

# Differential inhibition of T-cell receptor and STAT5 signaling pathways determines the immunomodulatory effects of dasatinib in chronic phase chronic myeloid leukemia

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Supplementary Materials:

Supplementary Method:

Immunophenotyping:

Surface staining was performed with the following directly conjugated antibodies (all purchased from Beckton Dickinson [BD], New Jersey): CD3 BUV395 (clone SK7), CD4 BV510 (L200), CD5 FITC (UCHT2), CD25 BV421 (M-A251), CD127 BV786 (HIL-7R-M21), CD45RA BV605 (HI100), CD56 FITC (NCAM16.2). Intracellular staining was performed using the following fluorochromes: pZAP70 PE-Cy7 (PY319), pSTAT5 AF647 (pY694), pLAT PE (pY171), TNF-alpha PE (MAB11), IFN-gamma BV421 (B27), IL-10 APC (JES3-19F1), IL-4 PE-Cy7 (8D4-8), IL-2 PerCP5.5 (MQ1-17H12) AND FoxP3 PerCP5.5 (PCH101) (BioLegend, San Diego).

Supplementary Results:

Correlation between Immune Cell RFI and Dasatinib Levels

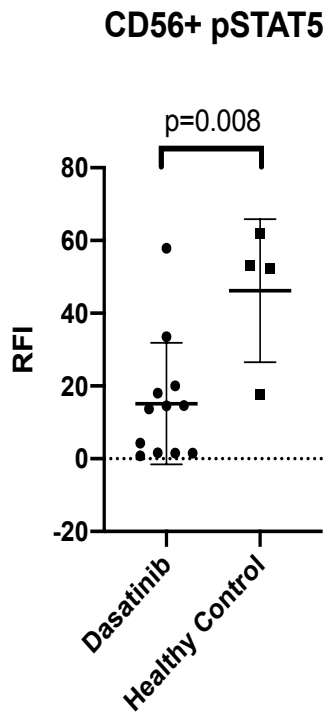
Patients on reduced dosage had dasatinib had plasma levels  $<5\mu\text{g/ml}$  and as such were assigned a value of  $3.5\mu\text{g/ml}$  for analysis [Limit of Detection= $5, 5/\sqrt{2}= 3.5$ ]. We observed moderate inverse correlation between dasatinib levels and immune cell RFI for pZAP70, pLAT and pSTAT5. Using Pearson correlation coefficient with pairwise deletion for missing values, correlation between dasatinib levels for pZAP70 CD3+ was  $r = -0.21$ , CD4+  $r = -0.3$ , CD8+  $r = -0.19$ , Treg  $r = -0.3$  and NK cells  $r = -0.28$ . For pLAT; CD3+  $r = -0.3$ , CD4+  $r = -0.34$ , CD8+  $r = -0.27$ , Treg  $r = -0.28$  and NK cells  $r = -0.26$ . For pSTAT5; CD3+  $r = -0.34$ , CD4+  $r = -0.4$ , CD8+  $r = -0.3$ , Treg  $r = -0.29$  and NK cells  $r = -0.36$ .

Supplementary Table 1. Clinical characteristics

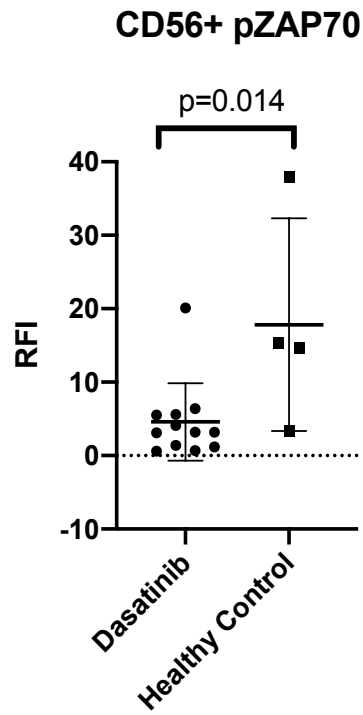
<b>Pt</b>	<b>Age</b>	<b>Sex</b>	<b>TKI</b>	<b>Dose (mg)</b>	<b>Response</b>
001	41	M	Das	100	MMR
002	49	M	Das	100	MR4
003	72	M	Das	100	MMR
004	37	F	Das	100	CCyR
005	59	M	Das	100	<CCyR
006	50	F	Das	100	CCyR
007	54	F	Das	100	MR4
008	29	M	Das	100	MR4
009	41	M	Das	100	MR4
010	49	F	Das	100	MMR
011	47	M	Das	100	MMR
012	56	M	Das	100	MR4
013	33	M	Das	50	MMR
014	39	F	Das	50	MMR
015	59	M	Das	50	MMR
016	45	M	Das	50	MR4
017	40	F	Das	50	CCyR
018	69	M	Das	50	MMR
019	40	M	Ima	400	MR4
020	41	F	Ima	400	MMR
021	40	M	Nil	600	MR4
022	38	F	Nil	800	MMR
023	72	M	Nil	600	MR4
024	47	M	Nil	600	MR4
025	36	M	Nil	600	MR4

Supplementary Figure 1.

a.

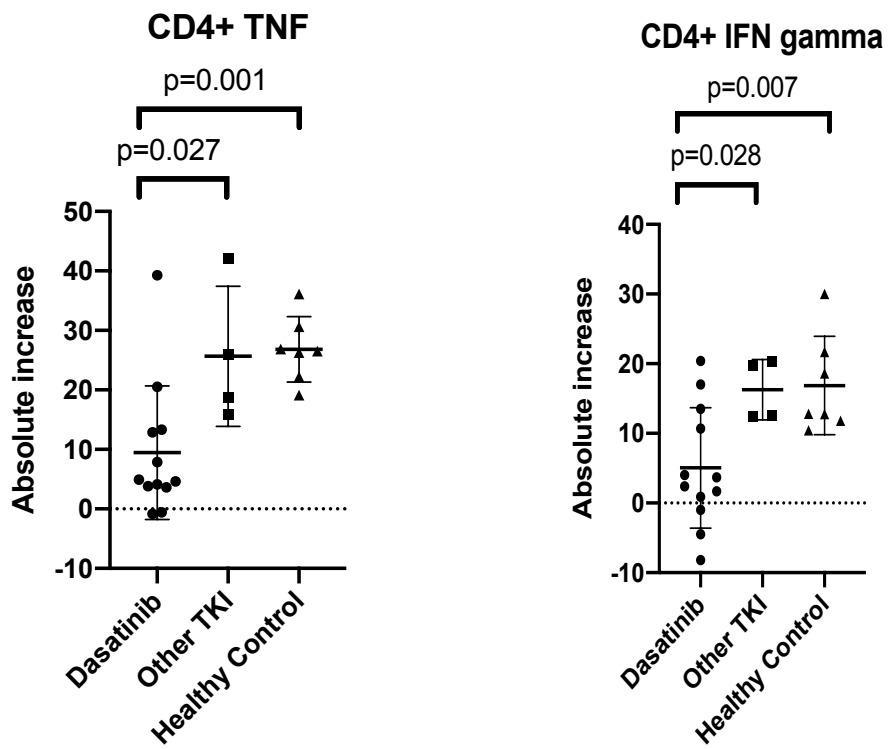


b.



RFI of a) pSTAT5 and b) pZAP70 within CD56+ cells in dasatinib treated patients compared with healthy controls.

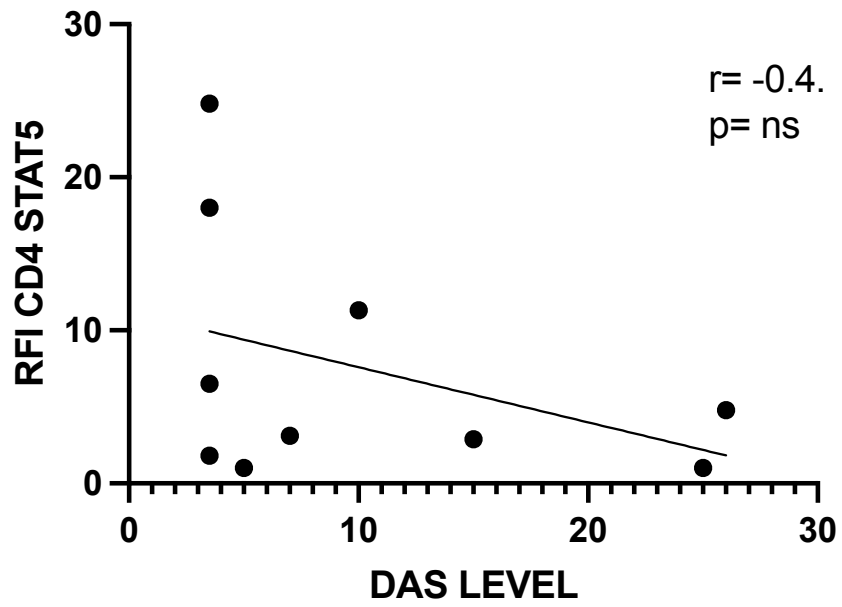
Supplementary Figure 2.



Absolute increase in a). TNF $\alpha$  and b). IFN $\gamma$  expression in CD4+ T cells after OKT3 activation in patients on dasatinib, patients taking other TKIs and healthy controls

Supplementary Figure 3.

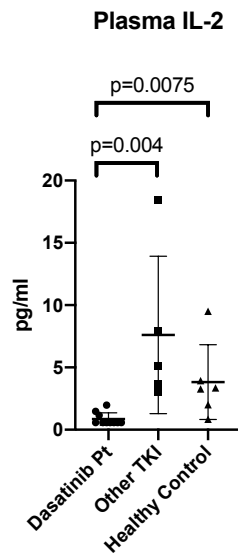
### Correlation pSTAT5 and Dasatinib Level



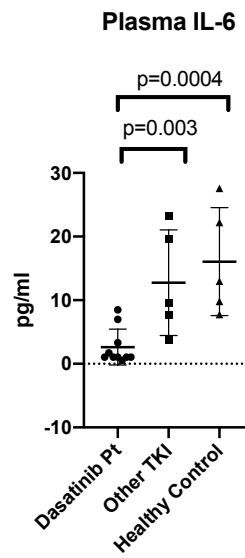
Correlation between plasma dasatinib levels and RFI of pSTAT5 in CD4+ cells

Supplementary Figure 4.

a.

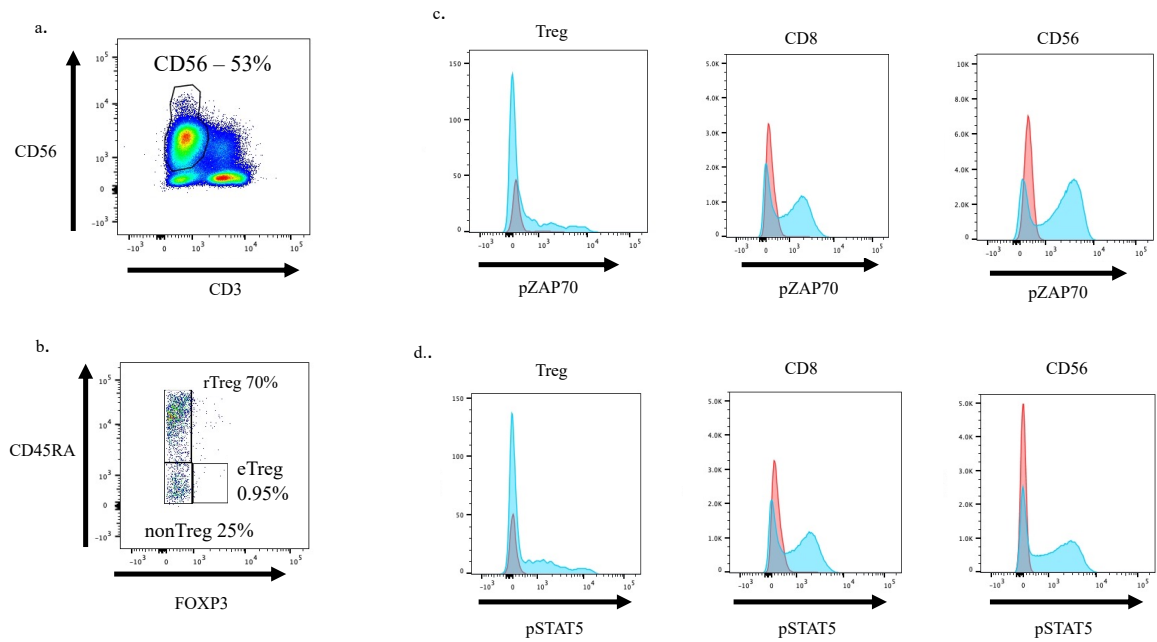


b.



a. Baseline plasma IL-2 levels in different groups. b. Baseline plasma IL-6 levels in different groups.

## Supplementary Figure 5



- Expansion of CD56<sup>dim</sup> NK cells in patient on 100mg dosage of dasatinib with acute viral infection.
- Reduction of eTregs in patient on dasatinib with acute viral infection.
- TCR signalling maintained in effector subsets in contrast to Tregs as shown by preserved pZAP70 in CD8 and CD56<sup>+</sup> cells in contrast to Tregs.
- STAT5 signalling maintained in effector subsets in contrast to Tregs as shown by preserved pZAP70 in CD8 and CD56<sup>+</sup> cells in contrast to Tregs.