92$), we compared the mutant allele burden in the different WHO subgroups. In CM, the median *KIT* D816V allele burden was 0.042% (range 0.005–0.530%), in MIS 0.084% (range 0.029–0.165%), in ISM 0.285% (range 0.006–34.585%), in SSM 5.991% (range 0.023–29.620%), in ASM 9.346% (range 8.816–32.480%), and in SM-AHNMD 3.761% (range 0.083–50.183%). These differences were highly significant (Kruskal–Wallis test; $P < 0.001$; Fig. 1A).

The *KIT* D816V allele burden correlated significantly with serum tryptase levels (Pearson's correlation; $R = 0.50$, $P < 0.005$) and age ($R = 0.56$, $P < 0.005$), but only roughly with mast cell (MC) infiltration in the BM ($R = 0.369$) or the percentage of MC in BM smears ($R = 0.334$) (Fig. S1). No significant differences in the allele burden were found when comparing patients with or without mediator-related symptoms (Fig. S2).

The *KIT* D816V mutation burden was a significant predictor for survival in our 92 *KIT* D816V+ patients (Cox regression, $P = 0.015$). Using a cutoff level of 2% mutant alleles, two prognostically distinct groups were separated. In the group with <2% mutant allele burden ($N = 66$), the median survival was not reached, whereas in patients with a *KIT* D816V allele burden of $\geq 2\%$ ($N = 26$), the median survival was 6.3 years (log-rank test; $P = 0.001$; Fig. 1B).

In 30 patients, the *KIT* D816V allele burden could be followed over a median observation time of 21 months (range 3–178 months). In patients with stable clinical course ($N = 19$), no substantial increase or decrease in the *KIT* D816V allele burden was observed (Fig. 2A,C,D; Fig. S3). In cases with advanced SM receiving cytoreductive therapy ($N = 11$), marked changes in the mutant allele burden were observed (Fig. 2B,E,F; Fig. S4). In patients with a good or partial response to cytoreductive treatment (cladribine $N = 4$,

hydroxyurea $N = 1$, interferon $N = 1$), a significant decrease in the *KIT* D816V burden (91.6% median reduction) was observed (paired t-test; $P = 0.027$).

Discussion

Recently, qPCR has been shown to be a reliable test for the quantification of *KIT* D816V in patients with mastocytosis (5–11). In line with these results, the median *KIT* D816V allele burden was 0.315% in our patients. The different subgroups of SM are considered to differ substantially with regard to the burden of neoplastic MC (12). Indeed, the lowest *KIT* D816V allele burden was observed in CM followed by ISM and SSM, the highest in ASM and SM-AHNMD. Similar results have recently been reported in a study investigating indolent SM but not cases with ASM (7). The higher *KIT* D816V allele burden in advanced SM, including SM-AHNMD, could also indicate that non-MC lineage cells harbor the mutation in these patients. In this regard, it is noteworthy that multilineage involvement is frequent in advanced SM (13, 14). Multilineage involvement may also explain why the *KIT* D816V allele burden correlated only moderately with serum tryptase levels and failed to correlate with the number of MC in BM biopsies and smears. Another explanation would be that other oncogenic mutations are involved in proliferation and/or survival of MC in the BM (15).

As the *KIT* mutation D816V triggers autonomous KIT activation, we are interested whether mediator-related clinical symptoms would correlate with the *KIT* D816V mutant burden. However, we are unable to demonstrate such a correlation, which also confirms the results obtained by Broesby-Olsen et al. (8) in a cohort including advanced SM. Together, these data suggest that mediator-related symptom occurs regardless of the WHO subgroup and independently of the burden of *KIT*-mutated MC.

Recently, an impact of the *KIT* D816V allele burden on survival has been suggested in a cohort with a very high percentage (52%) of advanced SM (6). In our cohort of

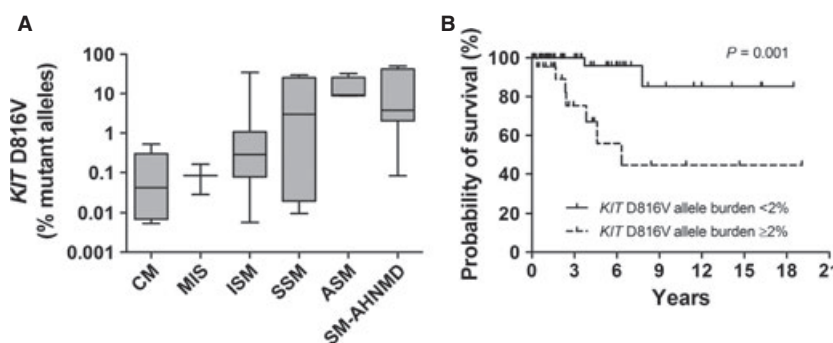


Figure 1 *KIT* D816V allele burden in different WHO subtypes of mastocytosis and its impact on survival. (A) Highly significant differences in the *KIT* D816V allele burden were found between patients with cutaneous mastocytosis (CM), mastocytosis in the skin (MIS), indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM), aggressive systemic mastocytosis (ASM), and

systemic mastocytosis with an associated hematologic non-mast cell lineage disease (SM-AHNMD) ($P < 0.001$). (B) Kaplan–Meier plot for overall survival of mastocytosis patients with a *KIT* D816V mutation burden <2% and patients with a *KIT* D816V burden of $\geq 2\%$ at diagnosis. The difference in the probability of survival was significant ($P = 0.001$).

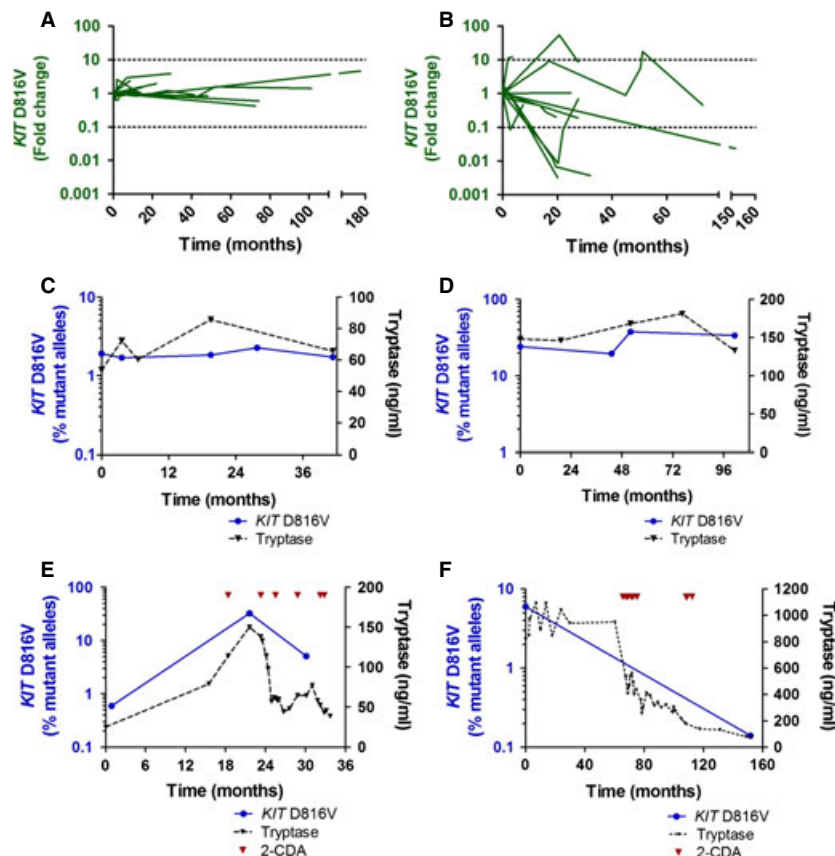


Figure 2 *KIT* D816V allele burden during the follow-up. Change in *KIT* D816V mutation-positive allele fraction during the follow-up in patients without (A; $N = 19$) and with cytreductive treatment (B; $N = 11$). The allele burden was normalized to the mutant burden at diagnosis. The value of 1 corresponds to no change in allele fraction over time, and the values of 0.1 and 10 (dotted lines) correspond to tenfold decreases and increases. (C–F) Follow-up of *KIT*

D816V allele burden (blue) and serum tryptase (black) in individual mastocytosis patients with an uneventful course (C, ISM; D, SSM), a patient initially diagnosed as ISM with disease progression to ASM-CMML and response to cytreductive therapy with cladribine (2-CDA) (E), and a patient with SSM receiving cytreductive therapy due to recurrent episodes of life-threatening vascular instability requiring epinephrine support (F).

consecutively diagnosed patients with only 17% advanced SM, thereby reflecting a less selected patient population, the *KIT* D816V allele burden was of prognostic significance regarding survival. Using a cutoff level of 2% mutant allele burden in our study, two prognostically different groups of patients with clearly different survival times could be separated. However, the value of a particular cutoff needs to be confirmed in future prospective trials.

At present, only a few laboratory parameters are available to monitor treatment response in advanced SM (4). So far, follow-up data of *KIT* D816V allele burden were only published for patients with ISM (8) and 4 patients with advanced SM (6). In our cohort, patients with indolent disease showed a stable allele burden during the follow-up, whereas in cases with disease progression, a marked increase in *KIT* D816V was found. Patients responding to cytreductive therapy showed a significant decrease in the *KIT* D816V burden. Thus, the *KIT* D816V allele burden may be employed for response evaluation in clinical trials.

Together, we confirm the diagnostic value of *KIT* D816V qPCR in mastocytosis. Moreover, our data show that the mutation burden differs significantly among patients with different WHO subgroups and is of prognostic significance concerning survival. Finally, the *KIT* D816V allele burden is a valuable follow-up parameter in untreated and drug-treated patients with advanced SM.

Acknowledgments

The authors would like to thank Matthias Mayerhofer (Hanusch Hospital, Vienna, Austria) for critical reading of the manuscript.

Financial support

This study was supported by Austrian National Science Fund (FWF) project P26079-B13 and SFB project F4704-B20.

Author contributions

G.H., G.E.D., G.G., G.M., and C.M. performed molecular tests and analyzed the data. G.H., K.V.G., F.W., M.K., E.H., P.V., and W.R.S. obtained and analyzed the clinical data. G.H., P.V., and W.R.S. designed the study and wrote the manuscript. All authors revised and approved the manuscript.

Conflicts of interest

P.V. is a consultant in a global Novartis trial investigating the effects of PKC412 in patients with advanced systemic mastocytosis. In addition, P.V. received honoraria and a research grant from Novartis. The authors declare no other competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Correlation of KIT D816V allele burden with disease-related markers of mastocytosis.

Figure S2. Association of KIT D816V allele burden with mediator symptoms.

Figure S3. KIT D816V allele burden during the follow-up of patients without cytoreductive treatment.

Figure S4. KIT D816V allele burden during the follow-up of patients with cytoreductive treatment.

Table S1. Patients' characteristics.

Supplementary material

Supplementary Tables

Table S1: Patients' characteristics

	<i>N</i>	f/m ratio		Age	Tryptase (ng/ml)	WBC (G/l)	Hb (g/dl)	Plt (G/l)	LDH (U/l)	AP (U/l)
CM	12	2.0	median	40	7	5.75	14.1	217	158	64
			min	24	3	4.23	11.7	170	136	44
			max	76	147	8.27	16.5	285	215	102
MIS	3	3/0	median	34	7	7.20	13.2	311	128	43
			min	28	6	4.89	11.8	243	82	42
			max	35	11	8.64	14.1	341	136	83
ISM	67	1.3	median	44	42	6.62	14.1	247	149	77
			min	20	6	3.67	11.7	123	88	41
			max	81	938	12.68	16.6	380	208	169
SSM	5	1.5	median	48	593	4.86	13.1	280	95	188
			min	34	153	3.98	11.2	201	81	82
			max	53	1090	30.09	14.4	405	157	215
ASM	7	0.2	median	59	213	5.77	9.2	115	162	345
			min	21	54	4.37	8.3	25	132	47
			max	73	1617	16.16	13.7	269	255	620
MCL	1	1/0	median	54	13	2.17	10.6	32	173	87
			min	54	13	2.17	10.6	32	173	87
			max	54	13	2.17	10.6	32	173	87
SM-AHNMD	10	0.3	median	55	89	7.25	10.2	86	216	109
			min	46	9	2.95	8.2	9	114	67
			Max	71	664	35.49	16.4	295	579	944
Total	105	1.1	Median	47	44	6.48	13.7	230	150	79
			Min	20	3	2.17	8.2	9	81	41
			Max	81	1617	35.49	16.6	405	579	944

CM cutaneous mastocytosis; min, minimum; max, maximum; MIS, mastocytosis in the skin; ISM, indolent systemic mastocytosis; SSM, smoldering systemic mastocytosis; ASM, aggressive systemic mastocytosis; MCL, mast cell leukemia; SM-AHNMD, systemic mastocytosis with an associated hematologic non-mast cell lineage disease; WBC, white blood cell count; Hb, hemoglobin; Plt, platelets; LDH, lactate dehydrogenase; AP, alkaline phosphatase; *N*, number of patients; f, female; m, male.

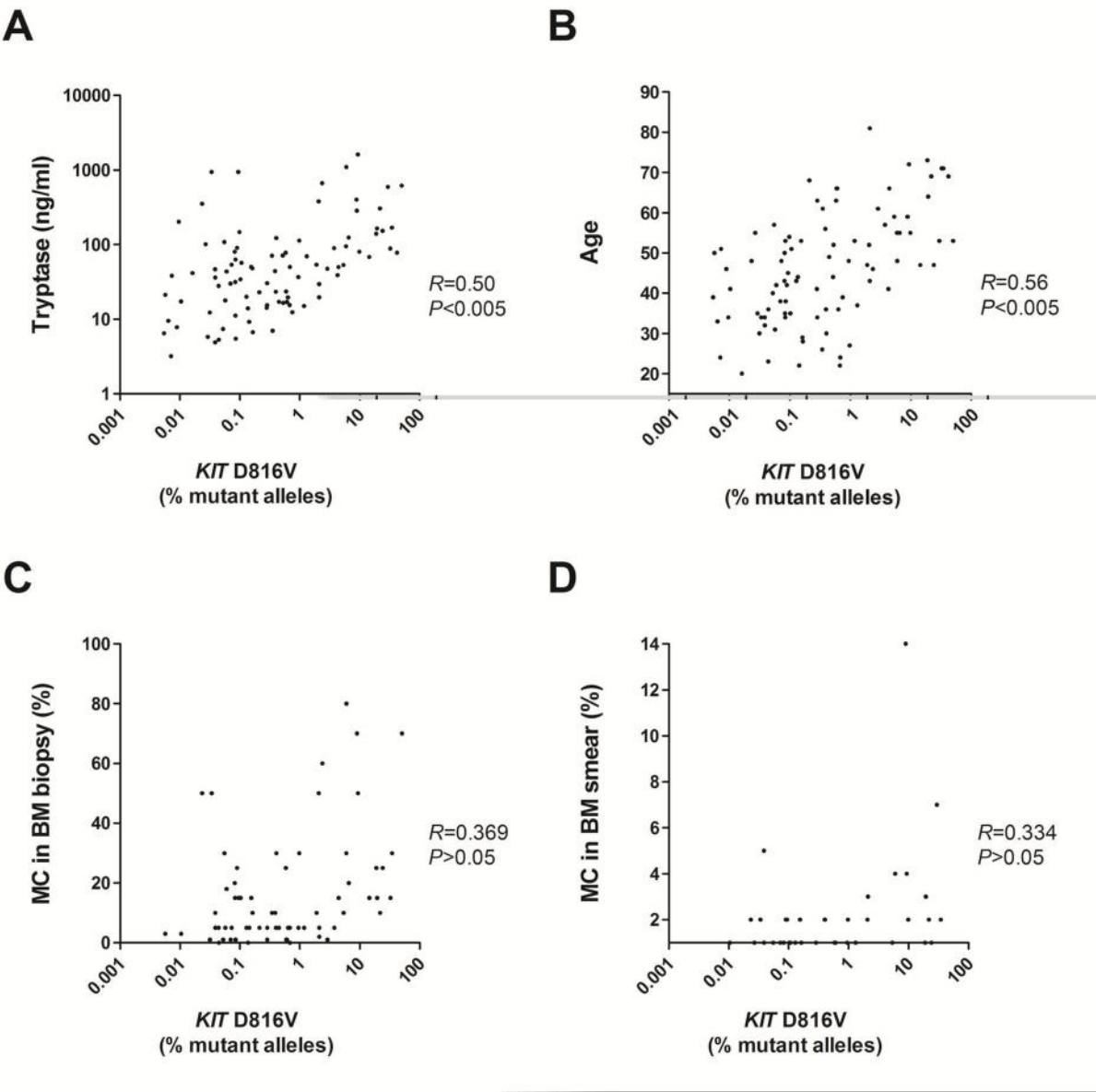
Supplementary Figures

Fig. S1. Correlation of *KIT* D816V allele burden with disease-related markers of mastocytosis. The *KIT* D816V allele burden was correlated with tryptase levels (A), age of disease onset (B), MC infiltration in the BM (C), or percentage of MC in BM smears (D). The *KIT* D816V allele burden correlated significantly with serum tryptase levels and age (Pearson's correlation).

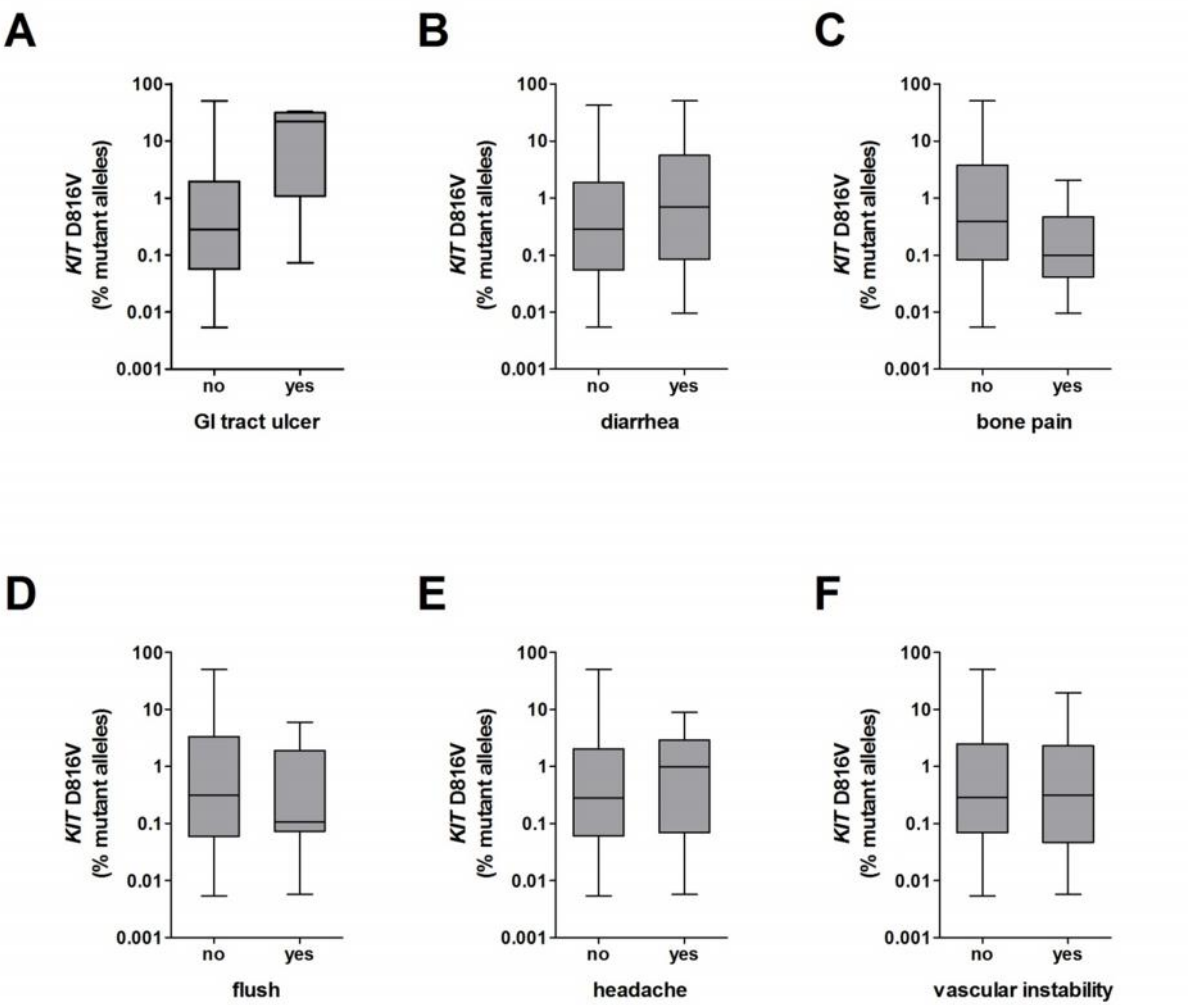
Fig. S2. Association of *KIT* D816V allele burden with mediator symptoms. Mutant allele burden was compared between *KIT* D816V positive mastocytosis patients with or without mediator symptoms like flushing, diarrhea, headache, bone pain, vascular instability and gastrointestinal (GI) tract ulcer. Marked differences were only found analyzing patients with or without gastrointestinal tract ulcer (A). However these differences were not statistically significant. No differences in the mutant allele burden between patients with or without the various other mediator symptoms were found (B-F).

Fig. S3. *KIT* D816V allele burden during the follow-up of patients without cytoreductive treatment. The follow-up of *KIT* D816V allele burden (blue) and serum tryptase (black) in eight individual ISM patients (A-H) who had a stable clinical course and did not receive cytoreductive treatment is shown. As visible the allele burden as well as the serum tryptase levels remained within the same range during the observation period.

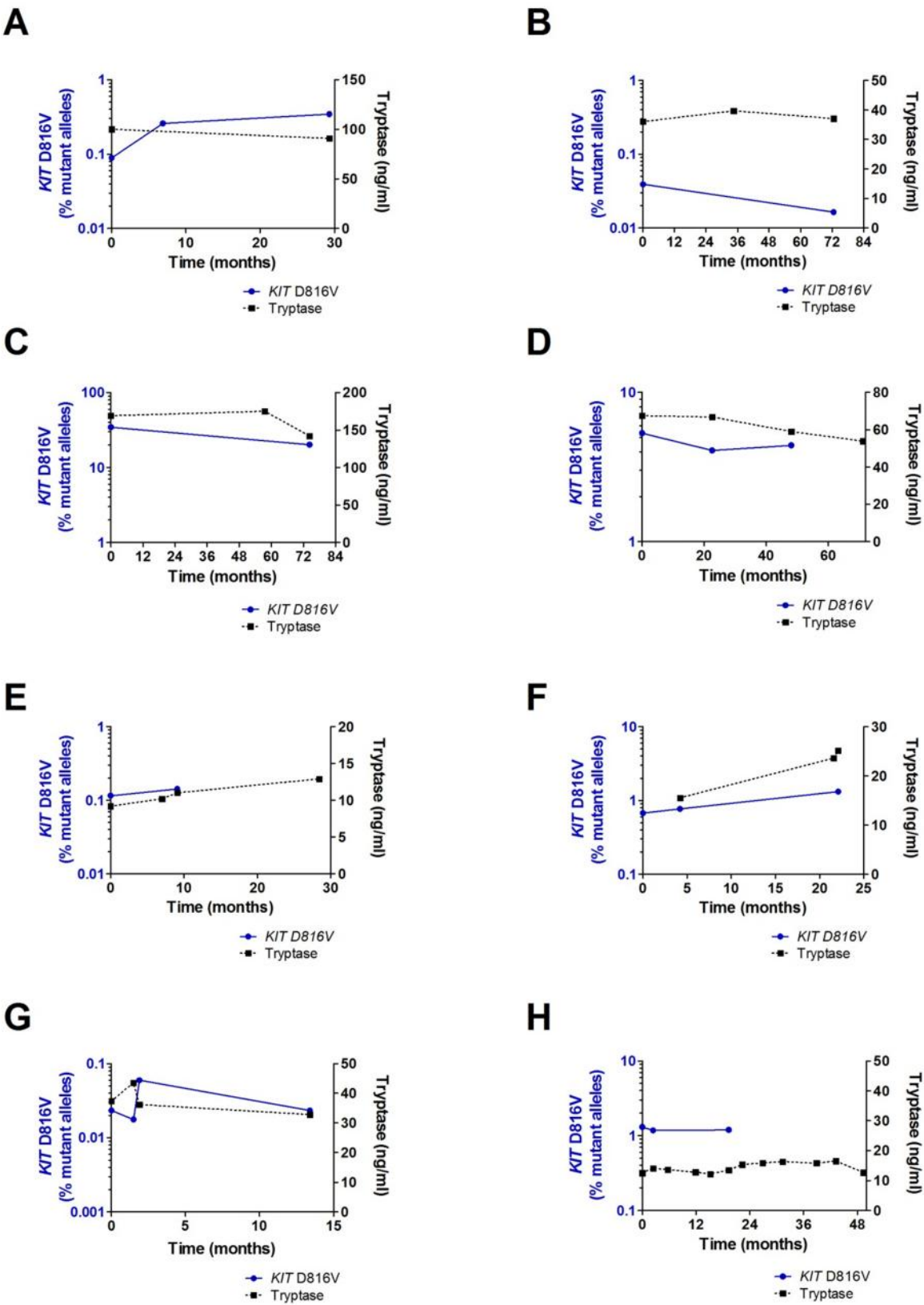
Fig. S4. KIT D816V allele burden during the follow-up of patients with cytoreductive treatment. The follow-up of *KIT* D816V allele burden (blue) and serum tryptase (black) in six individual patients with advanced SM (A-F) is shown. (A) ASM with response to treatment with interferon and cladribine (2-CDA) followed by an early relapse. (B) ASM with response to treatment with cladribine. (C) ASM with response to treatment with hydroxyurea. (D) SM-AHNMD (ASM-CMML) with response to interferon followed by developed a *KIT* D816V-negative secondary AML. (E) SM-AHNMD (ASM-CMML) responding to interferon followed by developed a *KIT* D816V-negative secondary AML. Polychemotherapy resulted in a regression of AML but a re-increase of the *KIT* mutation burden. (F) SM-AHNMD (ASM-CMML) with progression to secondary AML. The high allele burden (i.e. 50% of *KIT* D816V mutated alleles) argues for a *KIT* D816V-positive AML clone.



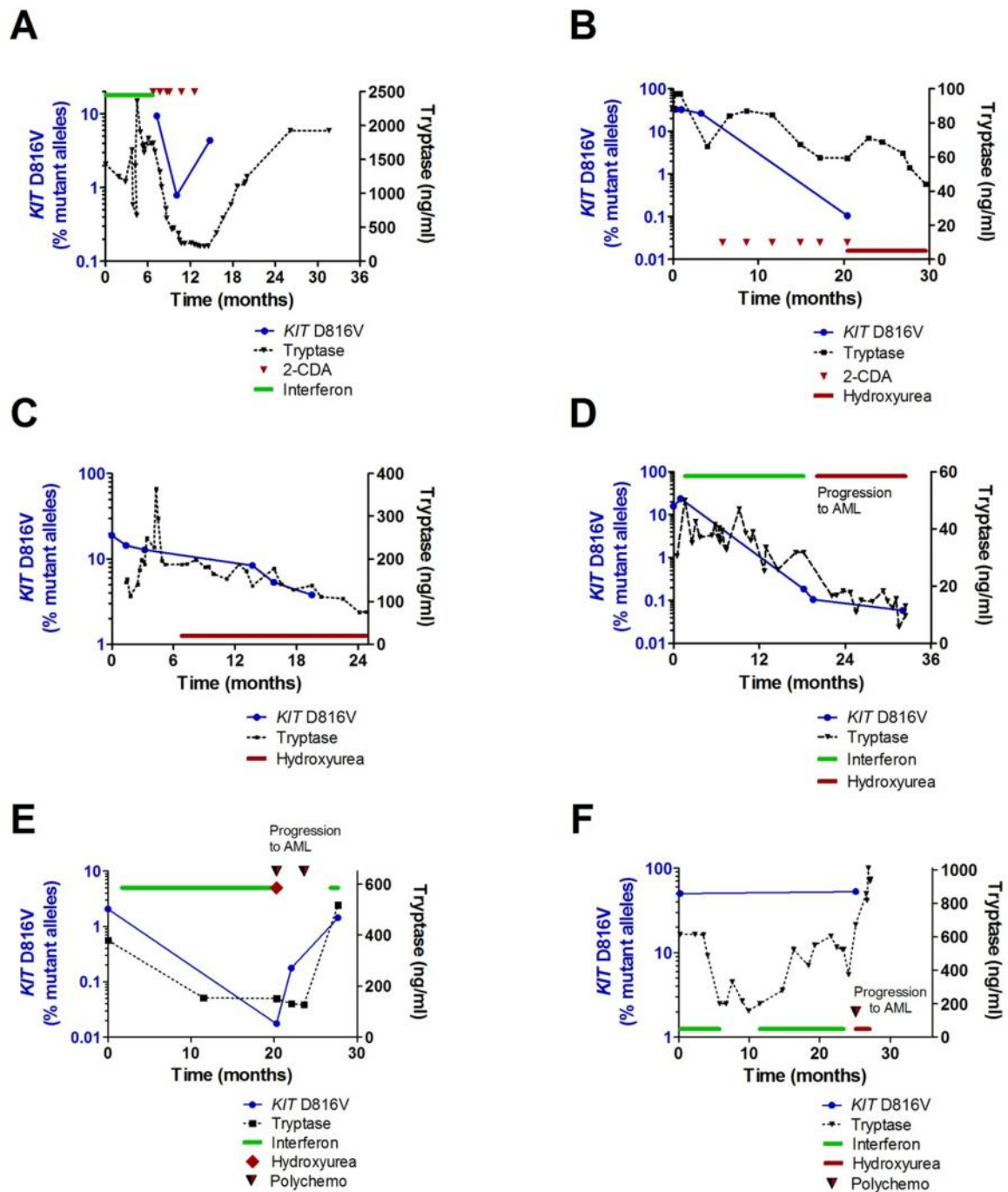
Hoermann et al, Fig. S1



Hoermann et al, Fig. S2



Hoermann et al, Fig. S3



Hoermann et al, Fig. S4