Supplementary Table 1. Summary of clinical, cytogenetics and molecular results for three CML patients with loss of 'normal' ABL1 and TKI resistance

Patient ID Sex/Age	Disease stage & treatment	Karyotype	FISH			Mutation in BCR-ABL1 domain
			Chromosomes	D-FISH (ABL1 and BCR)	ABL1 ex1b[1] ABL1 ex1a-11[2]	
Case 1 **M 34**	Presentation 10/2007	NA	NA	NA	NA	NA
	No CyR on STI by 02/2008	46,XY,t(9;22)(q34;q11.2)[12]/ 47,idem,+der(22)t(9;22)[3]	normal 9 der (9)t(9;22) Ph normal 22	(-) ABL1-BCR BCR-ABL1 BCR	(-) ABL1 ex1b ABL1 ex1a-11 ND	Not detected
	BMT 08/2008 CCyR & MR since 09/2008	46,XY	BCR-ABL1 negative			
Case 2 **M 41**	Presentation 10/2001	46,XY,t(9;22)(q34;q11)[20]	normal 9 der (9)t(9;22) Ph normal 22	ABL1 ABL1-BCR BCR-ABL1 BCR	ABL1 ABL1 ex1b ABL1 ex1a-11 ND	NA
	2001-2004 on INF, BU and STI BP 04/2005 BMT 07/2005	Partial cytogenetic response	Varying levels of BCR-ABL1 (+) BM cells with classical FISH pattern			Not detected
	2nd BP post BMT 08/2005 Deceased 12/2005	49,XY,+8,t(9;22)(q34;q11), del(9)(q31?q34),add(21)(q22), +add(21)(q22),+der(22)t(9;22) [8]/ 46,XX[3]	normal 9 der (9)t(9;22) Ph normal 22	(-) ABL1-BCR BCR-ABL1 x 2 BCR	(-) ABL1 ex1b ABL1 ex1a-11 x 2 ND	Not detected
Case 3[3] **F 25**	Presentation 05/2004; No response on STI BP lymphoid 10/2004 BMT 11/2004 without MR	46,XX[20][1] *Masked Ph by FISH due to t(9;22;16)(q34;q11;q13)*	normal 9 der (9)t(9;22) Ph der(16) normal 22	ABL1 ABL1 BCR-ABL1 BCR BCR	ABL1 ABL1 ex1b ABL1 ex1a-111 ND ND	NA
	Dasatinib 03/2005 with partial response 2nd BP lymphoid 12/2005 Deceased 05/2006	46,XX,t(1;3)(p36;q21), del(3)(p1?3), add(6)(p23), del(6) (q13q25), add(9)(q34), del(10)(p13), -16, add(18)(q23), +22 [cp6]/ 46,XX[94]	normal 9 der (9)t(9;22) Ph der(16) normal 22	ABL1 dim ABL1-BCR BCR-ABL1 BCR BCR	ABL1 ex1b ABL1 ex1b ABL1 ex1a-11 ND ND	Y253H

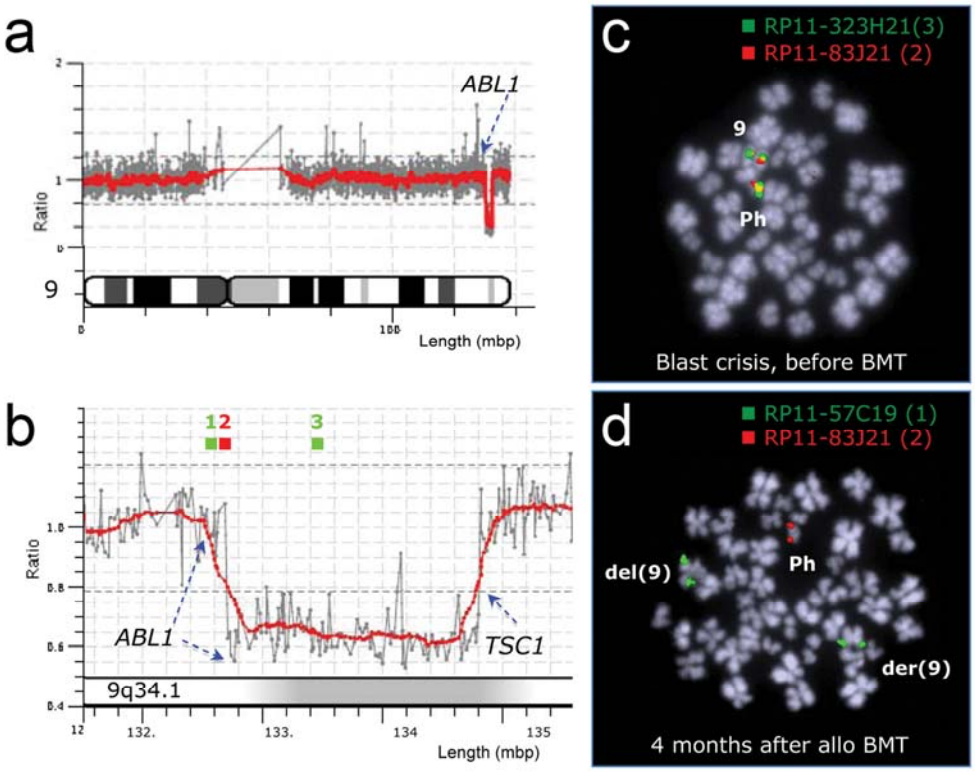
FISH was preformed with dual color/dual probe BCR/ABL1 (D-FISH, Vysis) and with BACs covering the ABL1 gene and flanking regions on both metaphase and quiescent cells. The whole chromosome painting (WCP) for chromosomes 9 and 22 was performed to assess rearrangements; ([1]) BAC probe RP11-57C19 @ 132470366-132643828 covers ABL1 exon 1b (hg18); ([2]) BAC probe RP11-83J21 @ 132641829-132818294 covers ABL1 exons 1a-11 (hg18); NA-not available; (-) missing FISH signal indicating deletion; CyR – cytogenetic remission; CCyR - complete cytogenetic remission; MR – molecular remission; BMT – bone marrow transplant; INF- interferon; BU- bisulfon; STI – imatinib; BP- blast phase. Mutations in *BCR-ABL1* kinase domain were not detected by real time PCR in case 1 and 2, while a low level clone with Y253H mutant was present in case 3, which does not encode for resistance to dasatinib (1). [3] Published before (2).

1. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BCR-ABL mutation status really matter? Blood 2009;114:5426-35.
2. Virgili A, Brazma D, Reid AG, Howard-Reeves J, Valganon M, Chanalaris A, et al. FISH mapping of Philadelphia negative BCR/ABL1 positive CML. Mol Cytogenet 2008;1:1-13.

Supplementary Table 2. FISH screening of ABL1 and BCR genes in Ph(+) CML-BP cell lines

Cell lines	Chromosomes	ABL1 (ex1b)[1] ABL1 (ex1a-11)[2]	D-FISH (Vysis) (ABL1 & BCR)	WCP (9 and 22)
MC3	normal 9	ABL1	ABL1	(+)[3]
	der (9)t(9;22)	(-)	(-)	(+)
	Ph	ABL1 (ex1a-11) x 2	BCR-ABL1 x 2	(+) x 2
	normal 22	ND	BCR	(+)
EM2	normal 9	(-)	(-)	(-)
	der (9)t(9;22)	ABL1 (ex1b) x 2	ABL1-BCR x 2	(+) x 2
	Ph	ABL1 (ex1a-11) x 4	BCR-ABL1 x 4	(+) x 4
	normal 22	ND	BCR	(+)
LAMA84	normal 9	(-)	(-)	(-)
	der (9)t(9;22)	ABL1 (ex1b) x 2	ABL1-BCR x 2	(+) x 2
	Ph	ABL1 (ex1a-11) x4	BCR-ABL1 x 4	(+) x 4
	normal 22	ND	BCR	(+)
KU812	normal 9	(-)	(-)	(-)
	der (9)t(9;22)	ABL1 (ex1b) x 2	ABL1-BCR x 2	(+) x 2
	Ph	ABL1 (ex1a-11) x 4[4]	BCR-ABL1 x 4[4]	(+) x 4
	normal 22	ND	BCR	(+)
BV173	normal 9	(-)	(-)	(-)[5]
	der (9)t(9;22)	ABL1 (ex1b) x 2	ABL1-BCR x 2	(+) x 2
	Ph	ABL1 (ex1a-11) x 2	BCR-ABL1 x 2	(+) x 2
	normal 22	ND	BCR	(+)
KCL22	normal 9	ABL1	ABL1	(+)
	der (9)t(9;22)	ABL1 (ex1b)	ABL1-BCR	(+)
	Ph	ABL1 (ex1a-11) x 2	BCR-ABL1 x 2	(+) x 2
	normal 22	ND	BCR	(+)

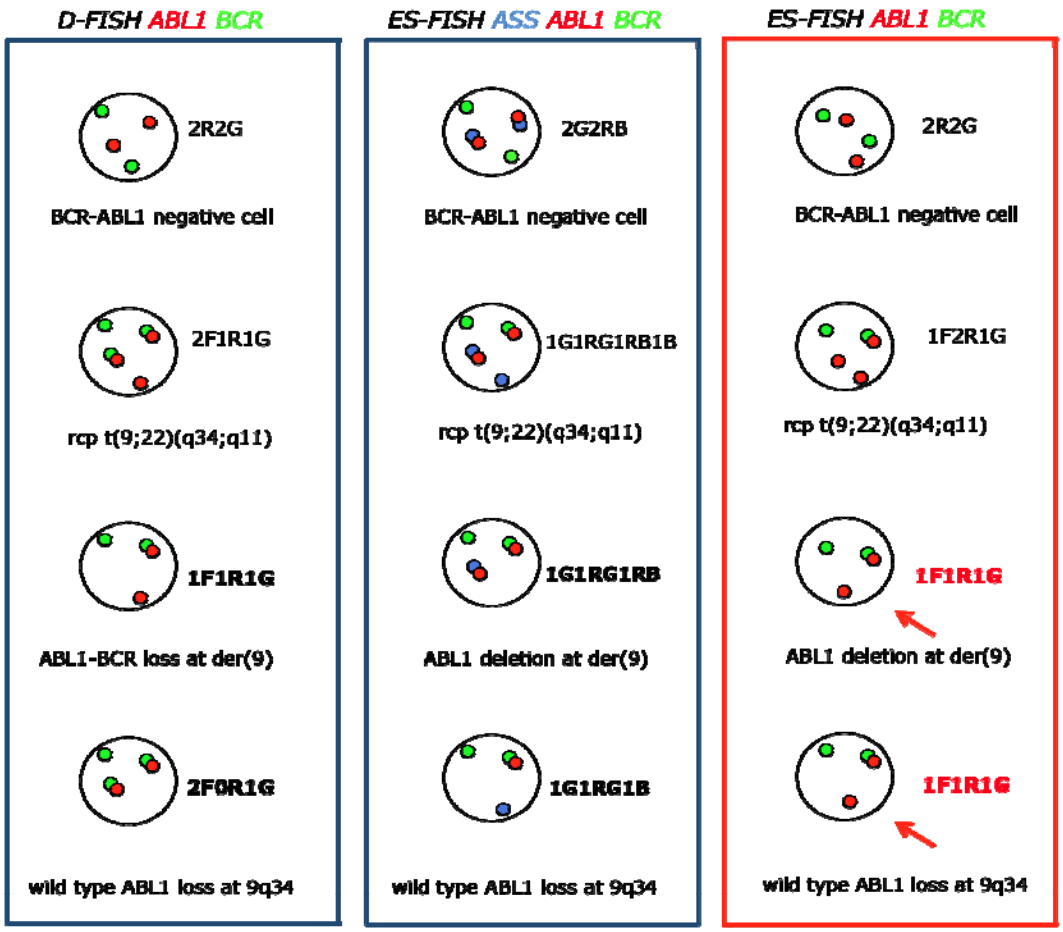
FISH was preformed with dual color/dual probe BCR-ABL1 (D-FISH, Vysis) and with BACs covering the ABL1 gene and flanking regions using metaphase cells. The whole chromosome painting (WCP) for chromosomes 9 and 22 was performed to assess rearrangements; ([1]) BAC probe RP11-57C19 @ 132470366-132643828 covers ABL1 ex 1b (hg18); ([2]) BAC probe RP11-83J21 @ 132641829-132818294 covers ABL1 ex1a-11 (hg18); ([3]) 9p11-pter in 3 copies due to t(9;16); Ph – der(22)t(9;22); ([4]) Ph tandem duplication; ND- not done; ([5]) 9p11-pter in 2 copies due to t(8;9)(q24;p11); ND-not done; (-) missing FISH signal indicating deletion; (+) FISH signal present.



Supplementary Figure 1

Deletion of the non-rearranged *ABL1* sequences in CML patient #3. A rare cryptic chromosome rearrangement was identified in this patient at presentation – a 'masked Ph' three way translocation described before(1). The patient did not achieve a complete cytogenetic remission on TKI treatment and eventually progressed to lymphoid blast crisis. Allogeneic bone marrow transplantation (BMT) was performed using a female sibling as a donor, but the patient relapsed 4 months later. **(a)** Array CGH profile of chromosome 9 showing deletion of the 9q34 region (arrow) developed during disease progression. The CGH was carried out using two BM samples of this patient taken at different time points: a sample obtained 4 months after BMT was tested against a blast crisis sample from the same patient before undergoing BMT, showing the appearance of the cryptic 9q34 deletion - i.e. the formation of the del(9) chromosome, in the former sample. **(b)** Close-up of the aCGH graph at the site of 9q34 deletion, highlighting the proximal breakpoint within *ABL1* and the distal breakpoint at *TSC1*. The deletion was estimated to be around 1.8 Mb long. The genome locations of the BAC probes used for FISH mapping are shown in green and red (1-3). **(c)** FISH on BM cells from the presentation blast crisis sample showed 2 signals from RP11-83J21 and RP11-323H21 on the normal 9 and an apparently 'normal' by G banding chromosome 22 (masked Ph). **(d)** FISH on BM cells collected 4 months after BMT, showed 2 signals from RP11-57C19 (5' *ABL1*) on both chromosomes 9 but only 1 signal from RP11-83J21 (3' *ABL1*) on the masked Ph (the expected signal on "normal" 9 is missing), confirming that the proximal breakpoint of the deletion falls within *ABL1* and validating the array results.

1. Virgili A, Brazma D, Reid AG, Howard-Reeves J, Valganon M, Chanalaris A, et al. FISH mapping of Philadelphia negative BCR/ABL1 positive CML. Mol Cytogenet 2008;1:1-13.



Supplementary Figure 2

Diagrammatic presentation of FISH signal patterns in non-dividing cells (from top to bottom) that are *BCR-ABL1* negative, Ph-positive without deletion, Ph-positive with deletion at der(9) or at the normal 9 chromosome obtained using commercial *BCR-ABL1* FISH probe sets (Vysis) – dual fusion/two color (D-FISH) on the left, single fusion/tricolor (ASS/ABL1/BCR) in the middle and extra signal system (ES-FISH) - on the right. Red arrow points to the identical signal pattern produced by the ES-FISH probe in cases with *ABL1* deletion at der(9) and with deletion of the 'wild' non-translocated allele, which renders it unfit for diagnostic purposes.