



A Novel Mechanism of Action of Midostaurin in Systemic Mastocytosis: Beyond KIT Inhibition



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Introduction

Constitutively active KIT tyrosine kinase harboring the D816V mutation is the major driver of systemic mastocytosis (SM) pathogenesis. The loss of function of SETD2 histone methyltransferase due to reduced protein stability is a common cooperating event in advanced SM (advSM).

Objective

We aimed to dissect the mechanisms of action of midostaurin in advSM and to explore the potential of SETD2 as a marker of response to therapy.

Methods

The mast cell (MC) leukemia cell line HMC-1 (negative for SETD2) and primary neoplastic MCs from 10 advSM patients, collected at regular time points during midostaurin treatment, were used for Western blotting (WB) and for evaluation of KIT D816V allele burden (AB) by droplet digital PCR (ddPCR).

Results

We found that Aurora Kinase A (AKA) is overexpressed and hyper-activated in patients with advSM compared to patients with indolent systemic mastocytosis (ISM) and to a pool of healthy donors. AKA hyper-activation is correlated with SETD2 loss (Figure1).

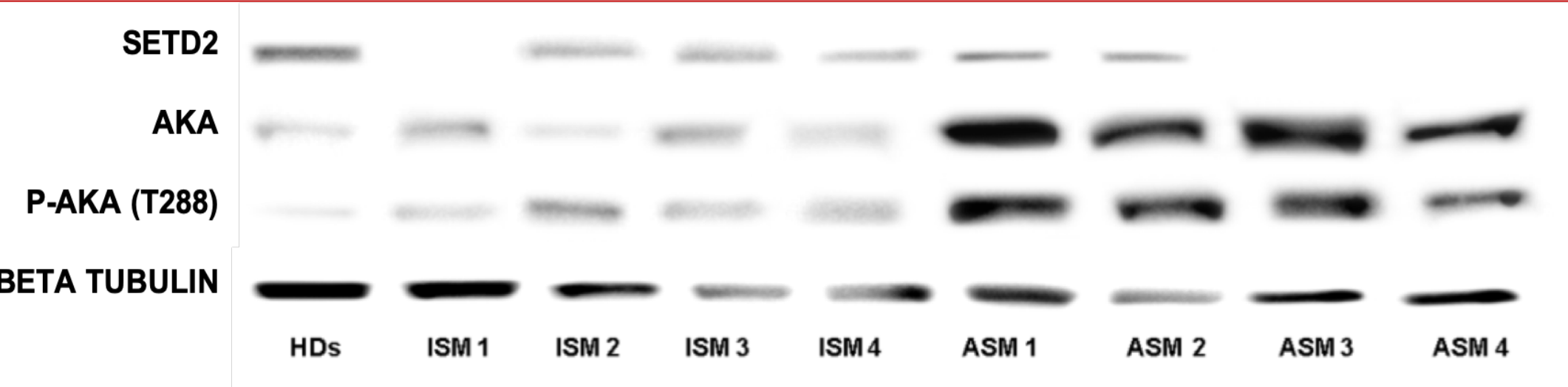


FIG.1

AKA inhibition by Danusertib rescued SETD2 expression and activity, raising the hypothesis that AKA might be implicated in SETD2 degradation (Figure 2).

The new standard of therapy in advSM is midostaurin that inhibits the activity of both wild-type and D816V mutant KIT, as well as of various other kinases including AKA. We found that midostaurin treatment (5 μ M) reduces AKA phosphorylation by 60%, partially restoring SETD2 expression and activity, in the HMC-1 cell line (Figure 3).

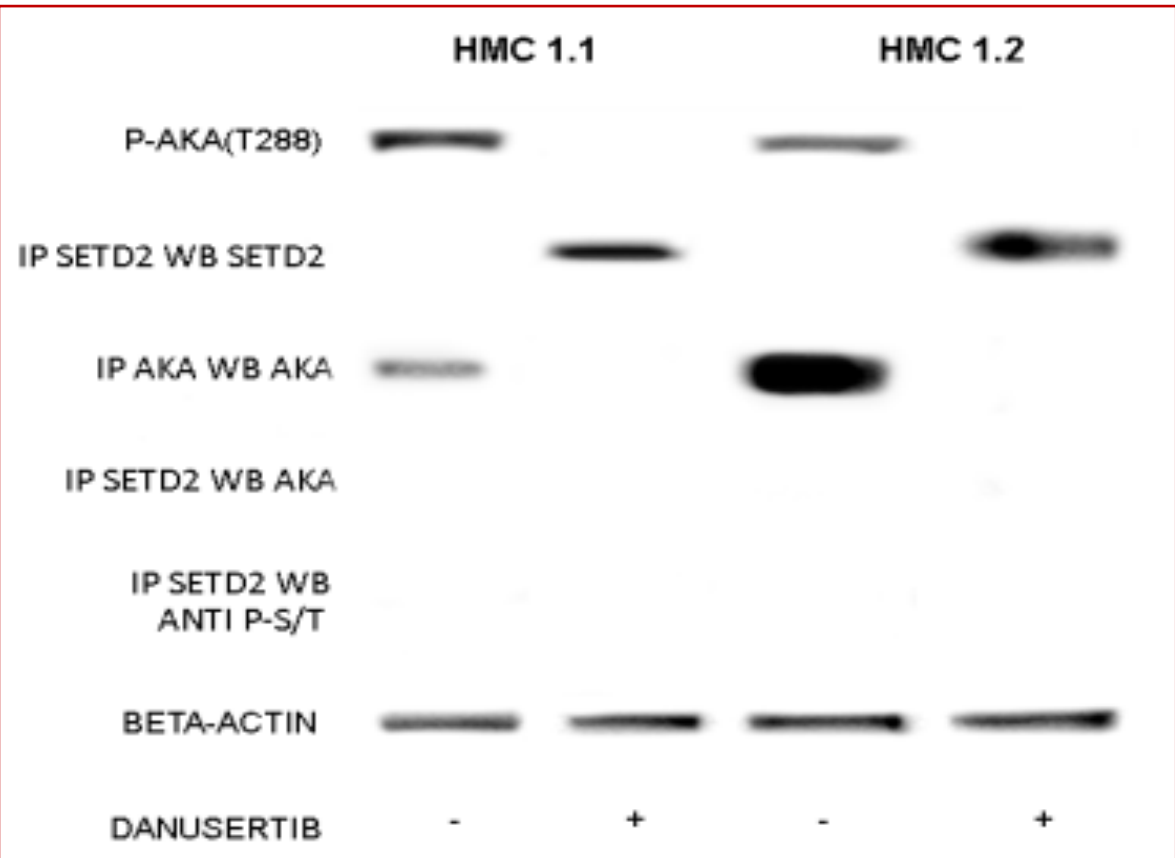


FIG.2

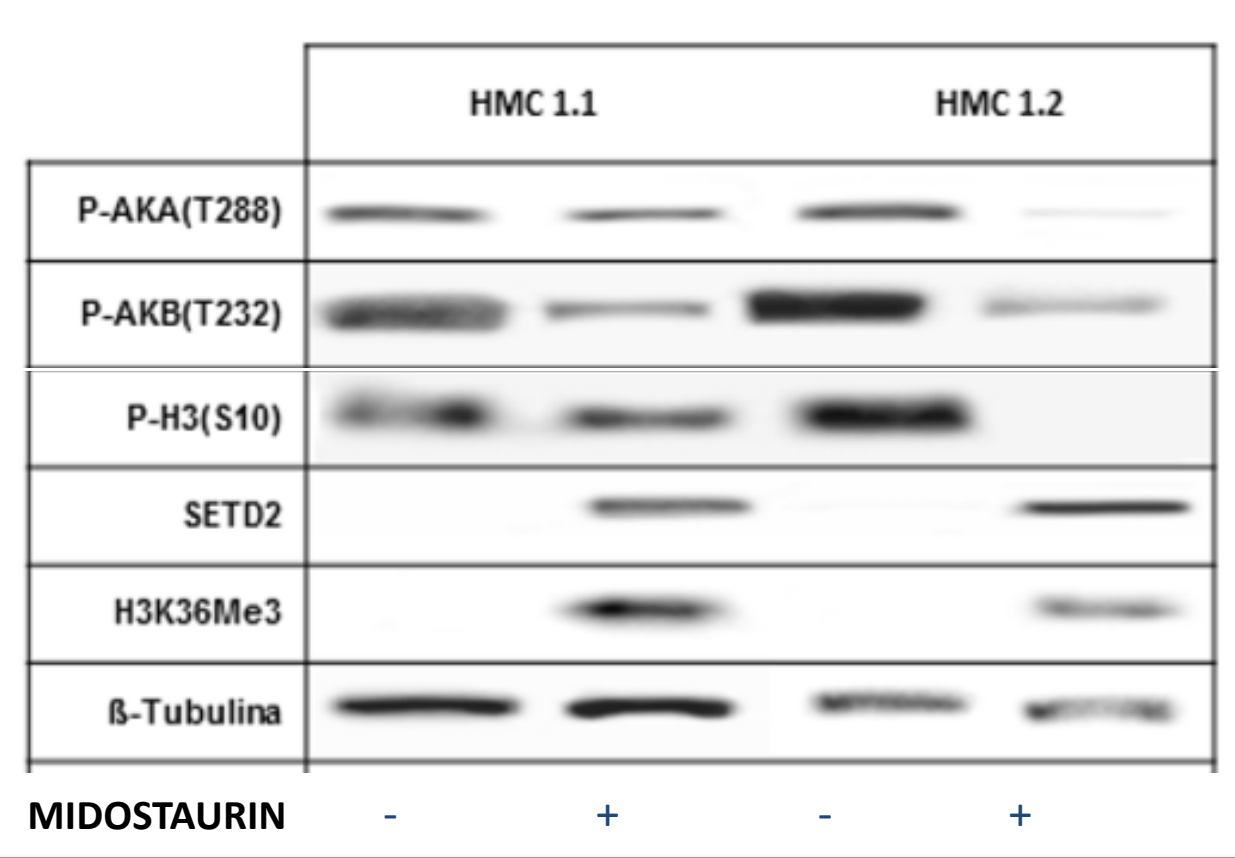


FIG.3

Our observations in cell lines were confirmed in neoplastic MCs collected from 8 patients with advSM, before and after midostaurin treatment (Figure 4).

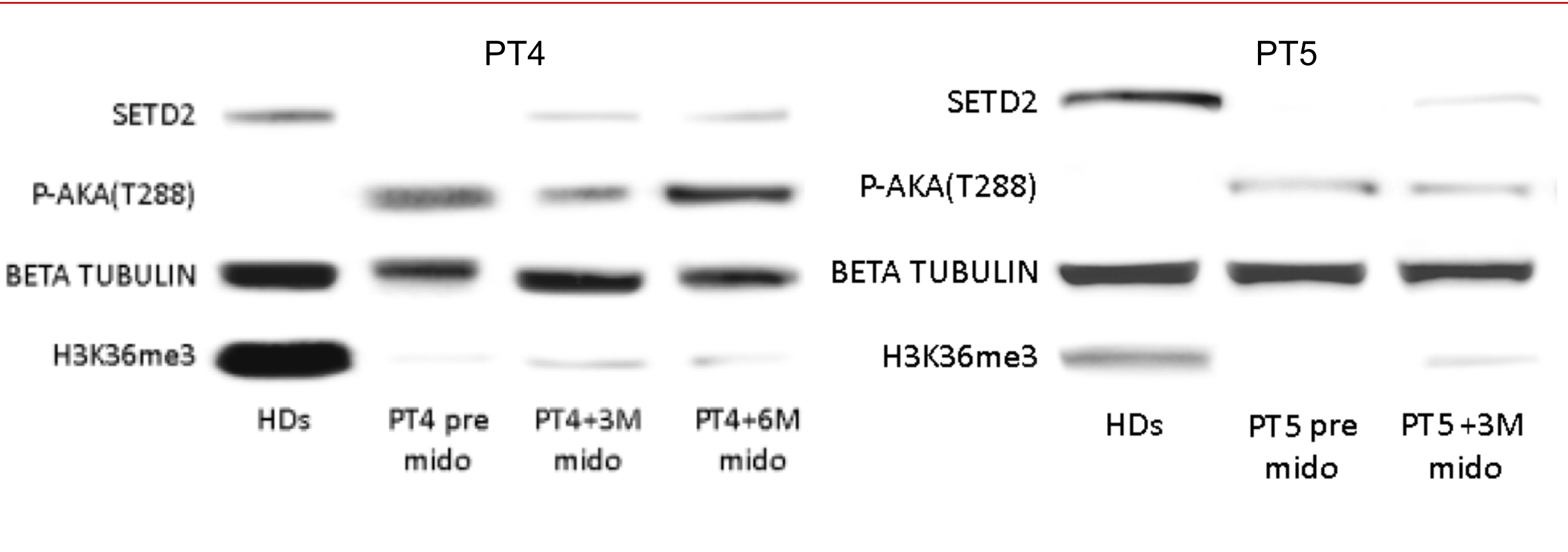


FIG.4

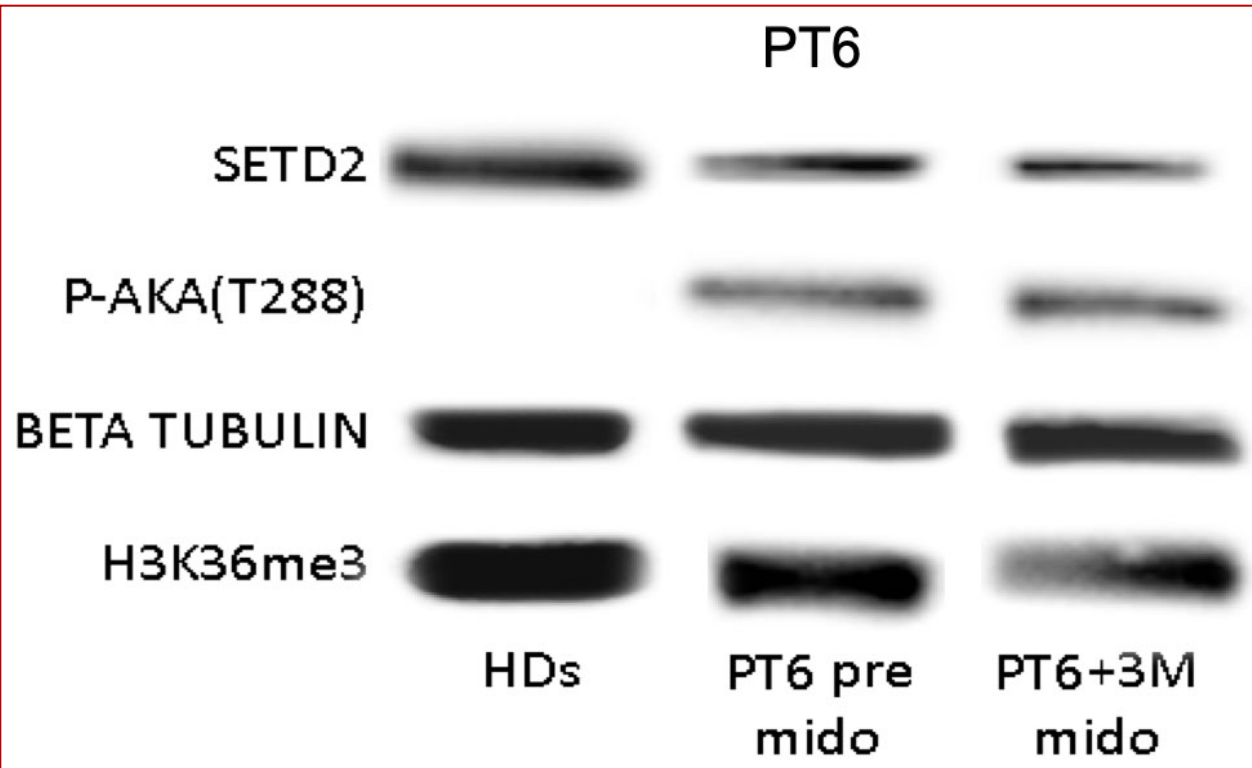
SETD2/H3K36me3 rescue correlated with clinical response to midostaurin and reduction of KIT D816V AB as assessed by digital PCR at 3 and 6 months of treatment (Figure 5).

PT4	PRE MIDOSTAURIN	+ 3M MIDOSTAURIN	+ 6M MIDOSTAURIN
KIT D816V Allele Burden	26,3 %	23,3 %	15,6 %
SETD2 levels	0,00	0,25	0,4
H3K36Me3 levels	0,01	0,04	0,03

PT5	PRE MIDOSTAURIN	+ 3M MIDOSTAURIN
KIT D816V Allele Burden	25,6 %	14,84 %
SETD2 levels	0,00	0,10
H3K36Me3 levels	0,00	0,10

FIG.5

In 2 refractory patients, no significant variations of the KIT allele burden or any increase in SETD2/H3K36Me3 levels were observed after midostaurin treatment (Figure 6).



PT6	PRE MIDOSTAURIN	+ 3M MIDOSTAURIN
KIT D816V Allele Burden	2,38 %	2,60 %
SETD2 levels	0,48	0,35
H3K36Me3 levels	0,85	0,69

FIG.6

Conclusions

- Midostaurin treatment inhibits AKA with consequent rescue of SETD2 expression and function *in vitro* and *in vivo*.
- SETD2/H3K36me3 deficiency by WB may serve as a biomarker of response to midostaurin.