

Neutrophil extracellular traps are increased in chronic myeloid leukemia and are differentially affected by tyrosine kinase inhibitors

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Background

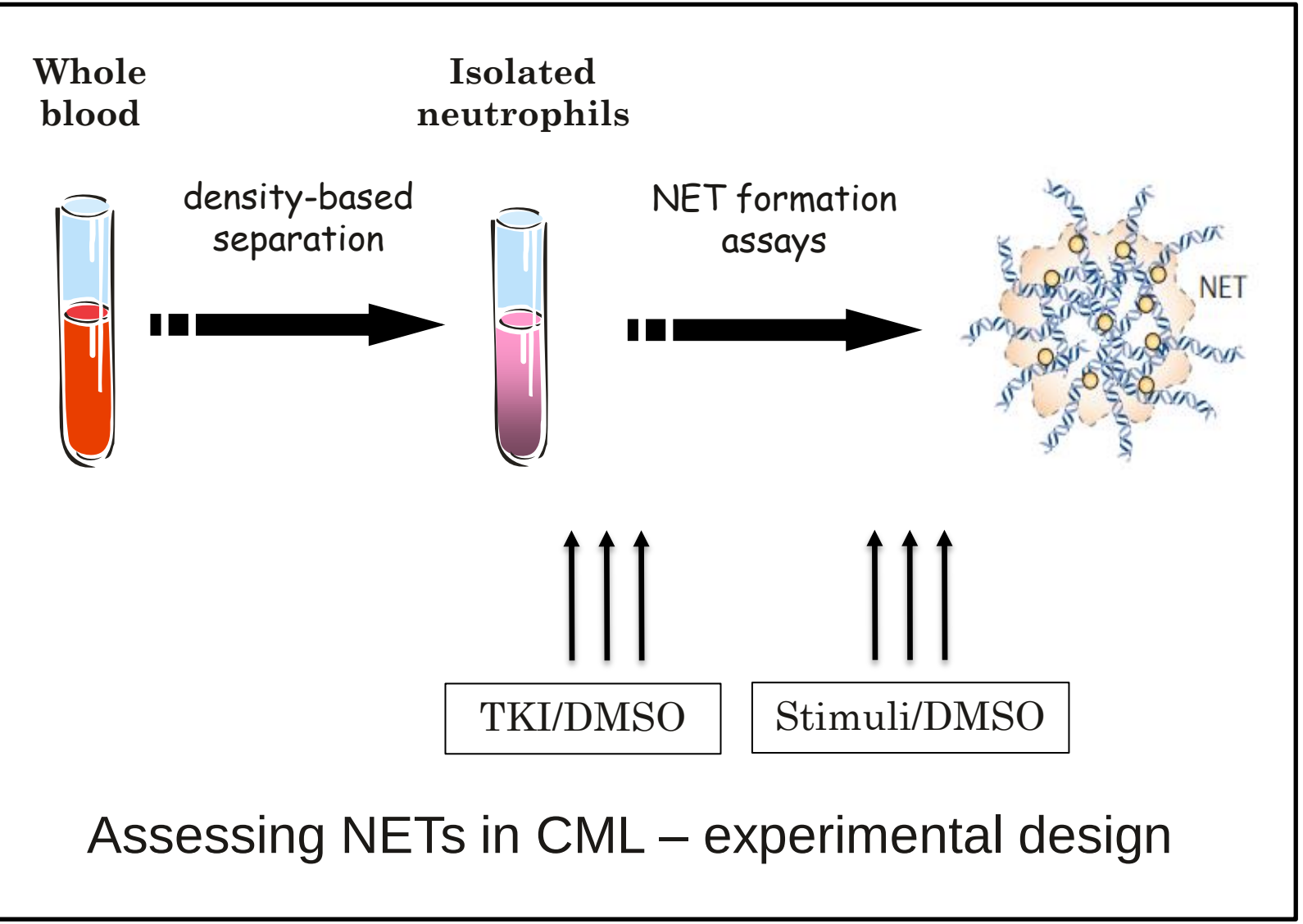
- Cardiovascular (CVS) complications are increasingly reported with the use of certain tyrosine kinase inhibitors (TKIs) to treat Chronic Myeloid Leukemia (CML) such as ponatinib and nilotinib.
- The reasons underlying these associations are not fully understood.
- In response to stimuli, neutrophils expel extracellular strands of decondensed DNA complexed with histones, myeloperoxidase (MPO) and other neutrophil granular proteins.
- Neutrophil extracellular traps (NETs) are able to ensnare and kill microbes but are also implicated in the pathogenesis of autoimmunity and thrombosis.
- Increased NET formation has previously been implicated in cancer-associated thrombosis.

Objective

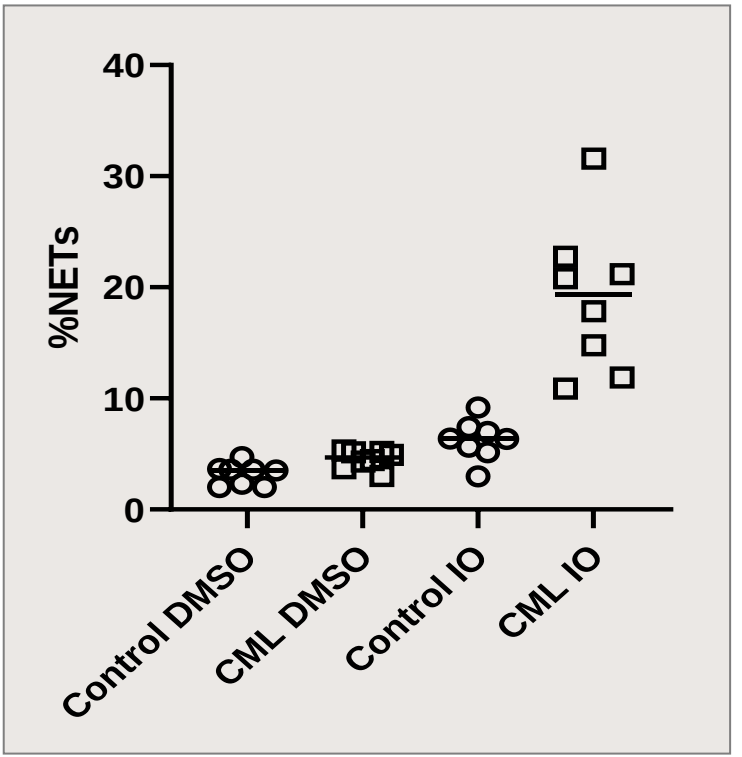
- To assess NET formation in neutrophils derived from patients with CML.
- To characterize the role of reactive oxygen species (ROS)/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase axis and of peptidylarginine deiminase 4 (PAD4) in NET formation in CML.
- To assess the effects of TKIs on NET formation in CML

Methods

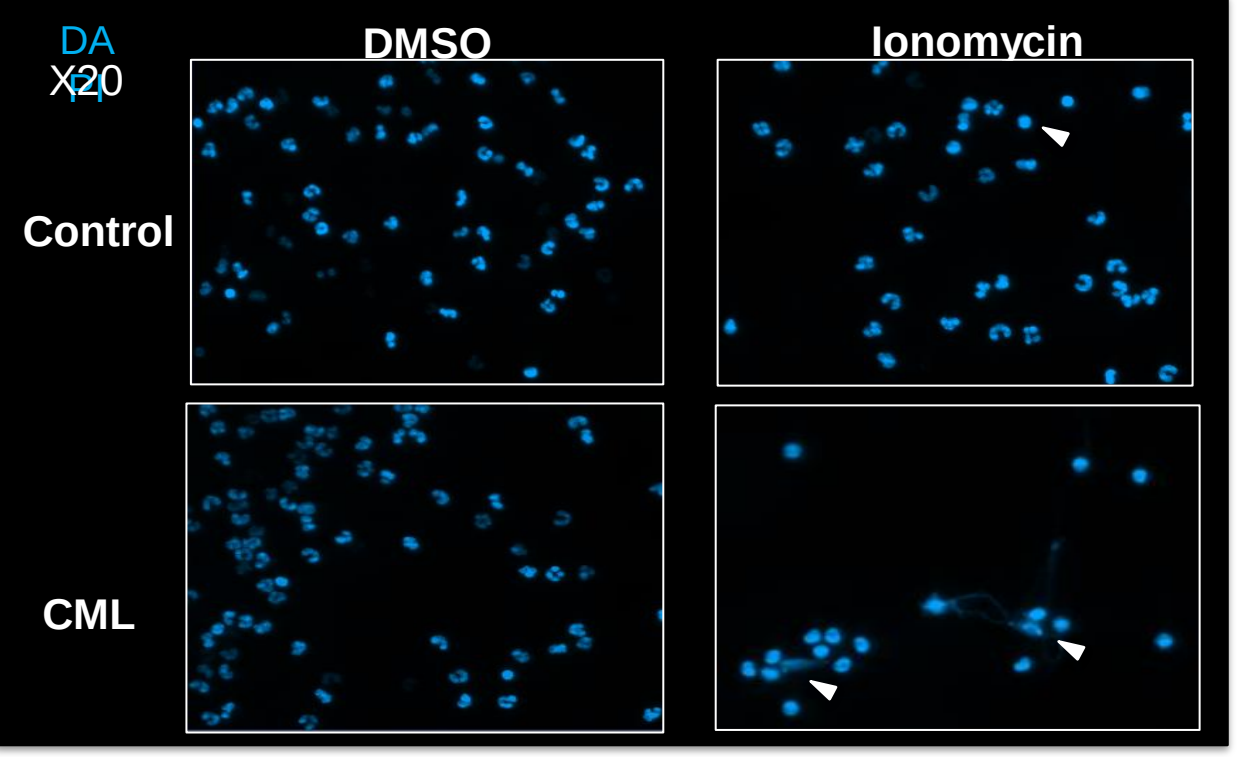
- Blood samples were obtained from patients with CML and form age/gender-matched controls.
- ER-*HoxB8* conditionally immortalized murine myeloid progenitor cell line was differentiated into functional neutrophils as previously described.
- BCR-ABL1* oncogene (or empty vector control) was transduced into ER-*HoxB8* cells using MSCV-(pBabe mcs)-human p210*BCR-ABL*-IRES-GFP-pcDNA.
- Isolated human neutrophils and differentiated ER-*HoxB8* neutrophils were incubated in the presence of 5.3 μ M imatinib, 0.17 μ M ponatinib and 0.22 μ M dasatinib for 4 hrs.
- Neutrophils were stimulated with phorbol 12-myristate 13-acetate (PMA) or 5 μ M ionomycin (IO) and NET formation was assessed by morphology, immunofluorescence (IF), the expression of NET-associated elastase and the expression of NET associated proteins.



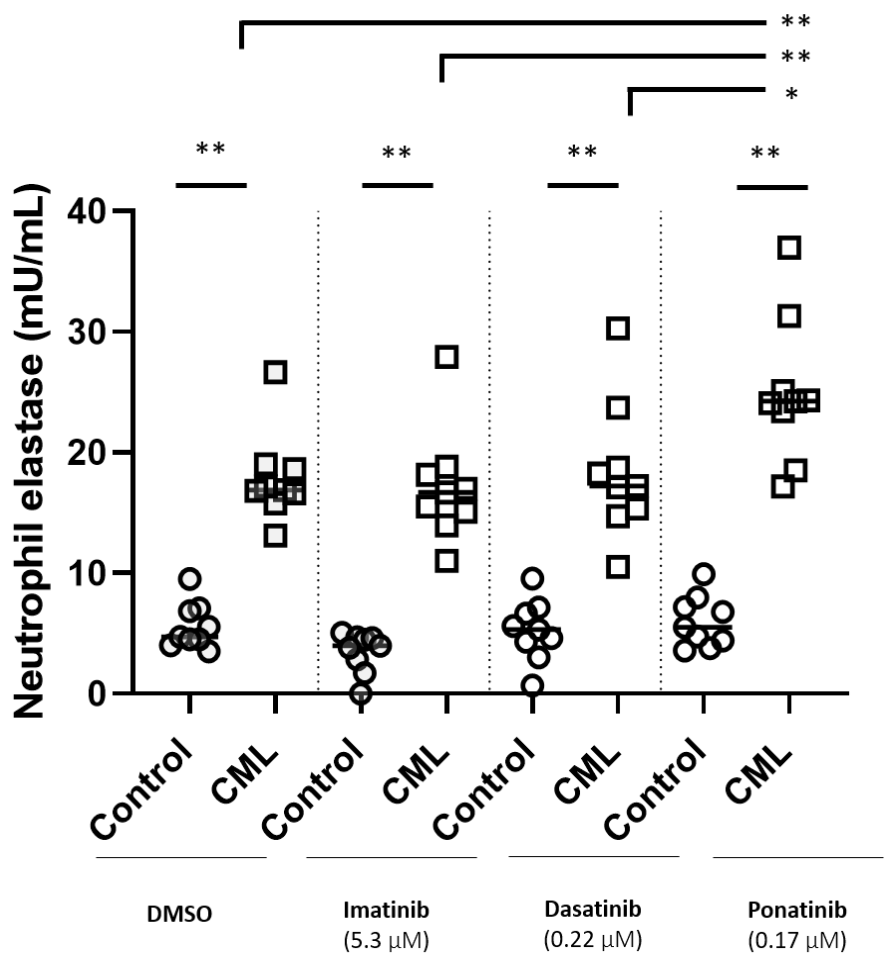
Results



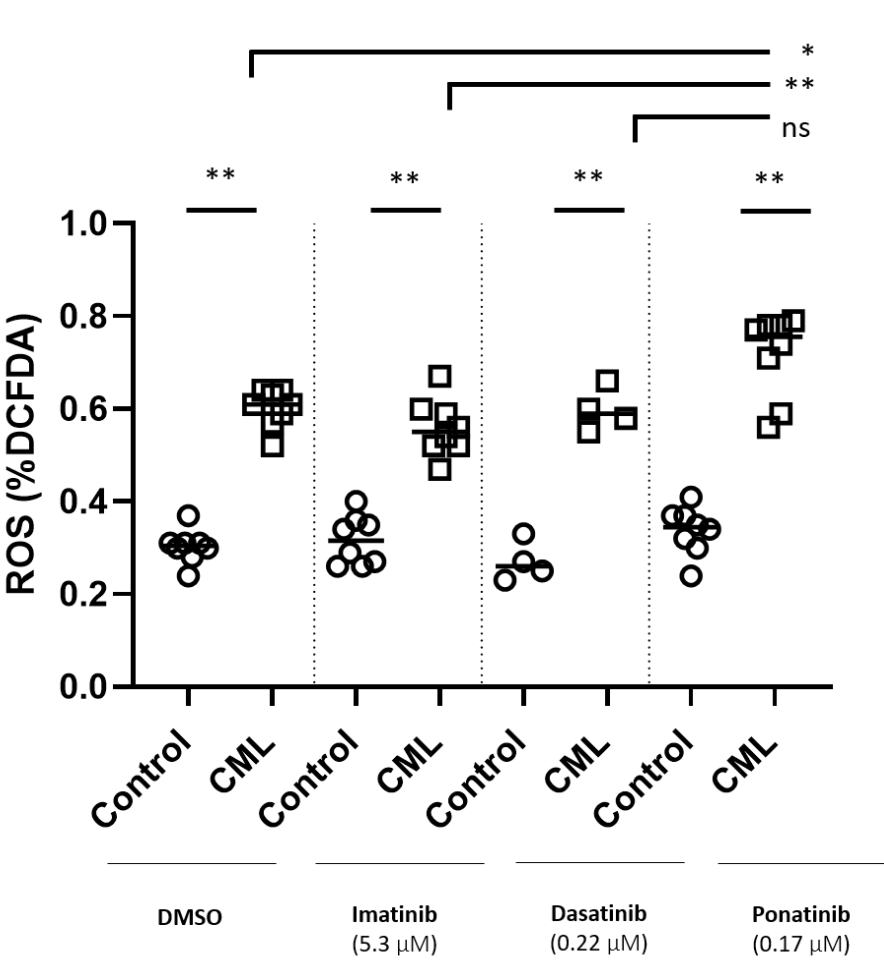
NET formation in patients with newly diagnosed CML compared to controls. Stimulated with 4 μ M IO/DMSO for 2 h. n=8 for each group.



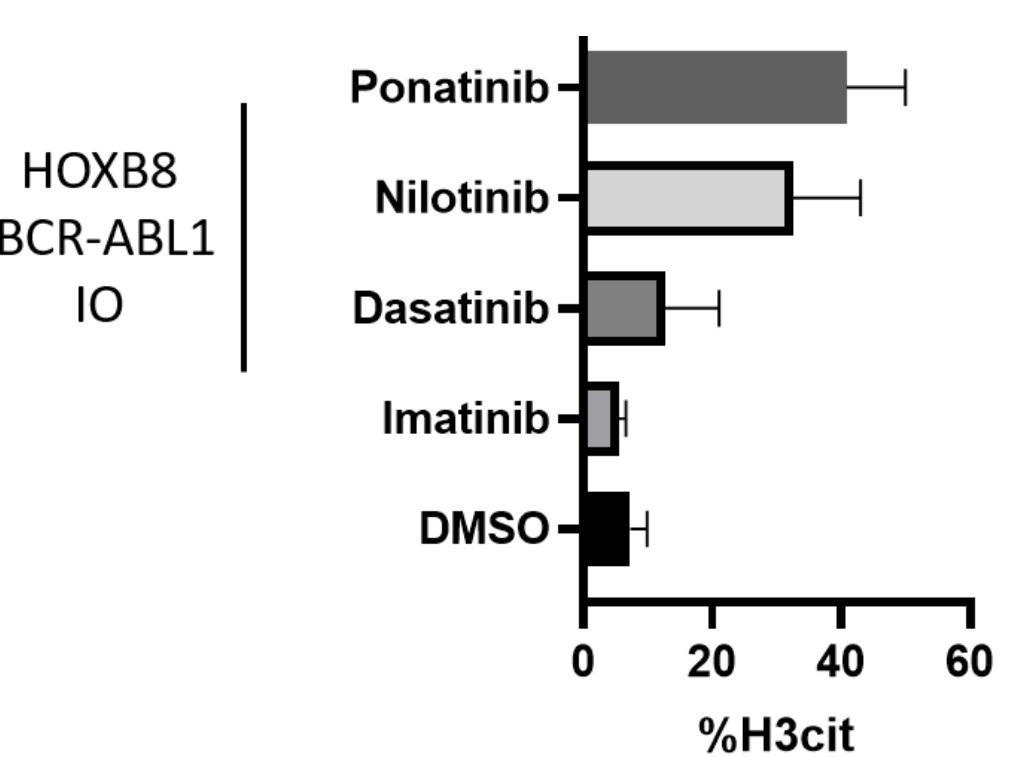
Representative IF images of neutrophils derived from patients with CML and controls DAPI is shown in blue. White arrowheads denote NET-associated morphologic changes.



Neutrophils from newly diagnosed CML patients and controls (n=9 for each group) were treated *ex-vivo* with various TKIs. Left: NET formation expressed as the amount of NET-bound elastase. Right: ROS levels measured by DCFDA-assay *p<0.05 **p<0.005.



Summary



Percent of H3cit in *HoxB8-BCR-ABL1* cells and *HoxB8* cells transduced with an empty vector stimulated with 4 μ M IO or DMSO and exposed to various TKIs. *p<0.05; ns – non-significant.

Discussion

- CML is associated with increased NET formation which is further augmented by ponatinib and nilotinib, suggesting a possible role for NETs in promoting vascular toxicity in CML.
- In the *HoxB8-BCR-ABL1* model, NET formation is PAD4, but not ROS dependent.

References

- Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014;123(18):2768-2776.
- Demers M, Krause DS, Schatzberg D, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. *Proc Natl Acad Sci U S A*. 2012;109(32):13076-13081.
- Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med*. 2018;10(436).