1-year treatment with ponatinib provides protection of CD4+ T cells against HIV that is maintained at least 1 year more after treatment interruption

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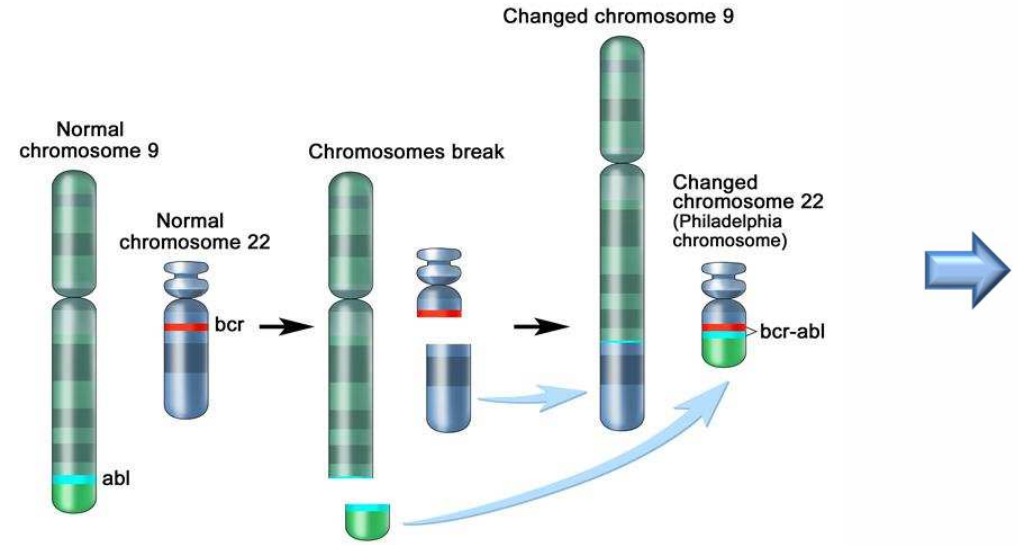
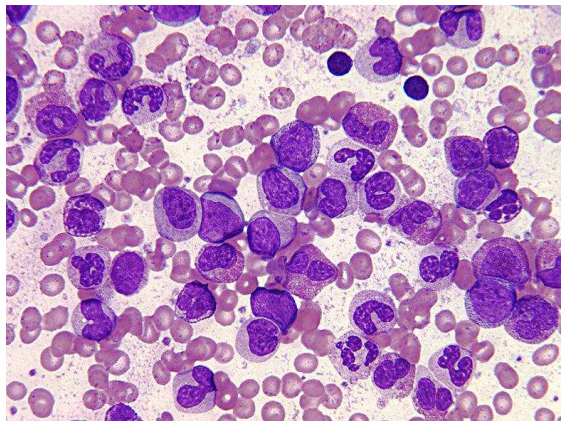
CONFLICTS OF INTEREST

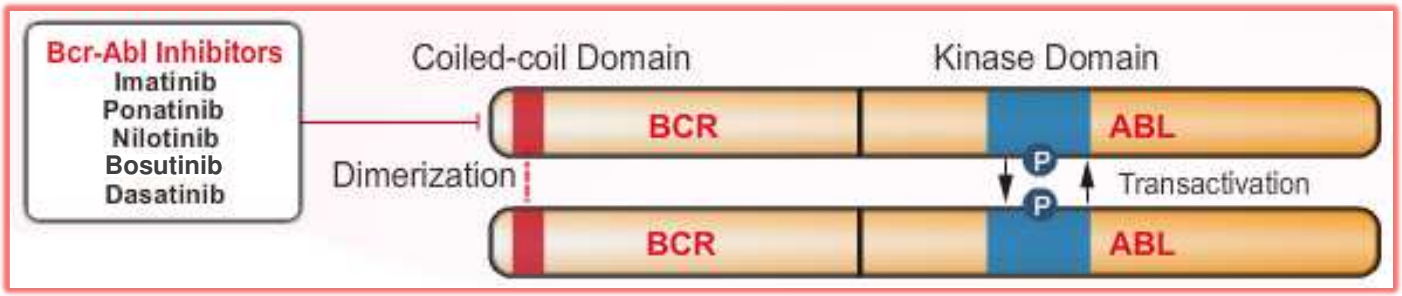
I have no conflicts of interest to disclose

Tyrosine kinase inhibitors



Chronic myeloid leukemia

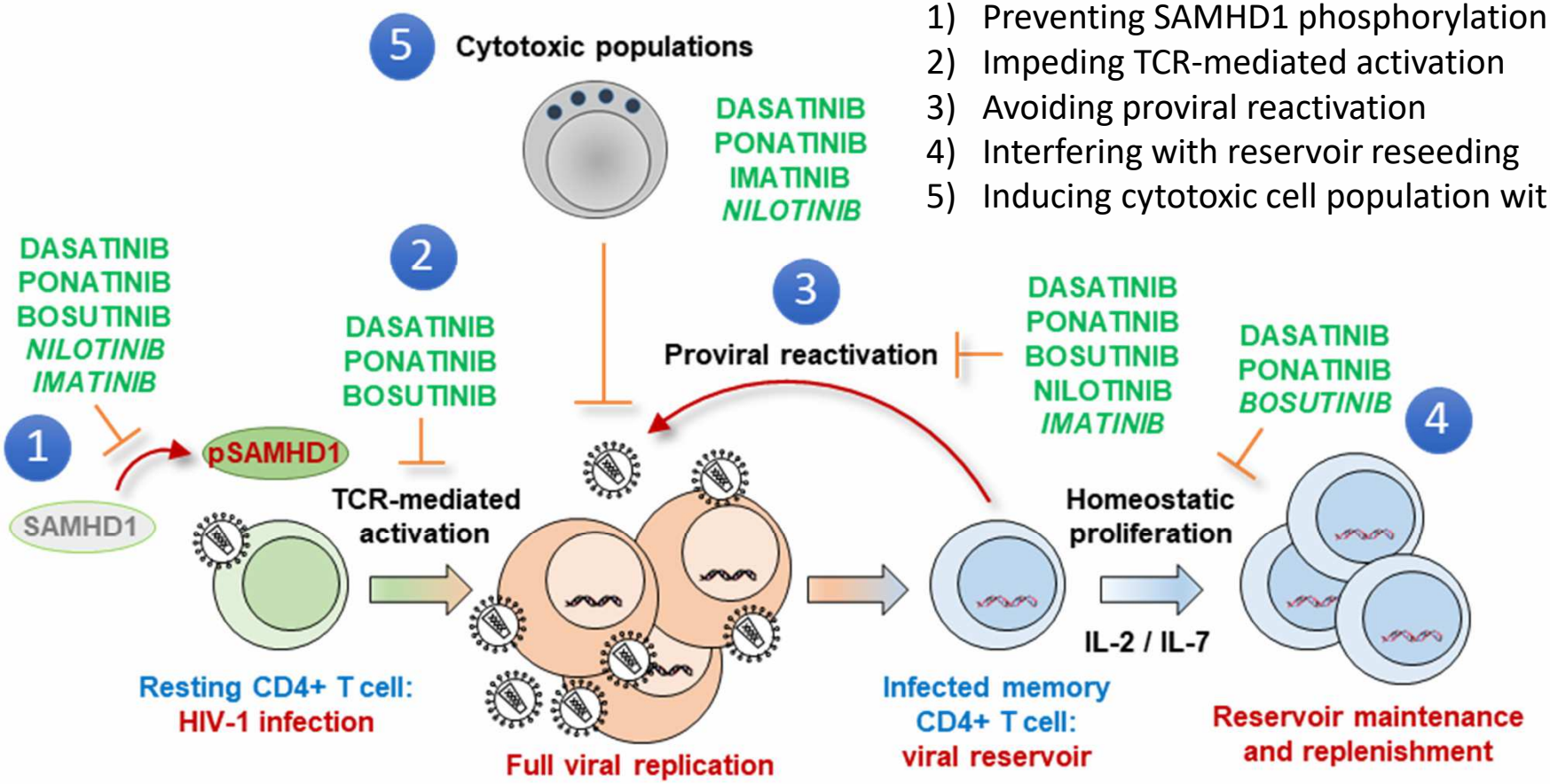




Bcr-Abl: quimeric fusion protein with uncontrolled tyrosine kinase activity

BACKGROUND

Mechanisms of action of TKIs for interfering with HIV-1 infection in CD4+ T lymphocytes



1) Preventing SAMHD1 phosphorylation
2) Impeding TCR-mediated activation
3) Avoiding proviral reactivation
4) Interfering with reservoir reseeding
5) Inducing cytotoxic cell population with antiviral activity

Bermejo et al., Biochem Pharmacol. 2018 Oct;156:248-264

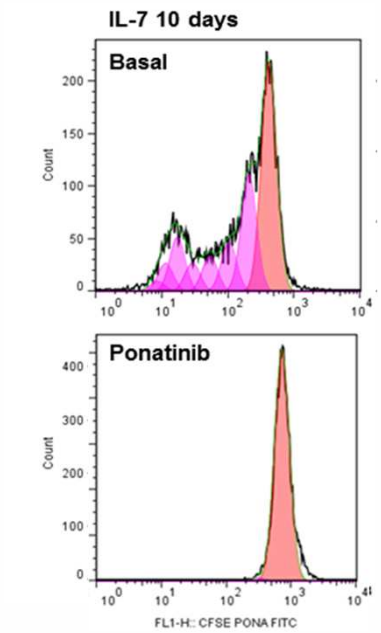
Ponatinib reduces T cell proliferation mediated by IL-7

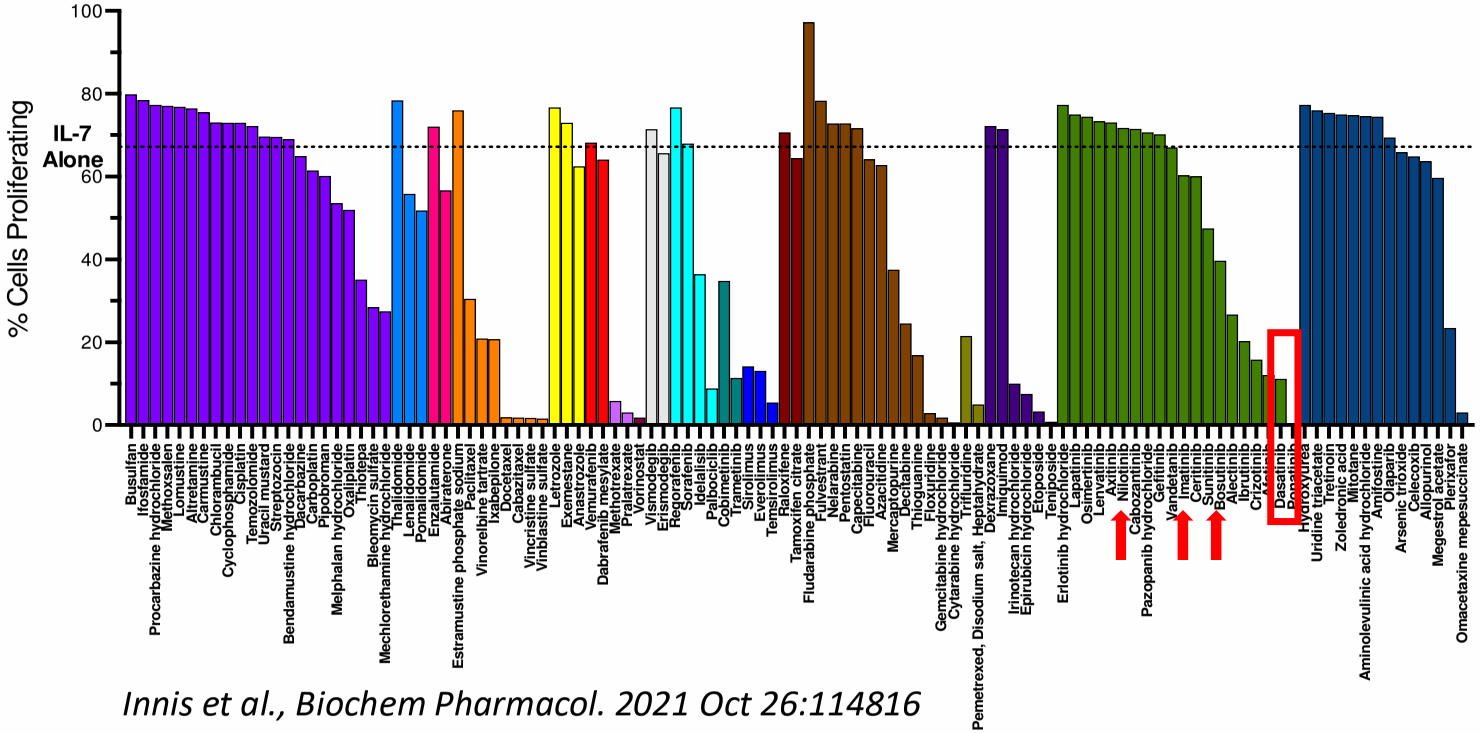
PBMCs from healthy donors

10 days → **Cell proliferation by flow cytometry**

+ Ponatinib + IL-7 **+ CFSE**

FDA approved anti-cancer drugs screened for their antiproliferative effect against homeostatic (IL-7 stimulated) proliferation: ponatinib is the most potent within the TKIs





Bermejo et al., Biochem Pharmacol. 2018 Oct;156:248-264

Innis et al., Biochem Pharmacol. 2021 Oct 26:114816

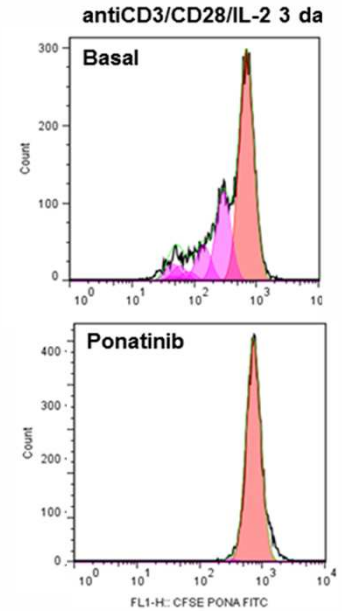
BACKGROUND

Ponatinib reduces T cell activation and proliferation mediated by TCR stimulation

PBMCs from healthy donors

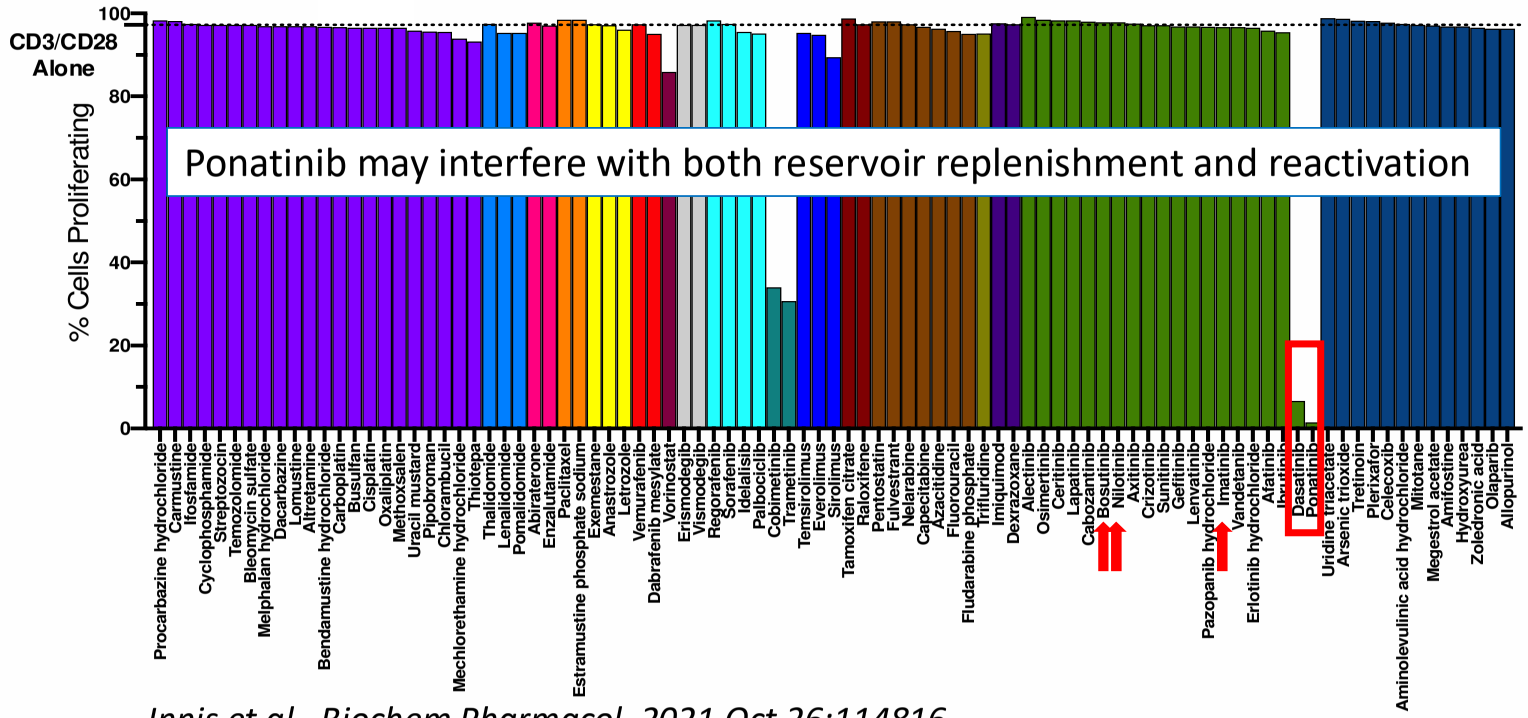
10 days → **Cell proliferation by flow cytometry**

+ Ponatinib + CD3/CD28/IL-2 **+ CFSE**



Bermejo et al., Biochem Pharmacol. 2018 Oct;156:248-264

FDA approved anti-cancer drugs screened for their antiproliferative effect against antigen-driven (aCD3/aCD28 stimulated) proliferation: ponatinib is the most potent of all the drugs assayed



Innis et al., Biochem Pharmacol. 2021 Oct 26:114816

BACKGROUND

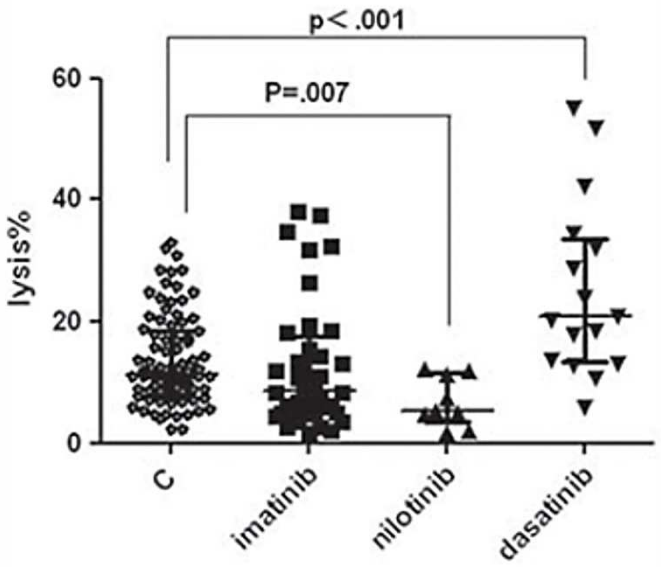
Ponatinib would need similar dose to interfere with HIV-1 replication than for the treatment of CML

	Imatinib	Nilotinib	Dasatinib	Bosutinib	Ponatinib
IC50	8.2 µM (4.07 µg/ml)	9.2 µM (4.91 µg/ml)	16.3 nM (8.26 ng/ml)	618 nM (327.28 ng/ml)	145 nM (77.46 ng/ml)
Cmax	2.35 µg/ml (400 mg once in healthy adults	1.59 µg/ml (400 mg twice in CML patients)	41.52 ng/ml (50 mg once in healthy adults)	120-141 ng/ml (600 mg once in CML patients and healthy adults, respectively)	54.7 ng/ml (45 mg once in healthy adults)
IC50/Cmax	1.7	3.0	0.2	2.3	1.4
Selectivity index	> 2.42	> 3.23	> 612	1.66	~ 68.68

Bermejo et al., Biochem Pharmacol. 2018 Oct;156:248-264

BACKGROUND

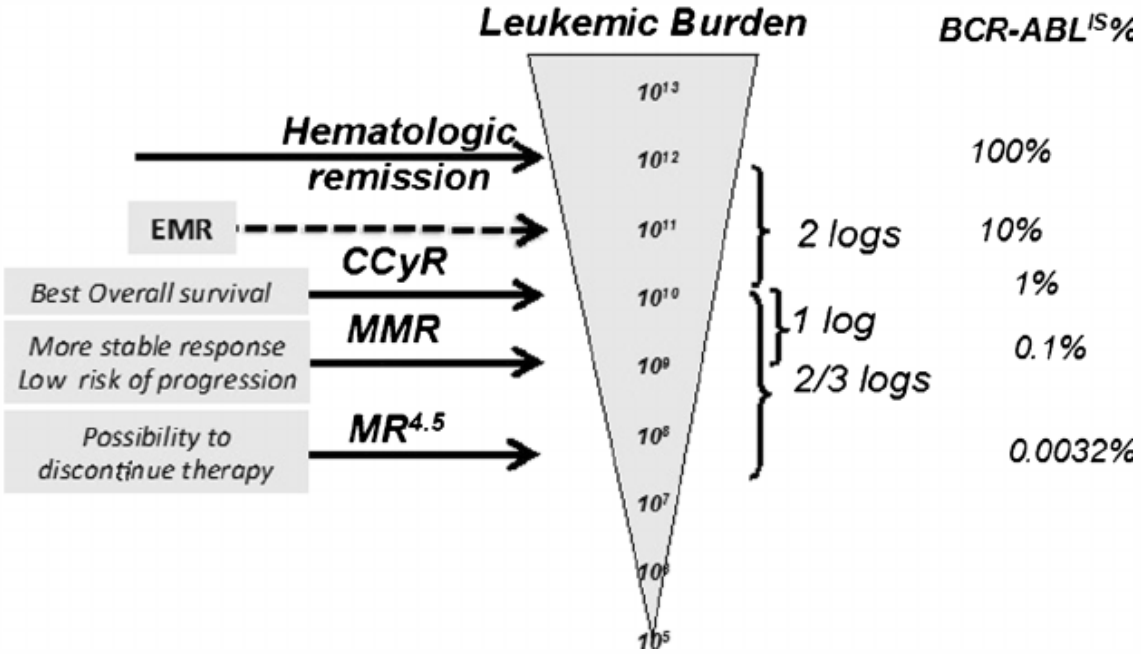
Treatment with TKIs stimulates the development of memory cytotoxic cell populations with capacity to control the residual leukemic load



NK-cell cytotoxicity among patients undergoing imatinib (*n* = 36), nilotinib (*n* = 9) and dasatinib (*n* = 17) treatment

Hayashi et al., Leukemia & Lymphoma 2012, 53, 1084-1089

Individuals with CML on treatment with TKIs for several years in DMR may attempt controlled treatment interruption, although ~50% may relapse of CML in the first year after discontinuation

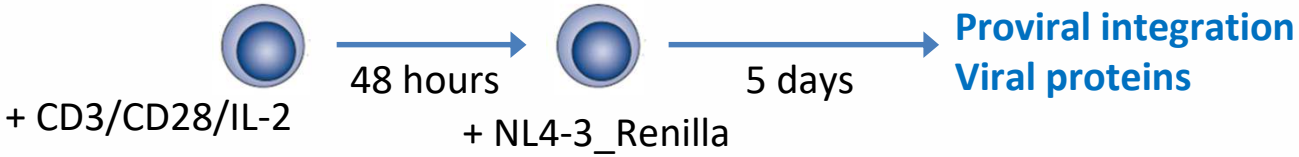


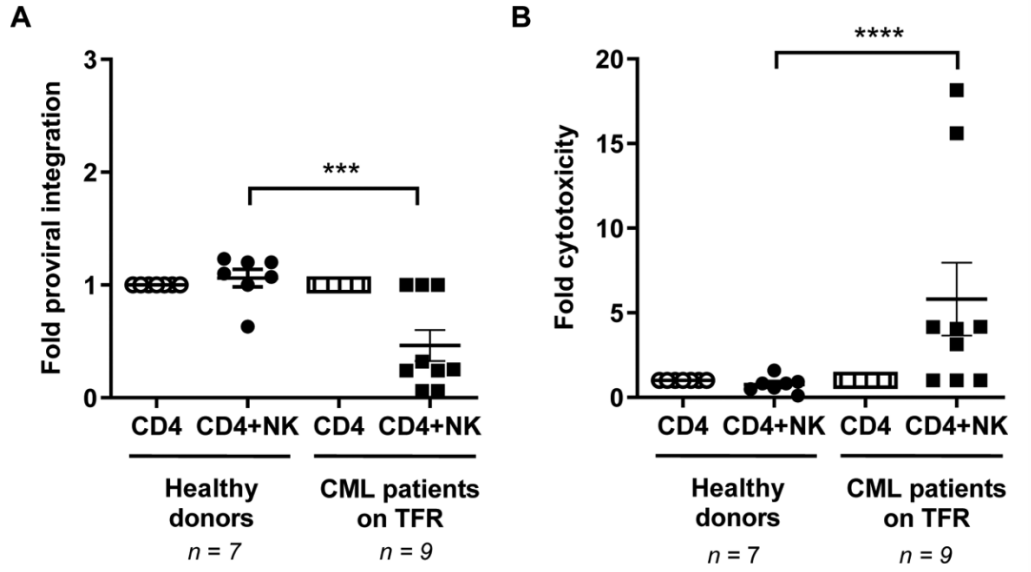
BACKGROUND

Memory cytotoxic cell populations may control the residual leukemic load after treatment discontinuation

CD4+ T cells from individuals with CML that withdrew treatment with TKIs more than 1 year ago are resistant to HIV infection

PBMCs from patients with CML off treatment with TKIs





Vigón et al., Biochem Pharmacol. 2020 Dec;182:114203

- Previous TKI treatment was with imatinib, nilotinib and/or dasatinib for 5.3 ± 0.4 years
- Mean time without treatment was 1.1 ± 0.2 years

May ponatinib be also able to protect CD4 from HIV infection one year after treatment discontinuation?

OBJECTIVES

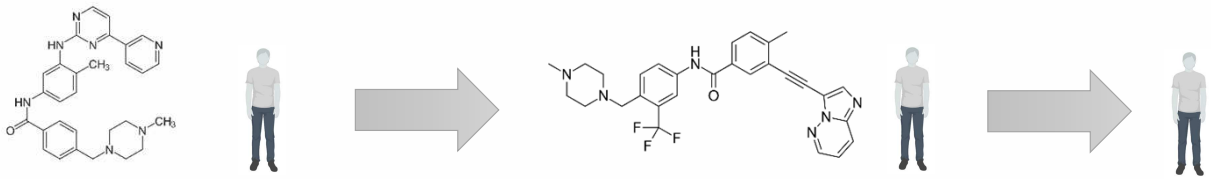
To evaluate the protective effect of ponatinib against HIV infection in CD4+ T cells of individuals with CML who were on treatment for one year and one year after discontinuation

To determine the effect of ponatinib in the antiviral response of cytotoxic cells (NK, NKT, TCRδγ+) after one year of treatment and one year after discontinuation

STUDY DESIGN

Multicenter, open-label, single-arm, Phase II exploratory clinical trial NCT04043676

Primary endpoint: To determine if 1 year-consolidation treatment with ponatinib avoids relapse of CML in individuals on DMR who have been on long-term treatment with imatinib



Individuals with CML on treatment with imatinib for 14 (IQR 5.5-15.5) years

Consolidation treatment with ponatinib 15mg/day for 1 year

Follow-up after discontinuation of ponatinib for CML relapse

Blood sampling:

- A: Before changing imatinib to ponatinib
- B: 1 year after ponatinib treatment
- C: 3 months after ponatinib discontinuation
- D: 6 months after ponatinib discontinuation
- E: 12 months after ponatinib discontinuation
- Pz_F: Relapse to CML
- Pz_G: 3 months after imatinib re-introduction

Participants

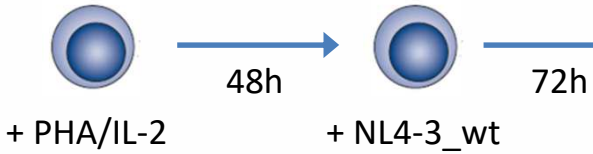
Participants (n=12) were recruited from 2018 to 2022 in 8 hospitals in Spain

	CML participants n (%)	**Gender** male, n (%)	**Years on treatment with TKIs,** median (IQR)
Completed treatment and did not relapse	5 (41.7)	4 (80)	14 (5.5 – 15.5)
Completed treatment and relapsed	4 (33.3)	2 (50)	14 (11.7 – 17.0)
Drop-out due to toxicity	3 (25.0)	3 (100%)	-

RESULTS

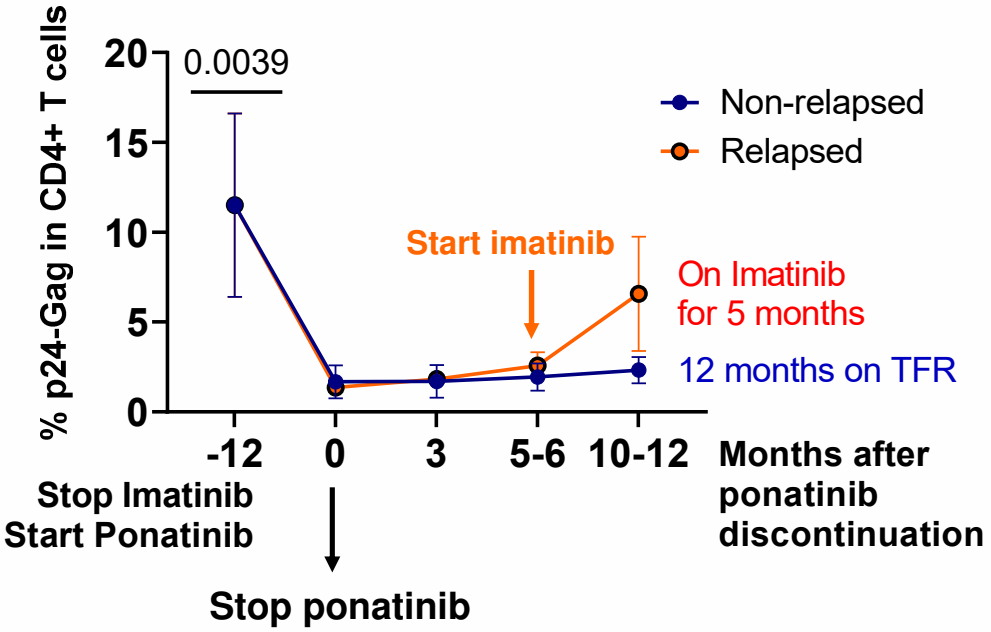
1-year treatment with ponatinib protects CD4+ T cells from HIV infection

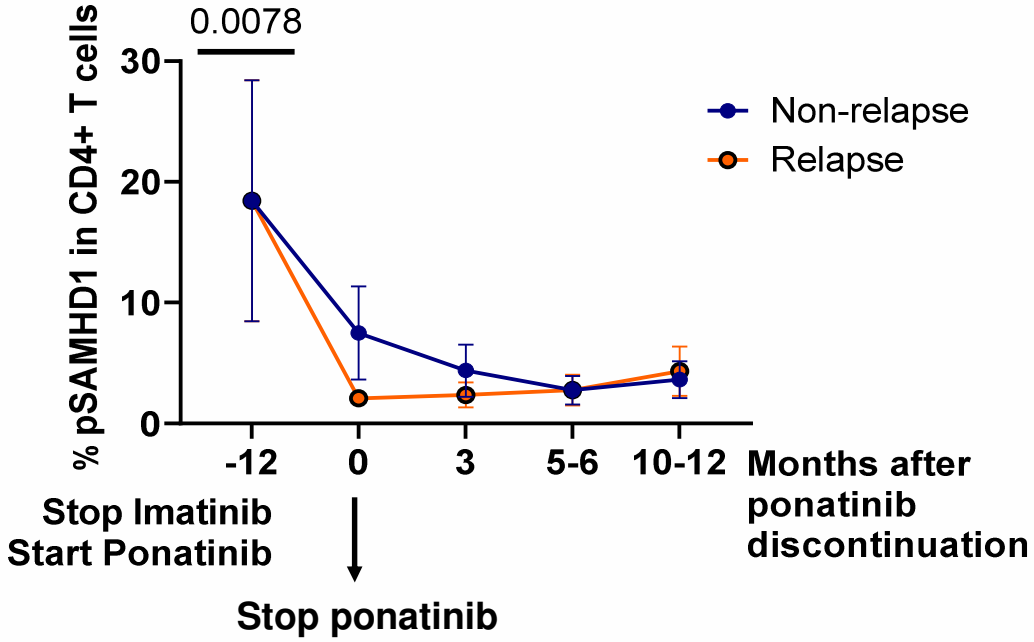
PBMCs from participants who completed the study (n=9)



Analysis by flow cytometry: **p24-Gag** **pSAMHD1**

1-year treatment with ponatinib preserves antiviral function of SAMHD1 in CD4+ T cells 1 year after treatment discontinuation



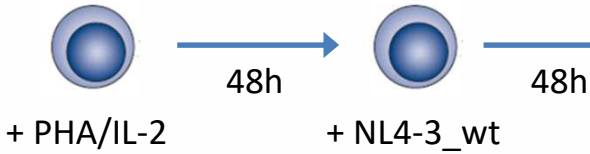


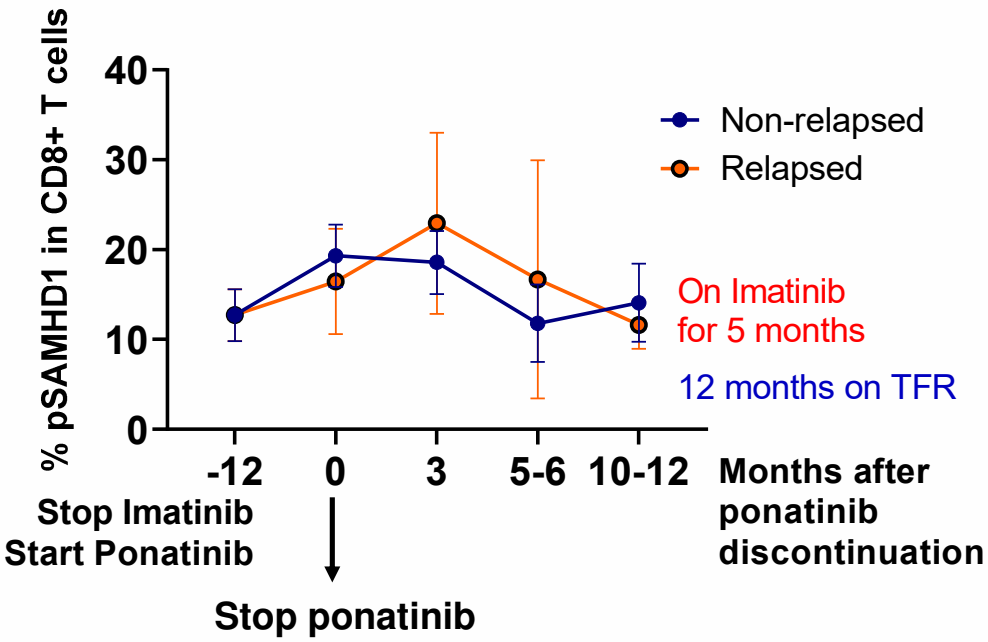
TFR: treatment-free remission

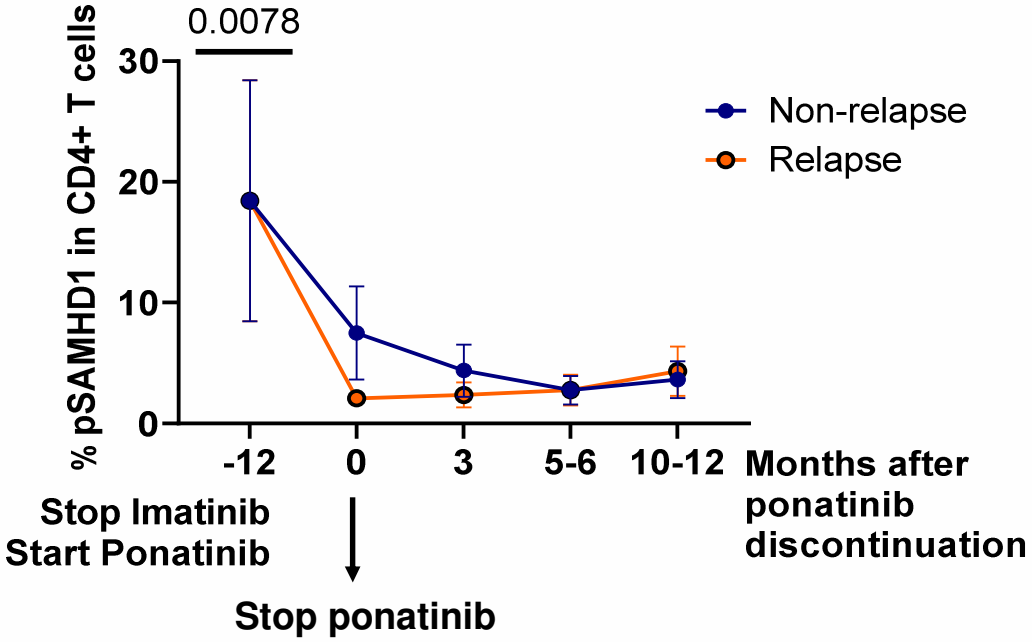
RESULTS

SAMHD1 phosphorylation was not affected in CD8+ T cells

PBMCs from participants who completed the study (n=9)



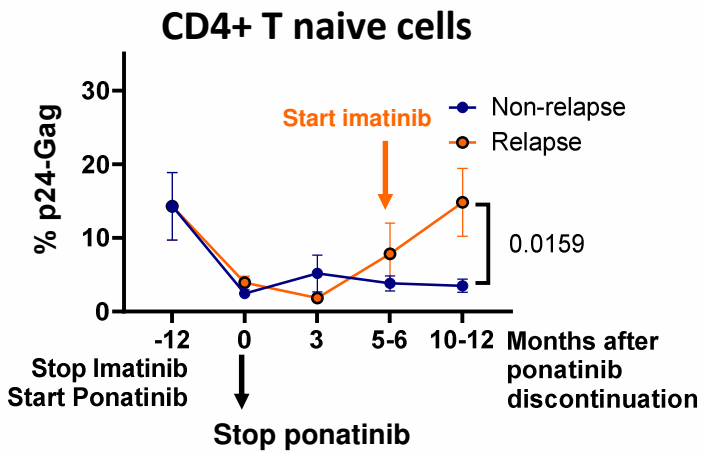


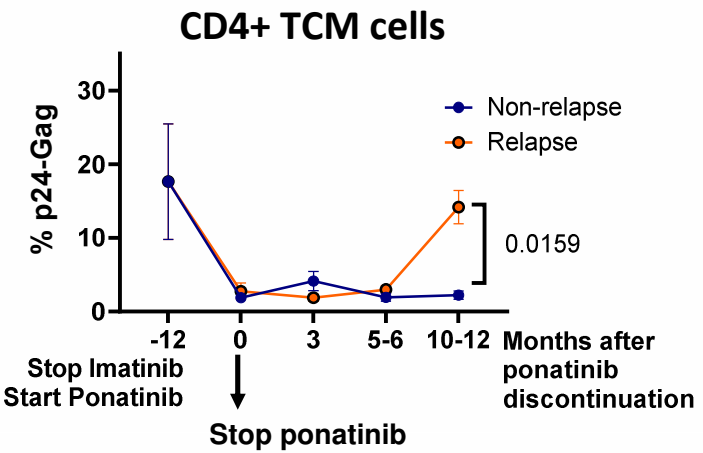


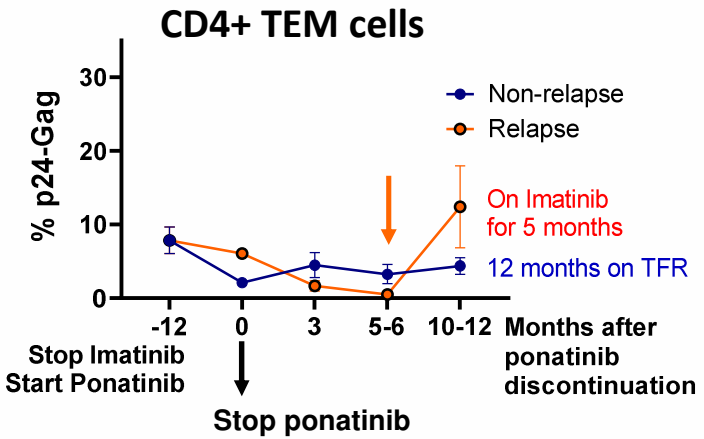
TFR: treatment-free remission

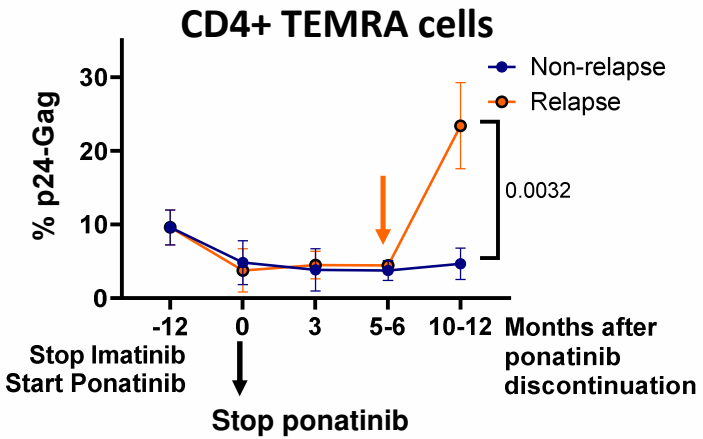
RESULTS

Protective effect of ponatinib was extended to all CD4+ T cells subpopulations









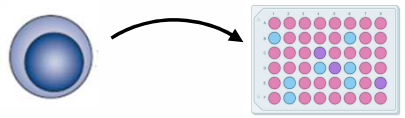
Protective effect against HIV infection is lost in those participants who relapsed of CML and after the re-introduction of imatinib

Could this difference be due to the formation of effective cytotoxic cell populations in those participants who did not relapse?

RESULTS

1-year treatment with ponatinib increased cytotoxic activity against HIV-infected cells

PBMCs from participants who completed the study (n=9)

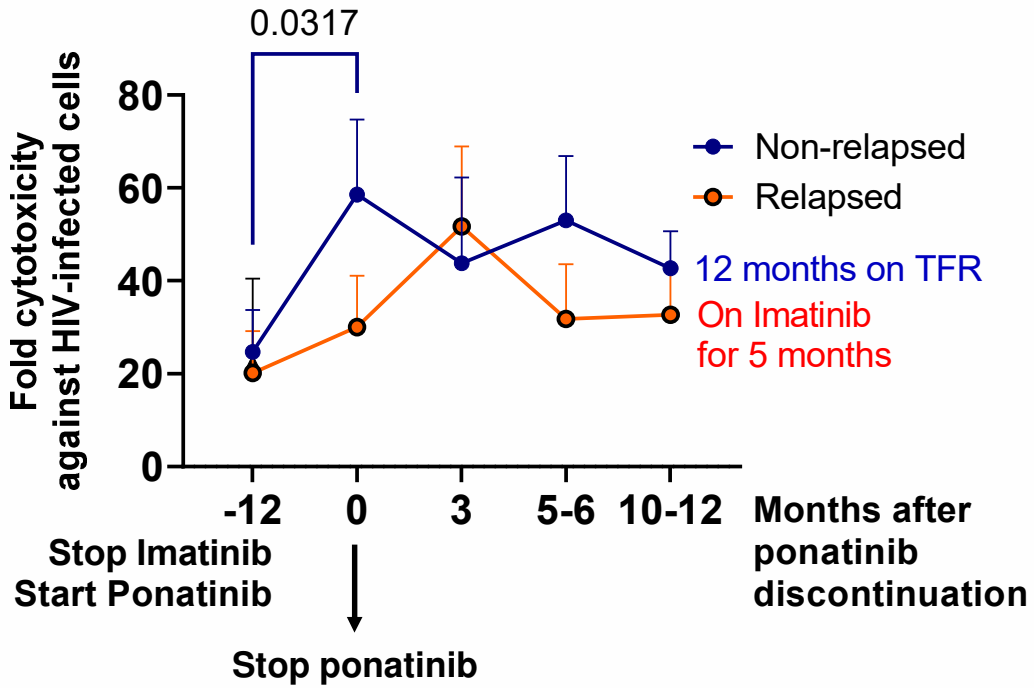


Basal

TZM + NL4-3_wt
48h

1h

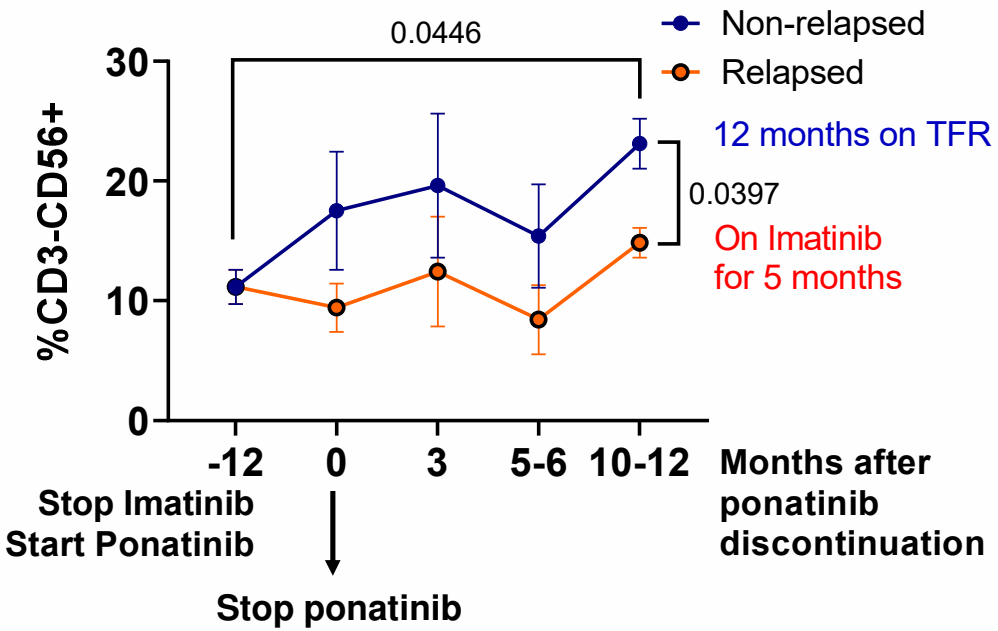
- **Measurement of caspase-3 activity in target cells**
- **Analysis of cytotoxic cell subpopulations by flow cytometry**

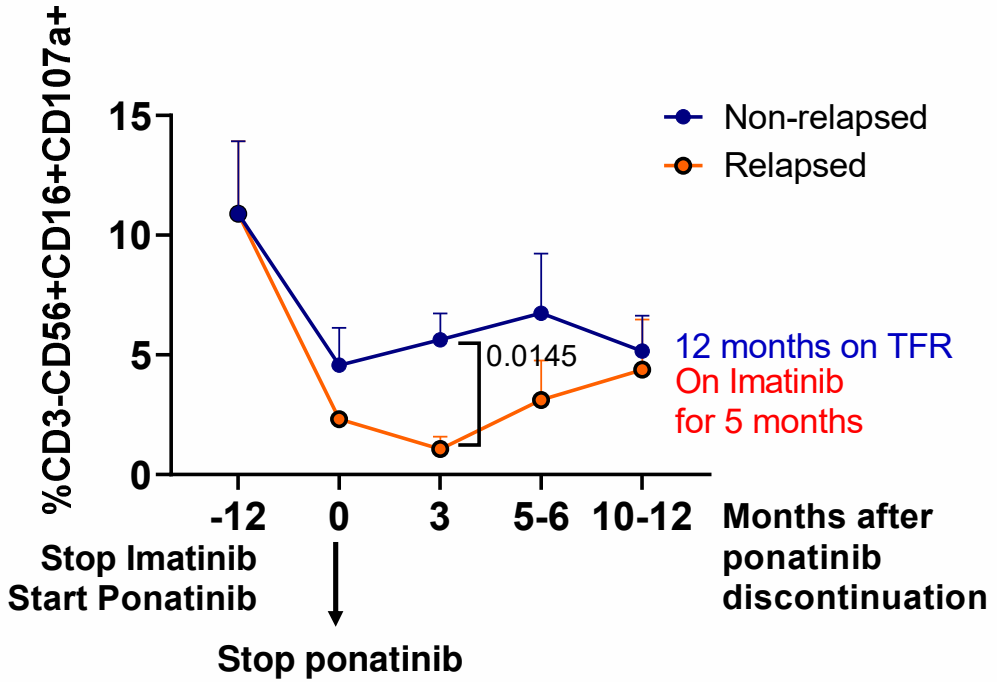


RESULTS

Levels of NK cells were increased in peripheral blood of individuals who did not relapse

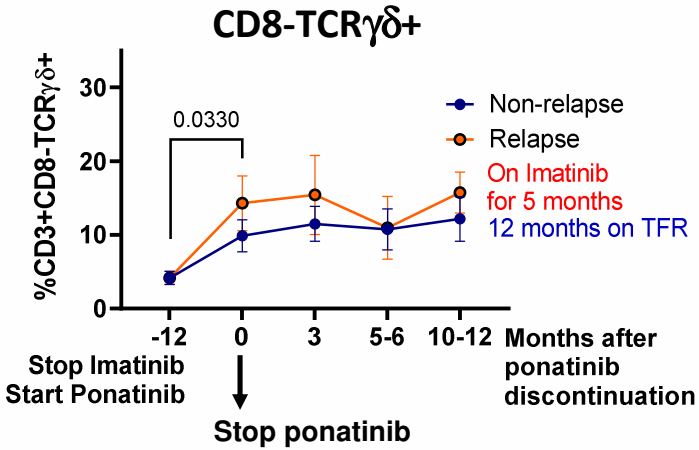
1-year treatment with ponatinib estimulates NK cell subpopulations with degranulation capacity that were sustained after treatment discontinuation in individuals who did not relapse

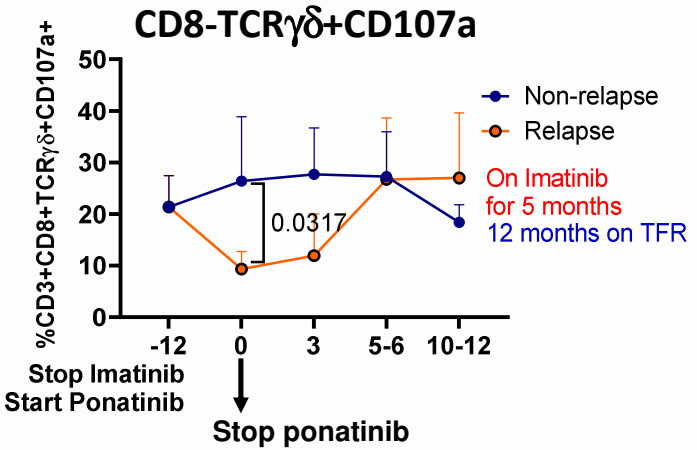


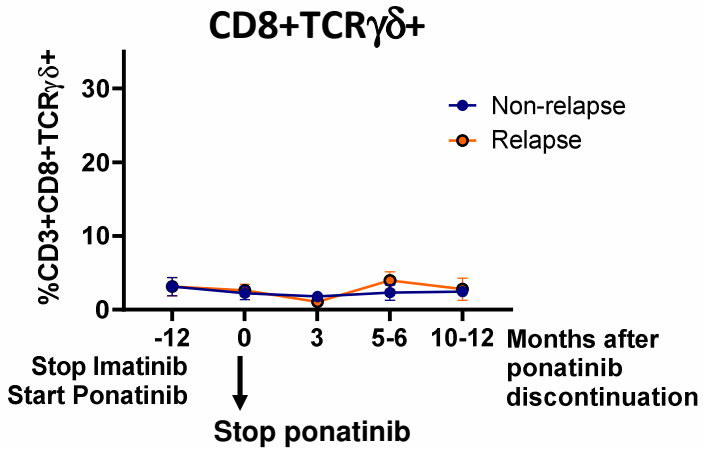


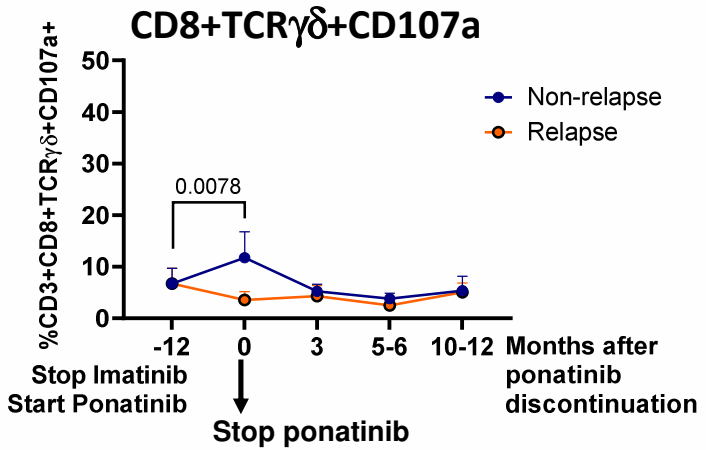
RESULTS

Tγδ+ cells showed transient increased expression of the degranulation marker CD107a





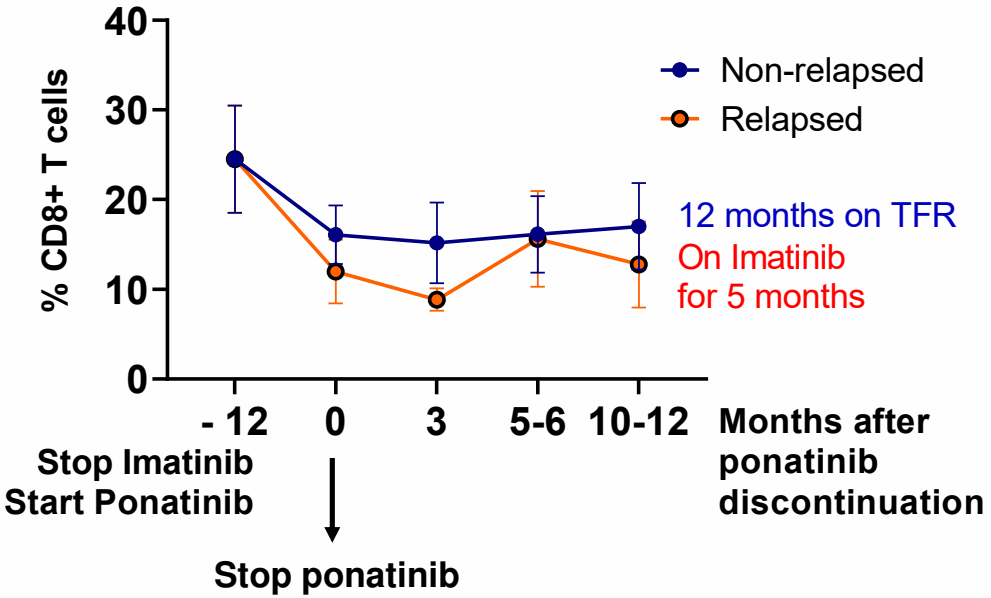


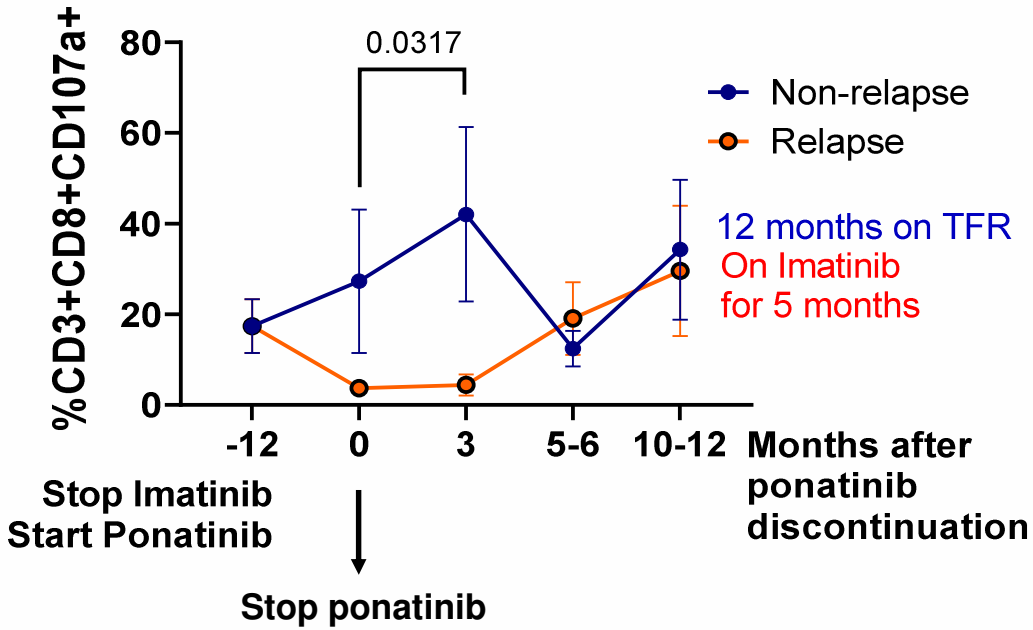


RESULTS

The expression of CD107a in CD8+ T cells was higher in non-relapsed participants, but it decreased after 6 months of treatment discontinuation

Ponatinib estimulates CD8+ T cells likely with anticancerous potential but unrelated to HIV infection





CONCLUSIONS

- Ponatinib interferes with both IL-7 and antigen-driven proliferation of CD4+ T cells
- One-year treatment with ponatinib induces a sustained interference with SAMHD1 phosphorylation in all CD4+ T cell subpopulations that protects them from HIV infection
- This protective effect is maintained for one year after treatment discontinuation in individuals with CML who did not relapse in the absence of treatment
- SAMHD1 phosphorylation is not affected in CD8+ T cells, which preserved their degranulation capacity
- Similarly to dasatinib, treatment with ponatinib enhances the antiviral cytotoxic response against HIV-infected cells, with an increased degranulation capacity of NK and Tγδ cells. CD8 also showed increased CD107a expression but it was unrelated to HIV infection
- Intensification therapy for 1 year with TKIs such as ponatinib or dasatinib could be a useful strategy to advance towards functional cure of HIV-1 infection

Key questions

- Could 1-year intensification treatment with dasatinib or ponatinib exert a long-term protection of CD4+ T cells from HIV infection?
- Could this short-term treatment induce the development of “memory” cytotoxic populations with antiviral activity against the viral reservoir?

Key findings

- 1-year treatment with a reduced dose of ponatinib exerts long-term changes in the anticancerous and antiviral immune responses in some individuals (50%), indicating the existence of specific features in the immune system that predispose to an effective protection against HIV infection

Next steps

- We will determine if the potential of ponatinib to induce a long-term protection against HIV relies on a sustained cytostatic effect on CD4+ T cells or an increased antiviral activity of essential cytotoxic cell populations
- We will try to characterize the specific features of the immune response of those individuals with CML who did not relapse after ponatinib discontinuation and showed a sustained antiviral response against HIV infection

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