Immunological Effects of Ponatinib Therapy in Patients with Chronic Myeloid Leukaemia

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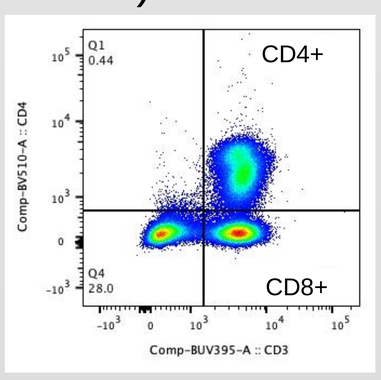
Introduction:

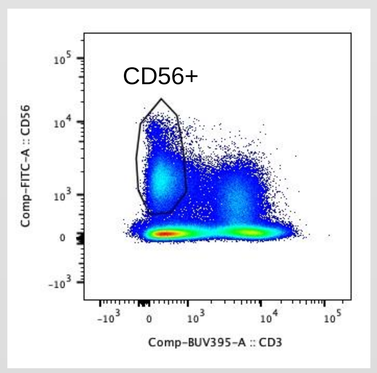
Ponatinib is the most potent tyrosine kinase inhibitor (TKI) currently available and retains activity against all currently recognised ABL1 kinase domain mutations. Ponatinib displays moderate SRC kinase inhibitory activity, with an IC50 of 5.4 nmol/L (O'Hare, Cancer Cell, 2009), however its potential immunosuppressive effect has not been studied.

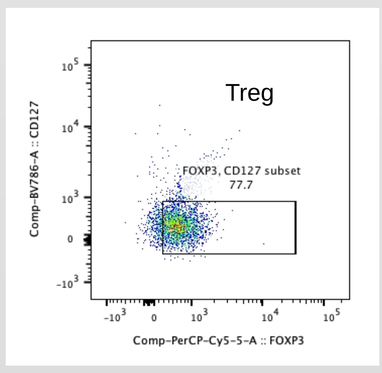
The SRC family kinase LCK plays a critical role in signalling from the T cell receptor (TCR) with immediate downstream targets including ZAP70 and LAT. NK cells also possess an abundance of SRC family kinases which play a pivotal role in signalling from activating NK cell receptors. We analysed immune cell function in a cohort of patients treated with this third generation TKI.

Methods:

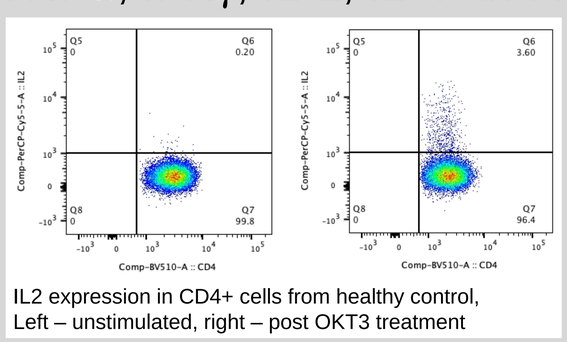
We performed a two-phase functional analysis of the immunomodulatory effects of ponatinib in 4 patients and 3 healthy controls. We performed phosphoflow cytometry in T effectors (Teff), T regulatory cells (Treg – CD4+/CD25+/CD127^{lo}/FOXP3+) and NK cells (CD3-CD56+).



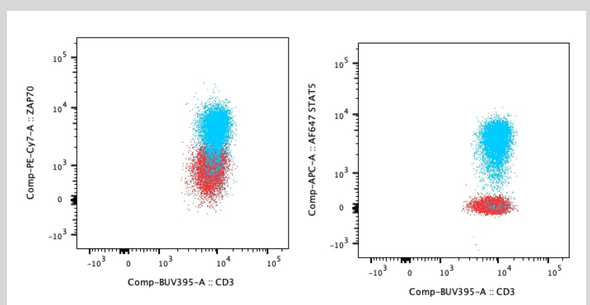




We analysed the effect of ponatinib on key signalling molecules downstream from the TCR and NK cell activating receptors, after incubation with H2O2, including phosphorylated ZAP70 and LAT, as well as the critical signalling molecule in T cells and NK cells, STAT5. 10 colour intracellular flow cytometry was then performed assessing the impact of ponatinib on T cell cytokine production including TNFα, IFNγ, IL-2, IL-4 and IL-10 after stimulation with OKT3.



IL2 expression in CD4+ cells from healthy control,
Left – unstimulated, right – post OKT3 treatment



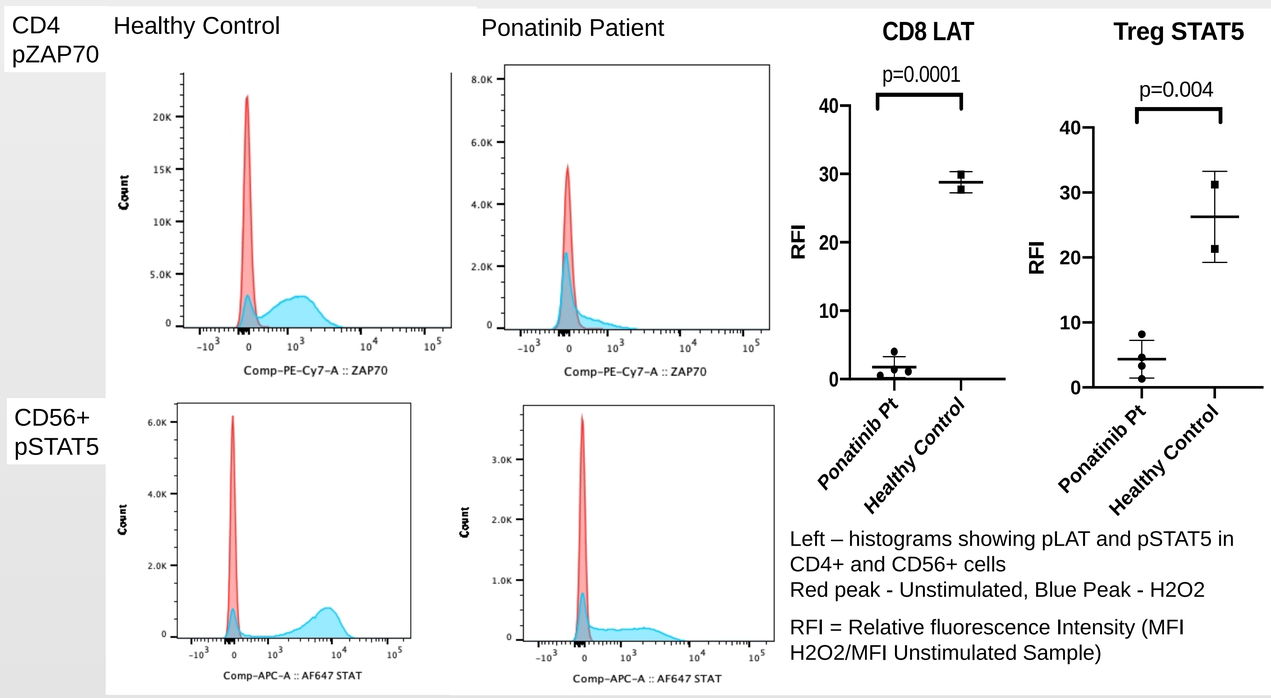
pZAP70 (left) and pSTAT5 (right) in CD3+ cells from control
Red events unstimulated blue events H2O2 treatment

Results:

Patients on ponatinib had significantly reduced phosphorylation of LAT compared with controls, with a mean increase in median fluorescence intensity (MFI) of 1.9 vs 19.1 in CD4+, 1.8 vs 28.8 in CD8+ and 5.7 vs 24 in CD56^{bri} NK cells (p=0.005, p=0.0001, p=0.006). Reduced phosphorylation of ZAP70 was also seen in all cell subsets analysed in patients on ponatinib but statistical significance was not reached.

Results Cont.

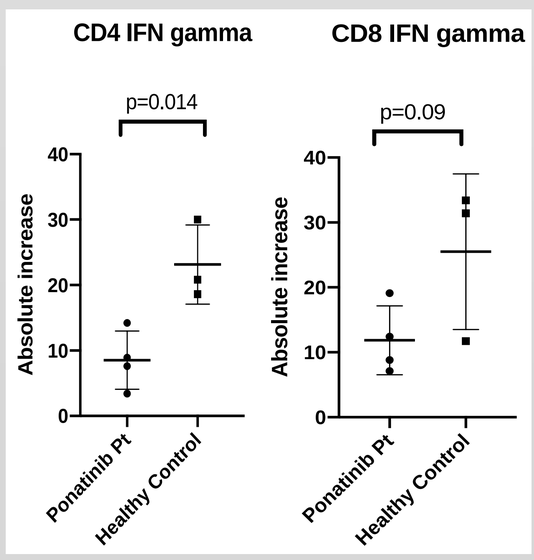
Patients on ponatinib also had significantly reduced phosphorylation of STAT5 with a mean increase in MFI of 4.3 vs 32.2 in CD3+, 3.4 vs 26.3 in CD4+, 5.1 vs 41.6 in CD8+, 3.7 vs 26.3 in Tregs and 9 vs 43.2 in CD56^{bri} cells (p=0.0001, p=0.0001, p=0.0001, p=0.004, p=0.004).

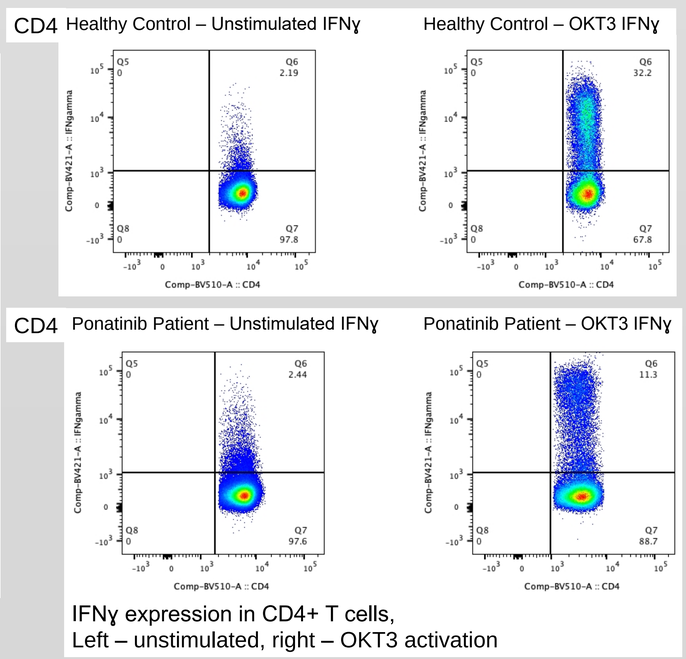


Left – histograms showing pLAT and pSTAT5 in CD4+ and CD56+ cells
Red peak - Unstimulated, Blue Peak - H2O2

RFI = Relative fluorescence Intensity (MFI H2O2/MFI Unstimulated Sample)

Patients on ponatinib had a reduced increase in expression of TNFα in CD4+ and CD8 cells when compared with controls, with an absolute increase in expression of 16.3 vs 33.3 and 10.2 vs 42.8 respectively (p=0.039, p=0.032). Patients on ponatinib also had a reduced increase in expression of IFNγ when compared with controls, with an absolute increase in expression of 8.5 vs 23.1 in CD4+ cells and 11.9 vs 25.5 in CD8+ cells (p=0.014, p=0.09).





IFNɣ expression in CD4+ T cells,
Left – unstimulated, right – OKT3 activation

Conclusion:

Ponatinib inhibits signalling within immune effector cells with an associated resultant decrease in expression of proinflammatory cytokines in T cells. The strongest inhibition of signalling was seen against pSTAT5 which plays a key role in T cell proliferation through IL-2 signalling and also regulates NK cell function primarily via IL-15. This is to our knowledge the first study to report on the immunosuppressive qualities of ponatinib and has important implications with regards to potential combination therapy with immunomodulatory agents.

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