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

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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 among 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 ($P<0.03$). To conclude, the *KIT* D816V burden correlates with the variant of mastocytosis, predicts survival, and is a valuable follow-up parameter in SM.

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Author Contribution

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., E.H., P.V., and W.R.S. obtained and analyzed clinical data. G.H. and P.V. and W.R.S. designed the study and wrote the paper. All authors revised and approved the manuscript.

Conflict 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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Keywords

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

Introduction

The great majority of patients with systemic mastocytosis (SM) harbor the somatic point mutation *KIT* D816V (1–4). Kristensen et al. reported a highly sensitive quantitative real-time PCR (qPCR) for *KIT* D816V (5). 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 i.e. 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).

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 3.761% (range 0.083%-50.183%) in SM-AHNMD. 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 cut-off 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 ≥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 of 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 multi-lineage 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).

Since the *KIT* mutation D816V triggers autonomous KIT activation, we were interested whether mediator-related clinical symptoms would correlate with the *KIT* D816V mutant burden. However, we were unable to demonstrate such a correlation which also confirms results obtained by Broesby-Olsen et al (8) in a cohort including advanced SM. Together, these data suggest that mediator-related symptom occur 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 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 cut-off 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 cut-off 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 was 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

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disease progression a marked increase in *KIT* D816V was found. Patients responding to cytoreductive 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

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

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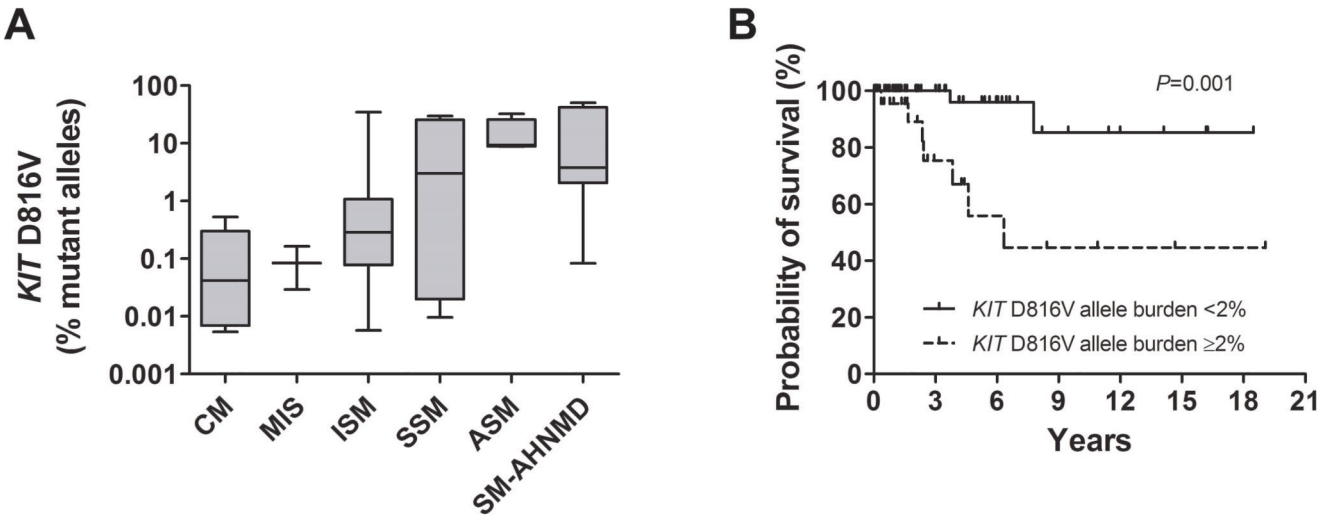

Fig. 1. *KIT* D816V allele burden in different WHO-subtype of mastocytosis and its impact on survival.

(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), mast cell leukemia (MCL), and systemic mastocytosis with an associated hematologic non-mast cell lineage disease (SM-AHNMD) ($P<0.001$, Kruskal Wallis test). (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 ≥ 2% at diagnosis. The difference in the probability of survival was significant ($P=0.001$).

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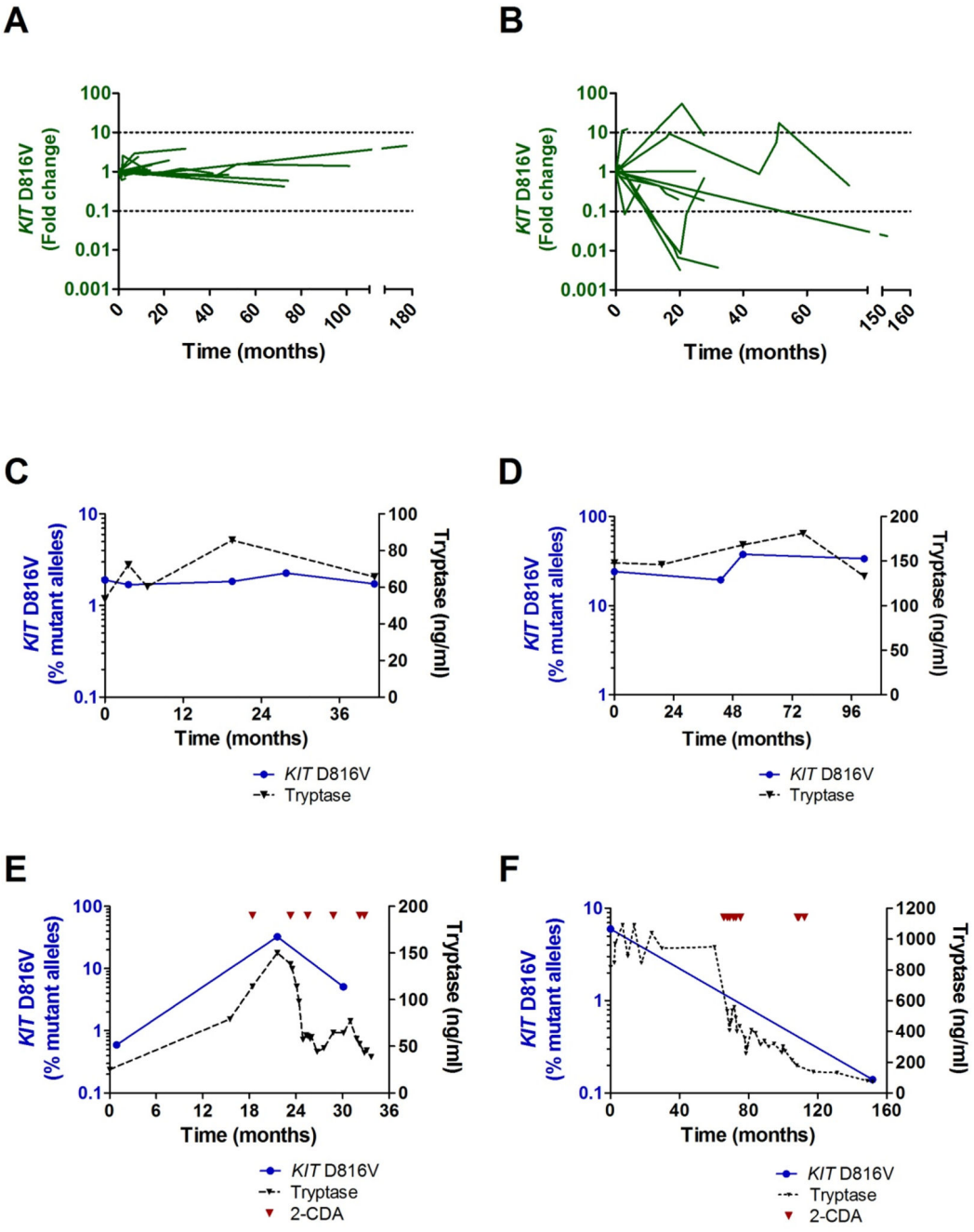

Fig. 2. *KIT* D816V allele burden during the follow-up.

Change in *KIT* D816V mutation positive allele fraction during the follow-up in patients without (A; *N*=19) and with cytoreductive 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 10 fold 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 cytoreductive therapy with cladribine (2-CDA) (E), and a patient with SSM receiving cytoreductive therapy due to recurrent episodes of life threatening vascular instability requiring epinephrine support (F).

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