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Letter to the Editor

Dasatinib reduces myelofibrosis by modulating pSTAT5 and NF-κB

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Myelofibrosis (MF) of the bone marrow (BM) can emerge de novo (primary myelofibrosis or PMF) or subsequent to a preceding myeloproliferative neoplasm. In chronic myeloid leukemia (CML), MF appears in 10% of cases at the time of diagnosis and is associated with poor outcome due to progressive BM failure [1]. So far, the only curative treatment option for this patient group is an allogeneic hematopoietic stem cell transplantation (HSCT) following the initial tyrosine kinase inhibitor (TKI) treatment. However, HSCT is associated with a high risk of morbidity and mortality and therefore limited to selected patients. For this, other treatment options are needed to reduce the risk of progression of MF in CML. Our group recently described the first report of a CML patient showing a significant reduction from MF-3 to MF-0/MF-1 with complete molecular response and marked regression of spleen size 28 months after starting dasatinib reduced-dose treatment (80 mg). Thus, an allogeneic HSCT could be avoided [2].

In this follow up study we now discuss immunohistochemical and western blot findings to further explore the underlying mechanism of dasatinib induced reduction of MF in this CML patient. Since MF is caused by activating mutations which imitate the effect of hematopoietic cytokines by prompting signalling via the JAK/STAT pathway, inhibition of JAK/STAT is critical for MF treatment [3]. Although disappearance of both BCR-ABL and JAK2 mutation was reported in one patient during dasatinib treatment [4], blocking this pathway by dasatinib has not been evidenced until now. Even though a JAK2 mutation was not present in our patient, the STAT pathway remains a promising target as new insights into molecular genetics of CML demonstrated that BCR-ABL can uncouple the canonical JAK2/STAT5 pathway, suggesting direct phosphorylation of STAT5 by BCR-ABL [5]. Indeed, dasatinib was shown to enhance apoptosis of K562 cells (CML cell line) by downregulation of STAT5 expression [6]. Another possible underlying pathway regarding MF regression in our patient could be the inhibition of NF-κB signalling. This pathway was recently shown to be abnormally activated in MF leading to enhanced release of transforming growth factor-beta1 (TGF-β1) in the BM microenvironment, one of the main mechanisms causing MF [7].

After informed consent was obtained from the patient, 3 μm paraffin-embedded BM sections were prepared from the time of diagnosis, the first evidence of MF reduction and the time of complete hematopoietic reconstitution and examined for phosphorylated STAT5 (pSTAT5) and NF-κB p65 subunit (p65) expression. Sections were dewaxed and antigen retrieval was done by heating in 10 mM citrate puffer (pH 6.0). Endogenous peroxidase was quenched by a 3% solution of hydrogen peroxide. Sections were then stained with anti-pSTAT5 respectively anti-p65, as well as anti-mouse isotype antibody. Incubation was made over night at 4 °C. Visualization was conducted using the universal immune-peroxidase polymer MAX-PO and Bright-DAB and sections were counterstained with Mayer's hemalum solution. Immunohistochemical analysis revealed a significant reduction of pSTAT5 and p65 expression during dasatinib treatment (01/2012–07/2014), thus implicating an involvement of the JAK-STAT5 pathway as well as the NF-κB signalling system. However, this effect was delayed and not detected at the first evidence of histological MF regression [2] (Fig. 1).

In general, two main reasons for MF regression in the course of dasatinib therapy need to be debated. One possibility is that the first event concerns remission of CML by TKI treatment, leading to a secondary reduction of MF (indirect effect). The second option is that, independent of BCR-ABL inhibition, dasatinib is able to induce MF regression directly. To further evaluate this aspect, we performed western blot analysis of BCR-ABL$^+$K562 cell and BCR-ABL$^-$L540 cell lysates 15 min, 30 min, 2, 3, 6, 12 and 24 h after application of 3,3 nM dasatinib, using antibodies against pSTAT5 and inhibitor of NF-κB (IκBa). Lysates from cells cultured in medium alone without adding dasatinib but respective amounts of dasatinib solvent (DMSO) served as controls and were collected after 2 h and 24 h.

As shown in Fig. 2, dasatinib reduced pSTAT5 expression and increased IκBa expression in both cell lines, however, in a different manner. In BCR-ABL$^+$K562 cells the effect could be demonstrated already after 15 min, lasting at least 24 h for pSTAT5 and only 2 h for IκBa. In BCR-ABL$^-$L540 cells the effect of dasatinib on STAT5 and NF-κB signalling was detected after 2 h, persisting until 24 h for pSTAT5 and 6 h for IκBa. These findings suggest that dasatinib is able to reduce MF, influencing different pathways in both, BCR-ABL dependent and independent conditions, but with a varied velocity pattern. Considering the duration of the effect, the STAT5 pathway seems to be predominantly involved.

In conclusion, we display here the first investigation of the mechanisms involved in TKI-induced reduction of myelofibrosis, showing in vivo (one case) and in vitro (cell lines) BCR-ABL dependent but also independent involvement of STAT5 and NF-κB pathway during dasatinib treatment.

These new findings should be the motivation to further evaluate the detailed mode of action how TKIs reduce MF. Thus, the molecular background of already described beneficial effects of dasatinib in PMF cases [8], could be established.

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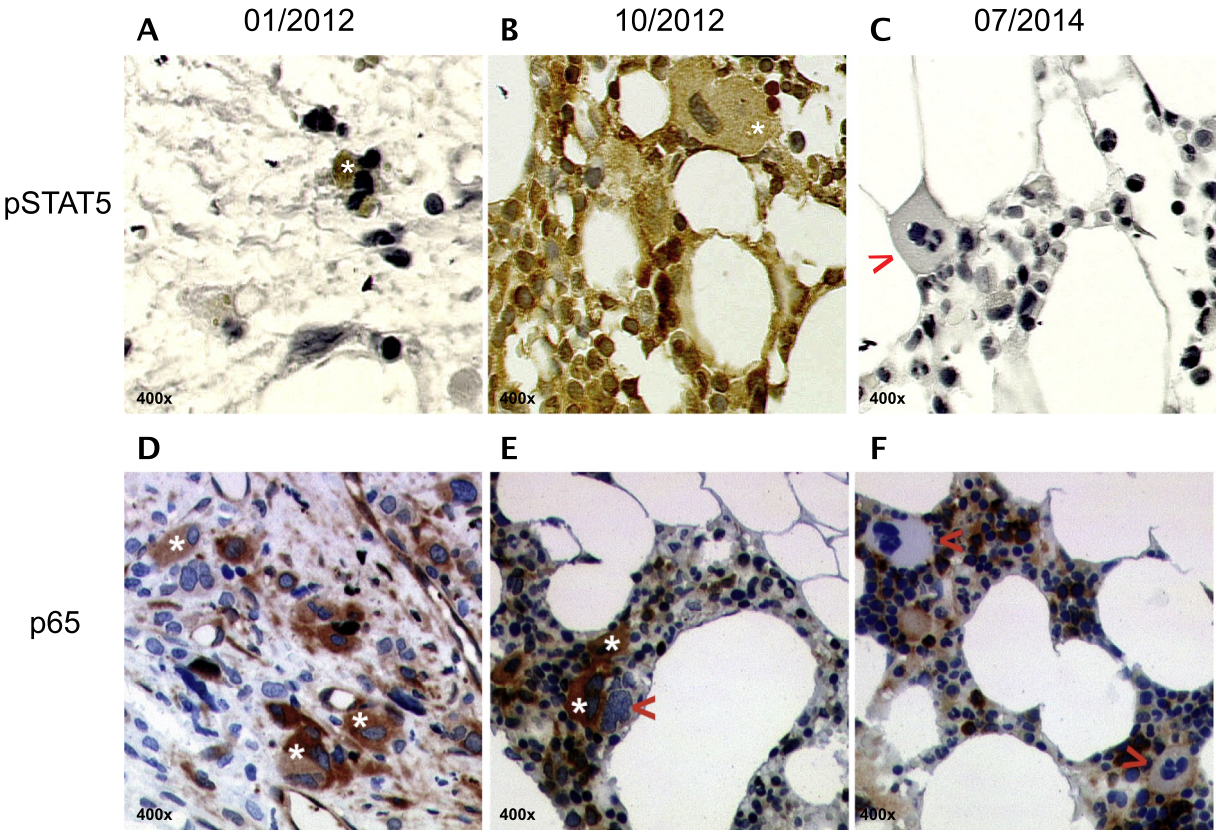

Fig. 1. In vivo STAT5 and NF-κB staining during dasatinib treatment in one patient with chronic myelogenous leukemia (CML) and myelofibrosis (MF). Immunohistochemistry analysis of bone marrow biopsy specimens from the time of diagnosis (01/2012), first evidence of MF reduction (10/2012) and complete hematopoietic reconstitution (07/2014) showing pSTAT5 (A–C) and NF-κB p65 subunit (D–F) expression. Red arrow: megakaryocytes with low/absent expression; Asterix: megakaryocytes with high expression (magnification 400 ×). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

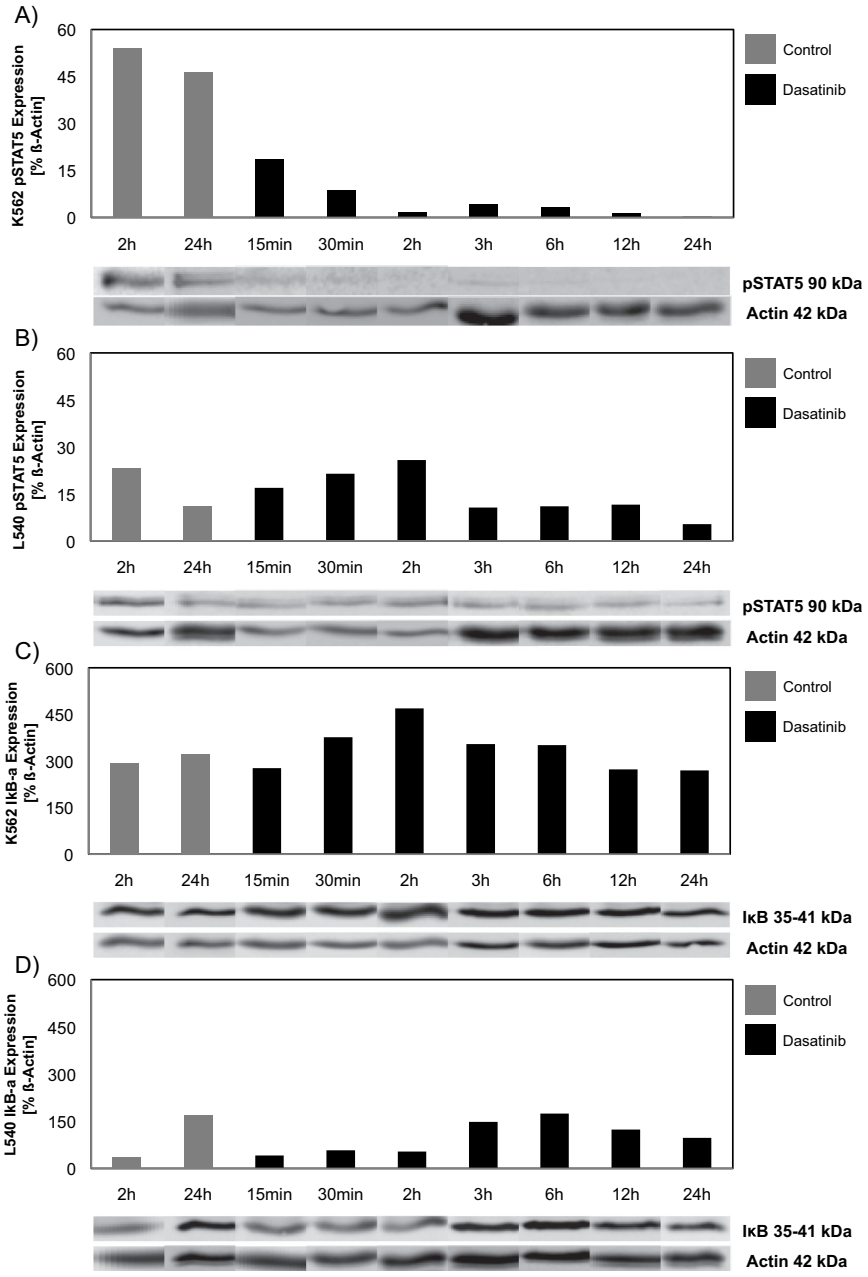

Fig. 2. In vitro direct an BCR-ABL independent expression of STAT5 and NF-κB protein during dasatinib treatment. Immunoblot analysis of pSTAT5 in K562 (A) and L540 (B) cells presented as percentage of pSTAT5 expression related to β-Actin. Immunoblot analysis of IκBa in K562 (C) and L540 (D) cells. Percentage of IκBa Expression related to β-Actin. Measurement intervals: 15 min, 30 min, 2 h, 3 h, 6 h, 12 h and 24 h after addition of dasatinib (black). Control: dimethyl sulfoxide (DMSO) medium, (grey; measurement after 2 h, 24 h).

Conflict of interest

The authors declare no conflict of interest.

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