# Pathogenesis of Tyrosine Kinase Inhibitors (TKIs) Associated Vascular Toxicity in Chronic Myeloid Leukemia (CML)

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## Background

- Evidence has emerged demonstrating an association between treatment with the TKIs nilotinib, ponatinib and dasatinib (but not imatinib or bosutinib) and vascular adverse events (VAEs) in CML.
- We used an *in-vitro* endothelial cell (HUVEC) model and an *in-vivo* hindlimb ischemia mouse model to study TKI-associated VAEs.

## Results

- Ponatinib significantly abrogated HUVEC tube formation (50-87% reduction in tube & junction number and segment length. 255% increase in isolated segments) (Fig. 1). Ponatinib's suppressive effect was partially rescued by VEGFR2 overexpression, a known ponatinib off-target.
- Dasatinib significantly abrogated HUVEC tube formation (88-97% reduction in tube & junction number and segment length. 300% increase in isolated segments) (Fig. 1).
- Imatinib, nilotinib and bosutinib did not affect HUVEC tube formation (Fig. 1).
- Ponatinib increased HUVEC apoptosis by 97% and inhibited their VEGF-induced migration by 35% (not shown).
- HUVECs treated with ponatinib, dasatinib and nilotinib revealed a differential angiogenesis-related gene expression pattern (Fig. 2).
- In our *in-vivo* model femoral artery was excised to induce ischemia and stimulate angiogenesis. Mice were treated with clinically relevant doses of TKIs and perfusion was measured via Doppler imager for 28 days.
- Dasatinib-treated mice showed a significantly delayed recovery (Fig. 3) and a significant increase in toe necrosis (Table 1).
- Imatinib- and bosutinib-treated mice showed a similar recovery pattern to that of control mice (Fig. 3).
- The recovery pattern of ponatinib-treated mice was also similar to control mice, differing from the anti-angiogenic effect that was observed in-vitro (Fig. 3).

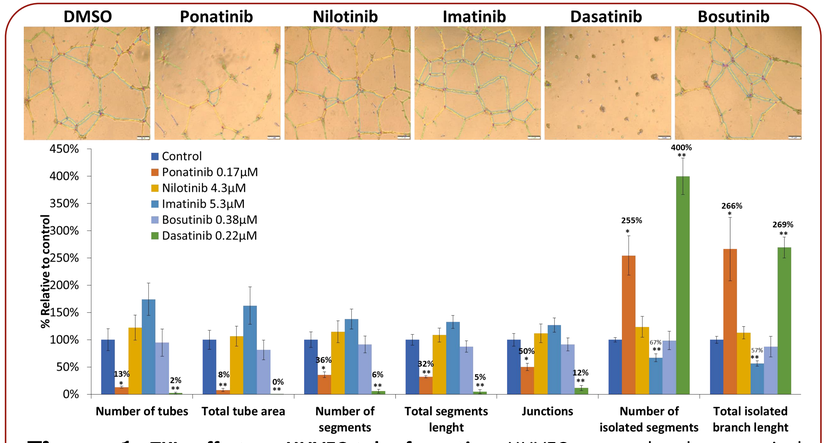



**Figure 1. TKIs effect on HUVEC tube formation**. HUVECs were plated on matrigel-coated plates w/wo TKIs. Tube formation was evaluated after 24h by light microscopy. Shown are representative fields (X4 mag.) of phase-contrast analyzed with Angiogenesis Analyzer software. The graph represents quantification of 3 experiments ± SEM using % for each treatment relative to control. *p≤.05, **p≤.01 (compared to control).

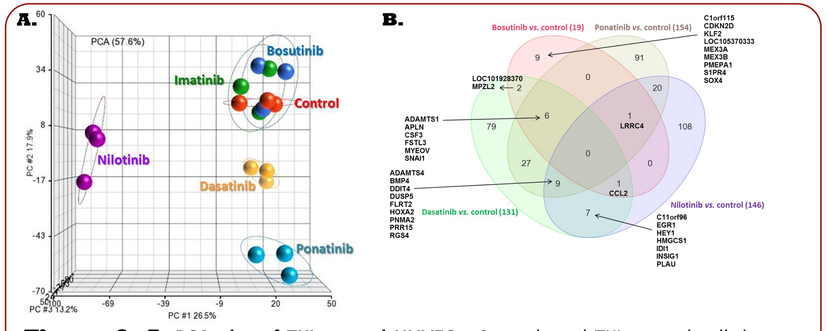



**Figure 2. A. PCA plot of TKI-treated HUVECs.** Control- and TKI-treated cell datasets were analysed to identify homogeneity between samples. Each group is represented in 3 replicates. **B.** Venn diagram of the differentially expressed (DE) genes.

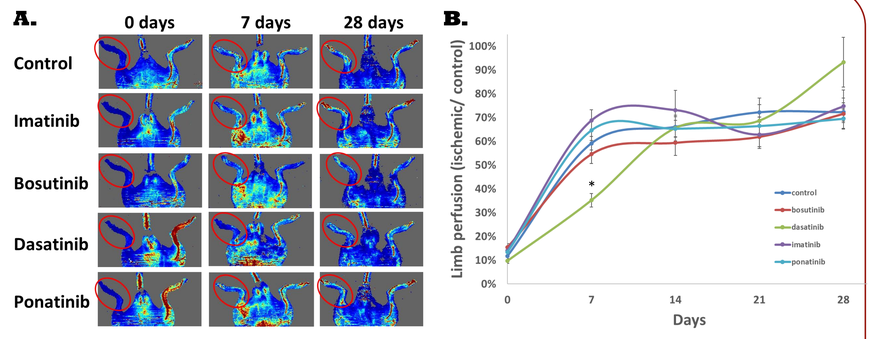



**Figure 3. Blood perfusion of TKI-treated ischemic mice.** Following ischemia, mice were treated daily with the relevant TKI. Perfusion was measured with a Doppler imager once a week for 4 weeks. **A.** Representative Doppler images. Control hindlimb on the right, ischemic hindlimb on the left (red circle). **B.** Perfusion expressed as mean±SEM analyzed by single ANOVA followed by TUKEY post-hoc analysis. *p<0.05 in dasatinib compared to all treatments.

**Table 1.** Toe necrosis in control- and TKI-treated mice

| Treatment | # of mice with necrosis |
|---|---|
| Control (n=30) | 2 (6.7%) |
| Ponatinib (n=30) | 3 (10%) |
| Imatinib (n=27) | 4 (14.8%) |
| Dasatinib (n=31) | 9 (29%) |
| Bosutinib (n=33) | 1 (3%) |

## Discussion

- The *in-vivo* and *in-vitro* results of dasatinib treatment are in line with its clinical VAE profile, implicating endothelial toxicity related to dasatinib and supporting a direct effect of dasatinib on vascular pathogenesis.
- Understanding TKI-induced VAEs pathogenesis could contribute to future development of safer next-generation TKIs and could potentially influence TKI-personalized tailoring for CML patients.

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