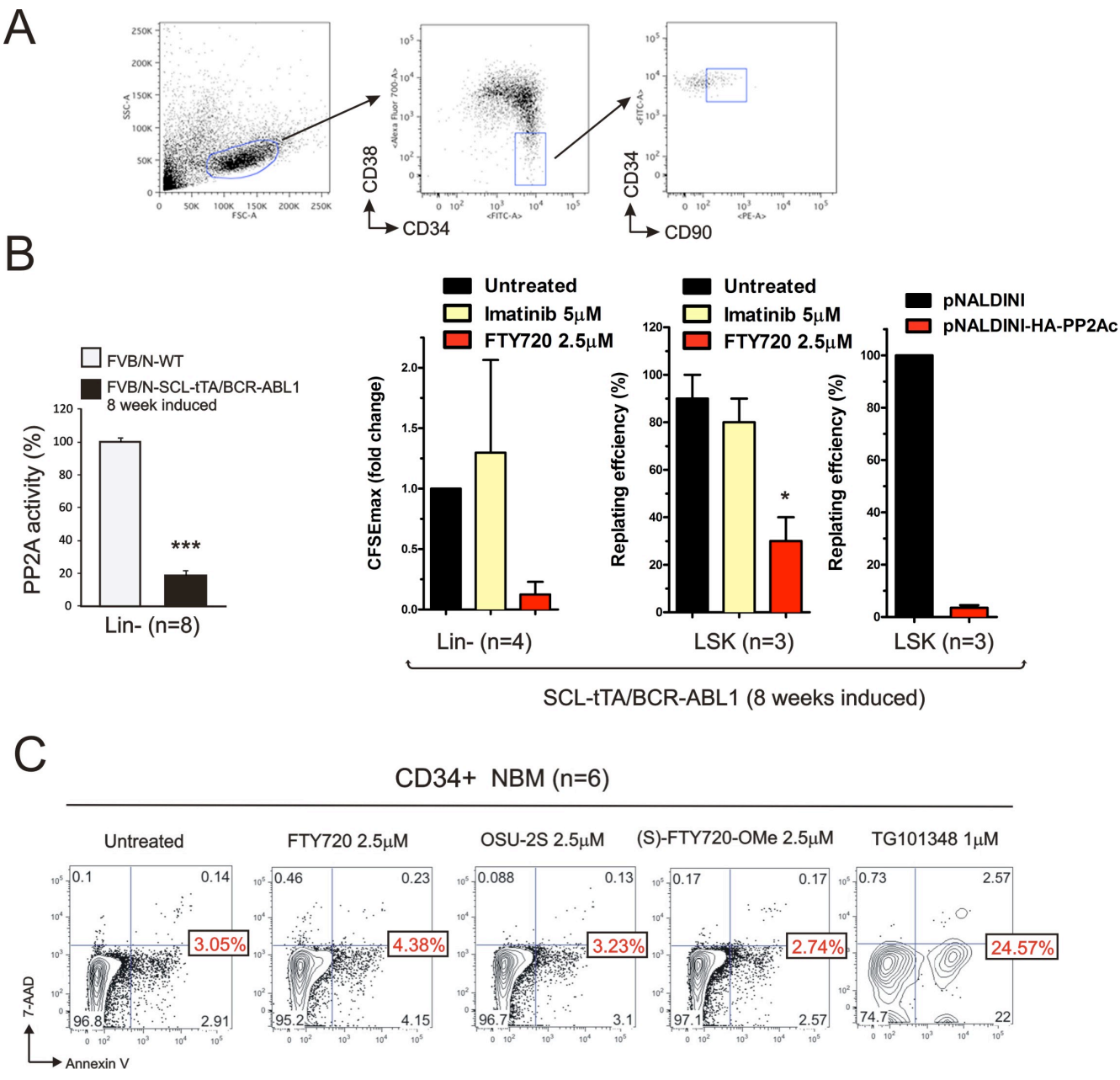
SUPPLEMENTARY MATERIAL

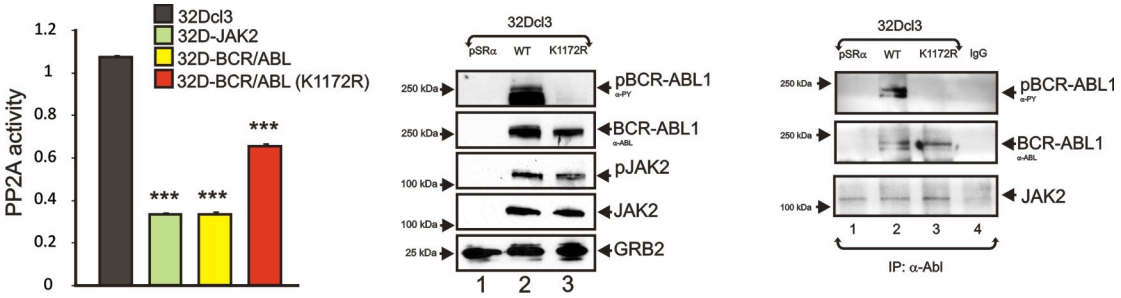
1. SUPPLEMENTARY FIGURES, LEGENDS and Table 1:

Figure S1



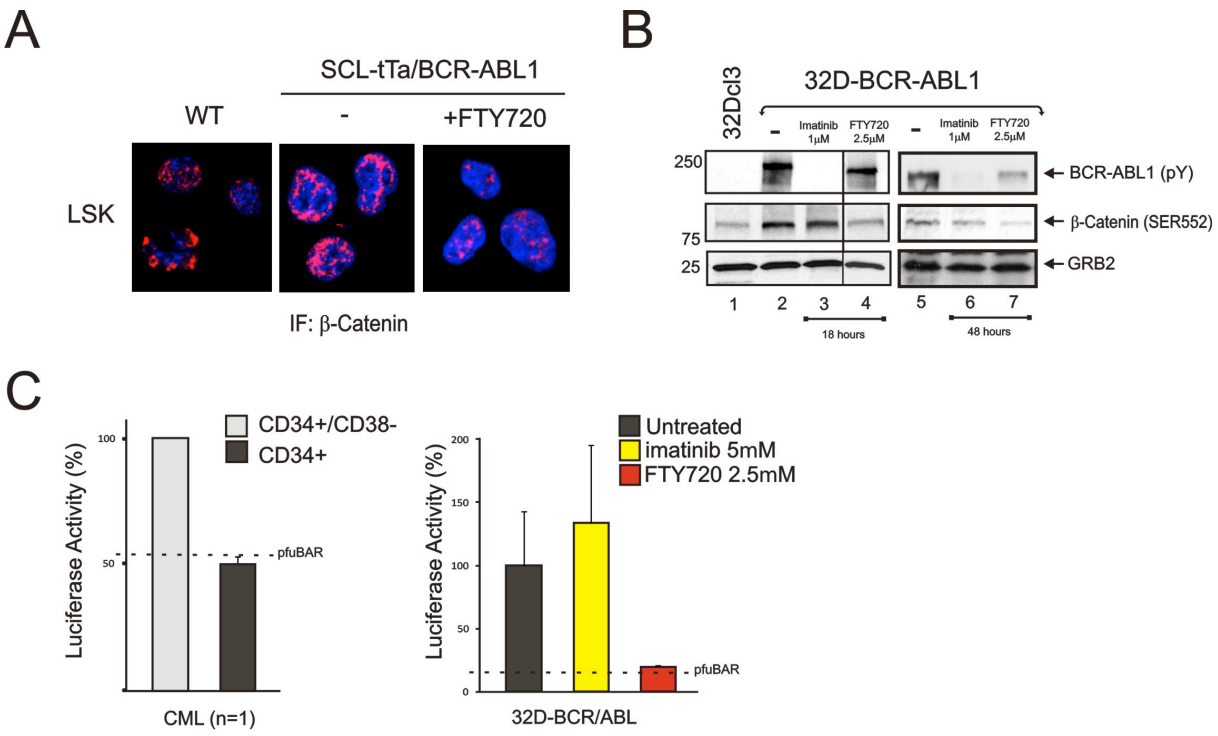
S1. ***Role of PP2A in mouse and human BCR-ABL1^{+} HSC and progenitor cells.*** **(A):** The dot plots depict the gating strategy utilized to isolate CD34+/CD38- and CD34+/CD38-/CD90+ stem cells from CML (chronic phase and blast crisis)(1) and NBM samples. **(B):** *First graph:* Phosphatase activity of the tumor suppressor PP2A(2) (Mean ± SD) performed on lineage-negative cells derived from the bone marrow of 8 week induced FVB/N SCLtTA/BCR-ABL1 mice compared to wild type mice. *Second graph:* The graph shows the proportion (± SEM) of quiescent CFSEmax cells(3) from CFSE/tracking assays of lineage-negative cells derived from the bone marrow of 8 week-induced FVB/N SCLtTA/BCR-ABL1 mice and treated as indicated. *Third and fourth graphs:* replating efficiency of single colonies plucked from colony forming assays (CFC) of LSK cells derived from the bone marrow of 8 week induced FVB/N SCLtTA/BCR-ABL1 mice and ectopically expressing HA-PP2Ac (right) or treated with FTY720 or Imatinib (left). **(C):** Annexin V staining shows levels of apoptosis in CD34+ NBM progenitors (n=3) untreated or treated with PP2A-activating (FTY720, OSU-2S and (S)-FTY720-OMe) or Jak-2 inhibiting (TG101348) drugs used at concentrations that induce apoptosis in quiescent CML but not normal HSCs.

FIGURE S2



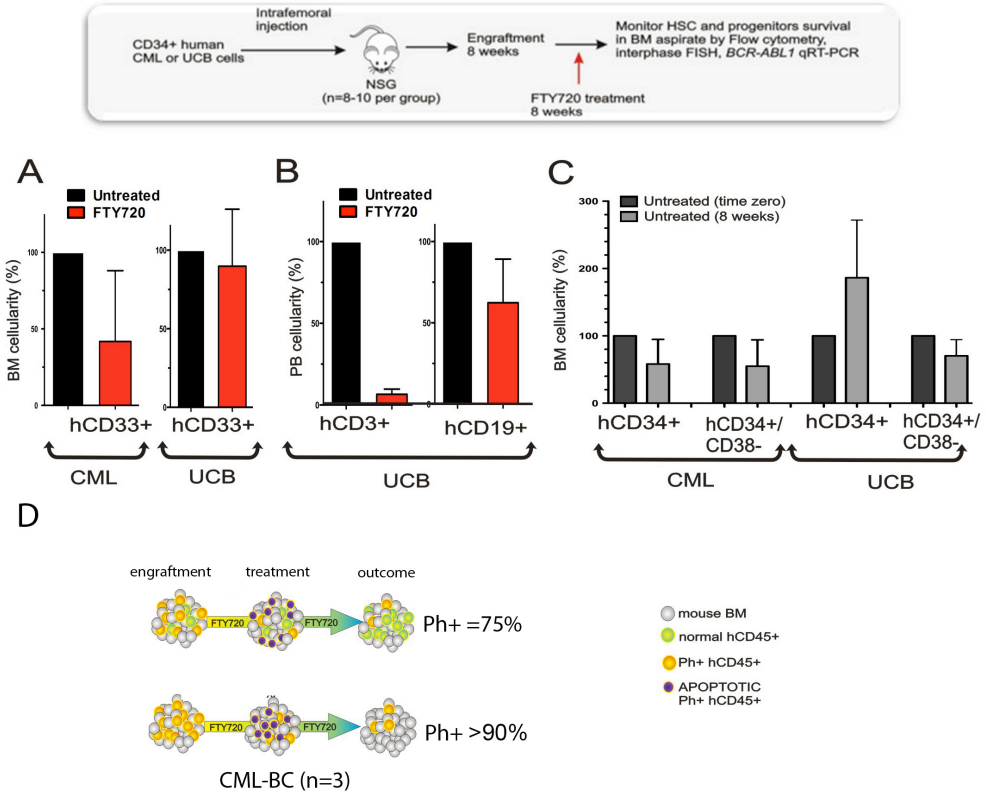
S2. ***BCR-ABL1 expression is sufficient to induce JAK2 activity and inhibit PP2A.*** *(left)* PP2A phosphatase activity assay (Mean ± SD) in parental, JAK2- and wild-type - or K1172R BCR-ABL1-expressing 32Dcl3 cells. *(middle)* BCR-ABL1 and JAK2 activity and expression in parental, wild-type and K1172R BCR-ABL1-expressing 32Dcl3 cells. GRB2 levels were detected as loading control. *(right)* association of endogenous JAK2 with wild-type and K1172R BCR-ABL1 in anti-ABL immunoprecipitates from 32Dcl3 cells ectopically expressing the wild-type or the kinase-dead mutant of BCR-ABL1.

FIGURE S3



S3. ***Effect of FTY720 on β-catenin activity in BCR-ABL1$^+$ mouse stem/progenitor cells.*** The independence of β-catenin activation from BCR-ABL1 activity was also evident in mouse LSK cells from SCL-tTA/BCR-ABL1 mice and in 32D-BCR/ABL myeloid precursors. **(A):** Confocal microphotographs show the expression levels of β-catenin (red) in FTY720-treated LSK cells derived from the bone marrow of 8 week induced FVB/N-SCL-tTA/BCR-ABL1 mice compared to wild-type mice; blue: nuclei. **(B):** In 32D-BCR/ABL cells, short-term (18 h) treatment with imatinib inhibited BCR-ABL1 without altering levels of activated (phospho-S552(4)) β-catenin. By contrast, FTY720, which requires at least 24 h to induce BCR-ABL1 inactivation/degradation in 32D-BCR/ABL cells(5), efficiently inhibited β-catenin (lane 4). As expected, long-term exposure (48 h) to FTY720 also suppressed BCR-ABL1 activity. **(C):** The graphs show the levels of LEF/TCF-dependent luciferase activity (Mean ± SEM) in 32D-BCR-ABL1 cells transduced with the lentiviral β-catenin reporter constructs and treated with either imatinib or FTY720; average luciferase levels of cells transduced with the negative control pfuBAR construct are indicated by the dashed line

FIGURE S4



S4. ***In vivo effect of FTY720 on normal and CML myeloid and lymphoid compartments.*** Schematic representation of the xenotranplantation of human CML-BC and UCB CD34+ cells into NSG mice (*Top Panel*). Graphs show the in vivo effect of 4 weeks FTY720 treatment (10 mg/kg/day) on human **(A)** myeloid ($CD45^+/CD33^+$) and **(B)** lymphoid T ($CD45^+/CD3^+$) and B ($CD45^+/CD19^+$) cells isolated from intrafemoral BM aspirates and peripheral blood, respectively, of NSG mice (8-10 mice per group) engrafted with human CD34+ cells obtained from BM of CML patients (n=3) or umbilical cord blood donors (UCB; n=3), the latter used as a source of normal hematopoietic cells. Red bars (mean ± SEM) indicate the number of cells in FTY720-treated animals expressed as percentage of the mean values (black bars) in untreated animals. **(C):** Human CML and UCB progenitor ($CD34^+$) and stem-enriched ($CD34^+/CD38^-$) cell fractions in BM aspirates from untreated animals at 8 weeks after engraftment (expressed as percentage of the mean values ±SE relative to the same fractions assessed at time of engraftment). **(D):** Schematic representation of the effect of FTY720 on mice engrafted with CML-BC patient samples with >90% and ~75% Ph^+ metaphases at time of sample procurement.

ID#	Phase	SEX/ AGE	Blasts	WBC (10^3/ul)	Phenotype	FISH	Karyotype
1	CP	M/48	0%	330.0		100%	46XY, t(9;22)(q34;q11)
2	CP	F/62	5% BM			100%	46XX, t(9;22)(q34;q11)
3	CP	F/60	0% PB; 0% BM	46.8		97%	46,XX,t(9;22)(q34;q11.2)[19]/46,XX[1]
4	CP	M/39	10% PB; 1% BM	653.7		99%	46,XY,t(9;22;10)(q34;q11.2;q11.2)
5	CP	F/53	1.9% PB; 1% BM	116.9		96%	46,XX,t(9;22)(q34;q11.2)
6	CP	M/56	2% PB; 0% BM	261.0		88%	46,XY,t(9;22)(q34;q11.2)[18]/46,XY[2]
7	CP	F/56	4% PB; 0% BM	50.1		96%	46,XX,t(9;22)(q34;q11.2)
8	CP	F/31	3-5%	200.0		90%	46,XY,t(9;22)(q34;q11)[25]
9	CP	M/63	1%	81.0		100%	46,XY,t(9;22)(q34;q11)[25]
10	CP	F/73	5-10% PB	173.5		92%	46,XX,t(9;22)(q34;q11)[25]
11	CP	M/46	1-2% PB	496.0		99%	46,XY,t(9;22)(q34;q11)[25]
12	CP	F/58	1%	28.3		100%	46,XX,t(9;22)(q34;q11.2)x7
13	CP	M/35	3%	49.2		100%	46,XY,t(9;22)(q34;q11.2)
14	CP	M/56	1%	8.6			46,XY,t(9;22)(q34;q11.2)
15	CP	F/38	3%	33.0	Myeloid	100%	46,XX,t(9;22)(q34;q11.2)x16
16	CP	M /32	16%	25.2		100%	46,XX,t(9;22)(q34;q11.2)x30
17	CP	M/ 48	10%	9.4			46,XY,t(9;22)(q34;q11.2)x28
18	CP	M/51	6%	18.2	Myeloid	100%	46,XY,t(9;22)
19	CP	M/39	2%	74.8		97%	46,XY,t(9;22)(q34;q11.2)
20	CP	M/35	3%	49.2		100%	46,XY,t(9;22)(q34;q11.2)
21	CP	M/33	3%	67.2			46,XY,t(9;22)(q34;q11.2)
22	CP	M/76	3% BM	231.0			46XY, t(9;18;22)(q34,q11,q11)
23	CP	M/61	2% BM	275.0			46XY, t(9;11;22)(q34,q13,q11)
24	CP	M/35	6% BM	400.0			46XY, t(9;22)(q34;q11)
25	CP	M/82	4% BM	47.5			46XY, t(9;22)(q34;q11)
26	CP	F/67	2% BM	92.0			46XX, t(9;22)(q34;q11)
27	CP	M/65	1%	138.2			46XY, t(9;22)(q34;q11)
28	CP	F/43	<5%	42.7			
29	CP	F/45	4% PB; 1% BM	389.9		87%	
30	CP	F/18	18%	18.4			
31	CP	F/31					46,XX,t(9;22)(q34;q11.2)[9]/46,XX[21]
32	CP	F/48	5% PB; 2% BM	120.8		95%	
33	CP						
34	CP						
35	CP						
36	CP						
37	CP						
38	CP						
39	AP	M/63	0%	20.7		90%	46,XY,t(9;22)(q34;q11.2)
40	AP	F/58	1%	37.2		100%	100% 46,XX,t(9;22)(q34;q11.2)x7
41	AP	M/58	2%	40.4	Myeloid		
42	AP	F/55	5%	88.3	Myeloid	85%	
43	AP	F/22					
44	AP					80%	46,XY,t(9;22)(q34;q11.2)[20]/45,-X,Y,t(9;22)(q34;q11.2)[5]
45	AP						
46	BC	M/32	47%	41.5	Myeloid	94%	t(9;22)(q34;q11.2); Trisomy 4,8, and 15, del(11), add(22), der(22)t(9;22)
47	BC	M/28	49%	12.4	Myeloid	95%	t(9;22)(q34;q11.2); Complex (4-way) PH translocation, del(5q), -Y, Trisomy 8,13, 19 and 20, t(2;16)
48	BC	F/53	24%	28.6	Myeloid	75%	t(9;22)(q34;q11.2); t(3;21), trisomy 19

49	BC	F/53	19%	7.7	Myeloid	72%	EVI1 64.5%
50	BC	M/24	21%	115.9	Myeloid	98%	t(9;22)(q34;q11.2); Inv(3q)
51	BC	F/27	35%	67.0	Myeloid	100%	46,XX,t(9;22)(q34;q11),del(17)(p13)[5]/46,XX,t(9;22)(q34;q11),add(17)(p?11),[4]/45,XX,dic,der(5)t(5;17)(q11.2;p13),t(9;22)(q34;q11),-17[3]/47-48,XX,+5,+6,der(9)add(9)(p11)t(9;22)(q34;q11),-9,-13,add(17)(p11.2),add(18)(q23),+der(22)t(9;22)(q34;q11)[6]
52	BC		63%	14.0	Myeloid	100%	48,XX,+8,t(9;22)(q34;q11),-17,+der(22),+r(mar)[15]/47,XX,+8,t(9;22)(q34;q11),-17,add der(22)(p1?2),+r(mar)[5]
53	BC	M/47	20%	235.0		100%	46,XY,t(9;22)(q34;q11)[25]
54	BC	M/46	10-15% PB	496.0		99%	46,XY,t(9;22)(q34;q11)[25]
55	BC	M/58	50% PB	303.0	Myeloid		47,XY,t(9;22)(q34;q11),+12,del(20)(q11)[4]/49,idem,+21,+der(22)t(9;22)(q34;q11)[2]/50,idem,+6,+21,der(22)t(9;22)(q34;q11)[4]
56	BC	M/51	35% PB	10.8	Lymphoid		46,XY,t(9;22)(q34;q11)[9]/46,idem,del(7)(p11.2p14),del(12)(p11.p12.1)[2]
57	BC	M/55	20-25% PB; 50% BM	43.1	Lymphoid	90%	46,XY,t(9;22)(q34;q11)[2]/46,idem,del(20)(q11)[2]/46,idem,t(13;21)(q10;q10),del(20)(q11)[3]
58	BC	M/54		447.0	Myeloid		46,XY,t(9;22)(q34;q11)[20]
59	BC	M/51	32% PB; 52% BM	97.3		96%	47,XY,+8,t(9;22)(q34;q11.2)
60	BC	F/47	25% PB; 87% BM	4.3		54%	46,XX,t(9;22)(q34;q11.2)[6]/46,XX[14]
61	BC	M/54		34.3	Myeloid	100%	46,XY,t(9;22)(q34;q11.2)
62	BC	M/51	56% PB		Myeloid		
63	BC	M/38	92%	103.1	Myeloid		
64	BC		77%	547.0	Myeloid	98%	46,XX,t(9;22)(q34;q11.2)
65	BC	M/58	>50% PB		Myeloid		
66	BC	F/55	50% BM	183.0	Myeloid		
67	BC	M/54	30% PB; 30-40% BM	60.0	Myeloid		
68	BC	M/56	75%	1.9	Lymphoid	90%	45,XY,t(9;22)(q34;q11.2),add(11)(q24),-18[9]; 45,XY,der(9)t(9;22)(q34;q11.2),add(11)q24),-18,der(22)idic(22)(q11.2)t(9;22)[2]; der(22)idic(22)(q11.2)t(9;22)[2] 45,XY,der(9)t(9;22)(q34;q11.2),add(11)(q24),-18,der(22)add(22)(p13)t(9;22)[6]; 46,XY[2]
69	BC	F/58	82%	12.5			45,XX,-7,t(9;22)(q34;q11.2),der(15)t(1;15)(q23;q24)[11]; 46,XX[9]
70	BC		93%				
71	BC	M/36	57%	18.3	Myeloid		46,XY,t(9;22;16;16)(q34;q11.2;q12;q22)[1];46,XY,der(9)t(9;22;16;16)(q34;q11.2;q12;q22),der(16)t(9;22;16;16),der(16)t(9;22;16;16),der(22)idic(22)(q11.2)t(9;22;16;16)[14]; Variant Ph+ clone exhibiting random changes[2]; 46,XY[2]
72	BC	F/56	69%	67.2			46,XX,inv(3)(q21q26),del(6)(q21q25),t(9;22;13;14)(q34;q11.2;q14;q31[20]
73	BC		80%				
74	BC	M/57					
75	BC						
76	BC	M/47		113.7	Lymphoid		
77	BC						
78	BC	F/91					

79	BC				
80	BC	F/45		256.0	Myeloid
81	BC				
82	BC				
83	BC				
84	BC				
85	BC				
86	BC				
87	BC	M/60	80% PB	40.9	Myeloid
88	BC				
89	BC				
90	BC				
91	BC				
92	BC				
93	BC	F/65			Lymphoid
94	BC				
95	BC				Lymphoid
96	BC				

Table 1. ***Available clinical data for the pre-treatment CML patient samples used in the manuscript.***

Abbreviations: CP: Chronic Phase; AP: Accelerated Phase; BC: Blast Crisis; WBC: White Blood Cell count.

2. REFERENCES TO SUPPLEMENTARY APPENDIX

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5. Neviani, P., Santhanam, R., Oaks, J.J., Eiring, A.M., Notari, M., Blaser, B.W., Liu, S., Trotta, R., Muthusamy, N., Gambacorti-Passerini, C., et al. 2007. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest* 117:2408-2421.