

SUPPLEMENTARY MATERIAL

Definition of time to event endpoints:

- Progression-free survival (PFS): defined as the time from the date of ponatinib discontinuation to the date of progression to accelerated phase (AP), blast crisis (BC) or death from any cause, whichever occurs first.
- Overall survival (OS): defined as the time from the date of ponatinib discontinuation to the date of death from any cause.

Treatment continuation guidance after second treatment discontinuation:

- A second attempt to undergo consolidation with ponatinib and subsequent treatment discontinuation after a patient failed the TFR phase was not allowed.

Materials for characterization of cytotoxic cell populations:

- EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) to collect samples.
- Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation through Ficoll-Hypaque gradient (Sigma Aldrich, St. Louis, MO) and cryopreserved until analysis.
- PBMCs were stained with CD3-Bv510, CD8-APCH7, CD56-Bv605, TCR $\gamma\delta$ -PE, and CD107a-PE-Cy7 (BD Biosciences) to evaluate the presence of NK, NKT-like, CD8, and T $\gamma\delta$ cells with degranulation capacity (CD107a+).
- Data acquisition was performed using spectral flow cytometer Cytex Aurora and SpectroFlo software (Cytex).
- Flow cytometry data was analyzed with GraphPad Prism v10.2.1 (GraphPad Software Inc., San Diego, CA)

Statistical analyses were performed with R (Core Team 2014; R Foundation for Statistical Computing, Vienna, Austria).

Figure S1. Study design.

Abbreviations: CML: Chronic myeloid leukemia; CP: chronic phase; MMR: major molecular response ($BCR::ABL1 \leq 0.1\%$ IS); MR: molecular response; MR4: $BCR::ABL1 \leq 0.01\%$ IS; PCR: polymerase chain reaction

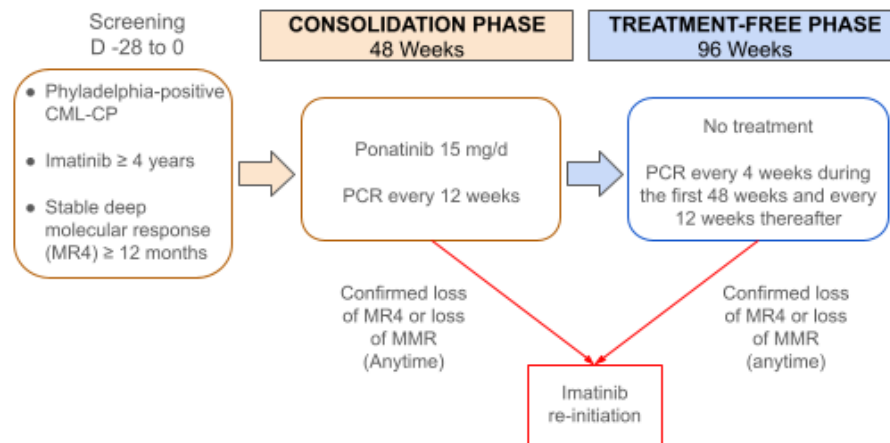


Figure S2. Molecular response recovery upon TKI restart. *BCR::ABL1* transcripts levels were centrally assessed by qPCR. Each line represents a patient. Visits were scheduled approximately every 4 weeks. Response IS thresholds are depicted in the background.

Abbreviations: UMR, undetectable molecular response (*BCR::ABL1* by qPCR as determined by central laboratories); MMR, major molecular response ($\leq 0.1\%$ *BCR::ABL1* IS); MR, molecular response; MR4.0, $\leq 0.01\%$ *BCR::ABL1* IS; MR4.5, $\leq 0.0032\%$; TFR, treatment-free remission.

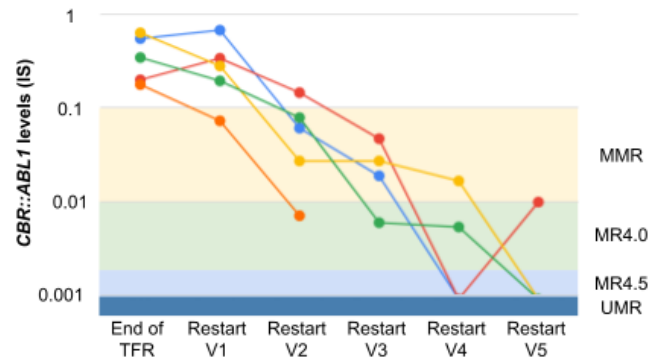


Figure S3. PFS after TKI discontinuation. PFS was analyzed by Kaplan Meier using the protocol definition (i.e. progression to accelerated phase (AP), blast crisis (BC) or death from any cause, whichever occurs first)

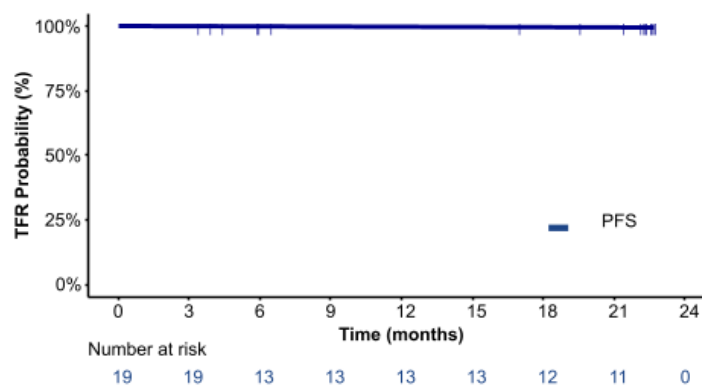


Table S1. qBCR::ABL (IS) Molecular responses during the study period for patients who completed ponatinib consolidation. Patients highlighted in orange lost major molecular response (MMR) or grade 4 molecular response and had to restart TKI. The patient in green experienced a grade 4 molecular response loss but did not restart TKI, as they maintained a sustained MMR, based on the physician's decision.. V0 is the baseline, before initiation of the ponatinib consolidation phase. V10 corresponds to the end of treatment, the departure point for the TFR phase. The visits 11 to EOS correspond to the TFR phase. Restart visits are recorded for patients who underwent retreatment with ponatinib.

	V0	V10 (EOT)	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	EOS	Restar t V1	Restar t V2	Restar t V3	Restar t V4	Restar t V5
02-01	0					0-0839		0-5499										0-0033	0-677	0-0606	0-0187	0	
02-02	0-0004	0-0006	0	0-0007	0-0009	0-0006	0	0	0-0013	0-0023					0			0-0005					
02-03	0-0053	0	0	0	0	0-0015	0-0046	0-0194	0	0	0	0	0	0	0	0	0	0					
03-01	0	0	0	0	0-0058	0-1992													0-3385	0-1453	0-0467	0	0-0098
04-01	0	0	0-0005	0	0	0	0	0-0027	0		0	0	0	0	0	0	0						
04-02	0	0	0	0	0-0037	0-0029	0-0252	0-0047	0-0095	0-006	0	0-0007	0	0-0032	0	0	0	0					
04-03	0	0-0004	0	0-0008	0	0	0	0		0	0-0004	0	0-0006	0	0	0	0	0-0013					
04-04	0-0014	0-0018	0-0057	0-0011	0-0065	0-0075	0-0046	0-0051	0-008	0-0113	0-0071	0-0206	0-008	0-0038	0-0179	0-0124	0-0653	0-0097					
04-05	0-0023	0-002	0-005	0-0039	0-0033	0-0251	0-1851	0-6342										0	0-2803	0-027	-	0-0165	0
04-06	0-0016			0-0022	0	0-0072	0-0054	0-0067		0-0904	0-0042	0-0075	0-0032	0-0032	0-0072	0-0012	0-001						
04-07	0-0099						0-0045	0-0069	0-0149	0-0141	0-0047	0-0106		0-0317	0-0765	0-0195	0-0136						
04-08	0			0-0423	0-2472	0-0405	0-1002		0-054	0-021	0-0306	0-0473	0-0618	0-058	0-114	0-0316	0-0266						
04-09	0-0041	0-0236	0-0063	0-0072	0-0097		0-0149		0-0179	0-0123	0-0094	0-0133	0-0019	0-0021	-	-	0-0015	0-0042					
06-01	0-0021	0-0086	0-0204	-	0-0062	0-0045	0-0239	0-012										0-014					
08-02	0	0		0		0	0	0	0	0	0	0	0	0	0	0	0	0					
09-01	0		0-0632	0-0011	0-0082	0-1294	0-3451											0	0-1941	0-0785	0-0059	0-0053	0
09-04	0-0075	0	0	0	0-0038	0-0487	0-0974	0-1772										0-0164	0-0725	0-007			
10-01	0-0012	0-0005	0	0	0-0008	0-0012	0	0-0002	0	0-0003	0-0006	0-0005	0	0	0	0	0	0					
11-02	0		0-0011	0	0-0019	0-0012	0-001	0	0-0079	0-0036	0	0	0	0	0	0	0	0					

Table S2. Most frequent AEs reported. Frequency >5% or grade 3 with any frequency. Note: a single patient may report more than one event.

n (%)	Overall (n=23)			Consolidation (n=23)			TFR (n=19)			TKI restart (n=5)		
	Any grade	Grade 1-2	Grade 3	Any grade	Grade 1-2	Grade 3	Any grade	Grade 1-2	Grade 3	Any grade	Grade 1-2	Grade 3
Constipation	8 (34.8)	7 (30.4)	1 (4.3)	8 (34.8)	7 (30.4)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Asthenia	7 (30.4)	7 (30.4)	0 (0)	6 (26.1)	6 (26.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)
SARS-CoV-2 infection	6 (26.1)	6 (26.1)	0 (0)	2 (8.7)	2 (8.7)	0 (0)	4 (21.1)	4 (21.1)	0 (0)	0 (0)	0 (0)	0 (0)
Myalgia	5 (21.7)	5 (21.7)	0 (0)	5 (21.7)	5 (21.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hypertension	5 (21.7)	4 (17.4)	1 (4.3)	4 (17.4)	3 (13)	1 (4.3)	1 (5.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	4 (17.4)	4 (17.4)	0 (0)	3 (13)	3 (13)	0 (0)	2 (10.5)	2 (10.5)	0 (0)	0 (0)	0 (0)	0 (0)
Arthralgia	4 (17.4)	4 (17.4)	0 (0)	3 (13)	3 (13)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Rash	4 (17.4)	4 (17.4)	0 (0)	4 (17.4)	4 (17.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Transaminitis	3 (13)	3 (13)	0 (0)	3 (13)	3 (13)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Back pain	3 (13)	3 (13)	0 (0)	3 (13)	3 (13)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)
Bone pain	3 (13)	3 (13)	0 (0)	3 (13)	3 (13)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Hypercholesterolemia	3 (13)	3 (13)	0 (0)	3 (13)	3 (13)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Anemia	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	1 (20)	1 (20)	0 (0)
Dysphonia	2 (8.7)	2 (8.7)	0 (0)	3 (13)	3 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pyrexia	2 (8.7)	2 (8.7)	0 (0)	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Heartburn	2 (8.7)	2 (8.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.3)	1 (5.3)	0 (0)	1 (20)	1 (20)	0 (0)
Anal Fistule	2 (8.7)	1 (4.3)	1 (4.3)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Blurred vision	2 (8.7)	1 (4.3)	1 (4.3)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Intermittent claudication	2 (8.7)	1 (4.3)	1 (4.3)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erectile dysfunction	2 (8.7)	1 (4.3)	1 (4.3)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Malaise	2 (8.7)	1 (4.3)	1 (4.3)	1 (4.3)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	1 (20)	1 (20)	0 (0)
Superficial thrombophlebitis	1 (4.3)	0 (0)	1 (4.3)	1 (4.3)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Muscular weakness	1 (4.3)	0 (0)	1 (4.3)	1 (4.3)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table S3. Pearson correlation was calculated between the levels of NK cells or NK CD107a+ cells and the molecular response (MR) against CML. The Pearson correlation coefficient (r) and p-values were calculated using a combination of Python libraries. Regression plots were generated using the Seaborn library

		Start ponatinib		Stop ponatinib		3mo TFR		6mo TFR		12mo TFR	
Correlation	Participants	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
NKs vs. MR	Non-relapse	0.79	0.02	0.24	0.57	0.09	0.84	0.06	0.88	0.20	0.63
	Relapse	0.63	0.37	0.05	0.95	-0.12	0.88	0.60	0.40		
NKs CD107a vs. MR	Non-relapse	-0.32	0.44	-0.13	0.75	-0.06	0.89	-0.24	0.57	-0.34	0.42
	Relapse	-0.34	0.66	-0.67	0.33	-0.50	0.50	0.50	0.50		