Onset of blast crisis in chronic myeloid leukemia patients in treatment-free remission

In 2022, the obtention of sustained deep molecular response (DMR) with successful subsequent treatment-free remission (TFR) is part of the new paradigm of treatment of chronic phase chronic myeloid leukemia (CP-CML).[1] Although generally known as being "feasible and safe", we observed six cases of blast crises (BC) after tyrosine kinase inhibitor (TKI) cessation, reported here.

Physicians of the French CML Study Group (Fi-LMC) were asked to report any cases of BC occurring in TFR patients; TFR being considered as ≥2 years of 4.5-log reduction (MR4.5) as recommended.[2] All patients were informed by the appropriate procedures approved by local ethical committees. Hematologic assessments were performed locally. *BCR::ABL1* transcripts were quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and reported as *BCR::ABL1/ABL1* in percent international scale (%IS),[1] TK domain mutations were screened by next-generation sequencing (NGS) and copy number variation (CNV) analyses were performed on marrow cell DNA at CP (all patients except patient 2) and BC (all patients) diagnoses. Panels included 48 "myeloid" genes (*Online Supplementary Table S1*) and 62 additional "lymphoid" genes for lymphoid blast crises (LBC) (*Online Supplementary Table S2*).

Chronic phase chronic myeloid leukemia descriptive analysis (Table 1)

Patient 1 is a 37-year-old male, with CP-CML diagnosed in 2000 treated with IFN-α+AraC from 2000-2001. He was switched to imatinib (IM) following IFN-α failure. After >5 years of IM and 2 years of DMR, he made a first cessation attempt, but lost major molecular response (MMR) at 12 months (M12). After IM resumption he switched to dasatinib 2011-2013 before attempting TFR for a second time. Patient 2 is a 56-year-old female, with CP-CML diagnosed in 2003 treated with IFN-α for 7 months and then with IM from 2003 to 2016. After 10 years of MR4.5 she stopped IM for TFR. Patient 3 is a 32-year-old female, partly reported in,[6] diagnosed in CP in 1996, treated with IFN-α+AraC from 1996 to 1999 who became intolerant while in DMR. She started IM in 2006, following molecular recurrence. After 3 years IM and 30 months MR4.5 she stopped IM in 2009 for a second attempt at TFR. Patient 4 is a 60-year-old female with CP-CML diagnosed in 2012, treated with nilotinib from 2012 to 2016. After nilotinib intolerance, while in sustained DMR, she switched to dasatinib that was also withdrawn 4 months later due to intolerance. Patient 5 is a 51-year-old male, CP-CML diagnosed in 2007 and treated with IM until 2013. After 6.25 years and 4.5 years of MR4, he stopped IM in 2013 for TFR. Patient 6 is a 58-year-old, CP-CML diagnosed in 2010 and treated with nilotinib from 2011-2014. Discovery of atherosclerosis resulted in nilotinib dose reduction from 2014 to 2016 and suspension after 5 years and >3 years in sustained MR4.5.

Blast crisis chronic myeloid leukemia descriptive analysis (Table 2)

BC occurred during the TFR phase in four of the six patients. MBC was observed in patients 5 and 6 whereas the remaining four patients were in B-lineage LBC. BC occurred in TFR phase for patients 1, 2, 4, and 6 (Figure 1A, B, D and F) whereas patients 3 and 5 experienced BC 9 months after IM resumption following MMR loss (Figure 1C and E). For patient 5, IM was switched to dasatinib after 4 months due to molecular progression and the emergence of ACA in Philadelphia chromosome-positive (Ph+) cells was demonstrated 5 months prior to BC. For patients with transformation during TFR, time to BC from TKI cessation varied from 6 to 63 months but the kinetics of BC followed similar dramatic patterns. The intervals from last MMR to BC were 1, 2, 3 and 6 months for patients 2, 5, 4, and 1 respectively. MMR loss occurred at the same time as BC for three of four patients (patients 1, 2 and 4) and belatedly following TKI cessation for these three patients (M48, M32 and M63 respectively). A Ph duplication was present at BC for patients 2 and 3. For MBC (patients 5 and 6) a 3q26 (involving *EVI1*), already present in accelerated phase prior to BC for patient 5, was identified again in BC. Patient 3 had a variant t(9;22)(q34;q11) and patient 1 a Y chromosome loss. CNV analysis detected *ABL1* amplification for patients 2 and 3. Double deletions of *IKZF1* (exons 2-8) were reported for patients 1, 2 and 4. *SETD2* deletions (whole gene or exons 16-21) were found for patients 2 and 4. Other CNV were also reported: *RhoA*, *CRBN*, *MYD88*, *CDKN2A*, *CDKN2B* deletions for patient 2, or *RUNX1*, *U2AF1* amplification for patient 3, and *XBP1* for patient 5. Of note, *XBP1* deletion was present in patient 5 whereas deletions of *SETD2*, *FLT3* and *TP53* with amplification of *IKZF1* and *MECOM* were reported in patient 1, all at CP diagnosis. Multiple gene mutations were found for MBC patients (patients 1, 4, 5 and 6). For patient 6, a nonsense mutation in *ASXL1* with a variant allele frequency (VAF) of 37% in exon 12 was observed. This clone was also present at CP (VAF: 33%) but not on a CCyR sample. For patient 5 a nonsense mutation *ASXL2* (VAF: 50%) in exon 12 was found with two *EP300* mutations. This clone was also present at CP (VAF: 25.2% for *ASXL2* and 52.7% and 50.8% for *EP300*). Interestingly, mutations were observed in *SETD2* for two patients (1 and 4) at

Table 1. Patient characteristics at diagnosis of chronic phase chronic myeloid leukemia.

Pt	Age at diag. (yrs)	WBC x10^9/L and blasts % at diag	Risk scores	Type of Trans.	Cytogenetics	NGS at diag. Mutated gene, (VAF % of mut.)	CNV	Treatment before 1st TFR	Duration of treatment before 1st TFR, mth*	Loss of MMR after 1st TFR	Treatment for 1st loss of MMR	2nd TFR	Loss of MMR after 2nd TFR	Follow-up of CP (Mo.)
1	37	57/3	Sokal low ELTS low	e14a2	46,XY,t(9;22)(q34;q11)[2]/idem,-Y[20]	No mutation	*SETD2, FLT-3, TP53* deletions. *IKZF1* and *MECOM* amplifications	IFN-α+AraC Imatinib	15+69	Yes (mth 12)	Imatinib+/-IFN-α Dasatinib	Yes	No	198
2	56	21.9/0	NA	e14a2	46,XX,t(9;22)(q34;q11)[20]	NA	NA	IFN-α Imatinib	7+157	No	/	No	/	196
3	32	67.8/0	Sokal low ELTS low	ND	46,XX[1]/46,XX,del(22)(q11),der(9)t(9;?),der14t(14;?)del(12q)[16]	No mutation	No	IFN-α +AraC	36	Yes	Imatinib	Yes	Yes (mth 10)	182
4	60	70.7/1	Sokal Int ELTS low	e14a2	46,XX,t(9;22)(q34;q11)[20]	No mutation	No	Nilotinib+ Dasatinib	39+4	No	/	No	/	107
5	51	391/2	Sokal Int. ELTS low	ND	46,XY,t(9;22)(q34;q11)[20]	***ASXL2*** (25.2%) ***EP300*** (52.7% and 50.8%)	*XBP1* deletion	Imatinib	75	Yes (mth 6)	Imatinib Dasatinib IFN-α	No	/	90
6	58	104.6/3	Sokal Int ELTS low	e14a2	46,XX,t(9;22)(q34;q11)[20]	***ASXL1*** (33%)	No	Nilotinib	60	Yes (mth 5)	/	No	/	68

Ara-C: Cytarabine; CP: chronic phase; diag: diagnosis; IFN: interferon; MMR: major molecular response; mth: months; Mut.: mutation; ND: not done; CNV: copy number variation; NGS: next-generation sequencing; Pt: patient; TFR: treatment-free remission; Trans.: transcript BCR-ABL1; VAF: value allele frequency; WBC: white blood cells. *These numbers refer to the number of months of treatments before 1st TFR mentioned in the previous column

BC, not detectable at CP. Patient 1 had two low-level *SETD2* variants whereas a stop-gain variant was estimated as 40.7% for patient 4. An additional *KMT2A* splice-acceptor variant was found for patient 1. In addition, patient 2 showed a T315I (VAF: 2%) + E255K (VAF: 5%) + Y253H (VAF: 5%) *ABL1* TK domain mutations at BC relapse (18 months after BC diagnosis). Patient 5 had three mutations at BC: F317L (VAF: 10.6%); T315I (VAF: 4.9%) and E255V (VAF:23.6%), but the patient had been on TKI for 9 months.

Allogeneic stem cell transplantation (allo-SCT) was performed for patients 3, 5, 6, and autologous-SCT (auto-SCT) in CR for patient 1. Patient 2 received EWALL induction

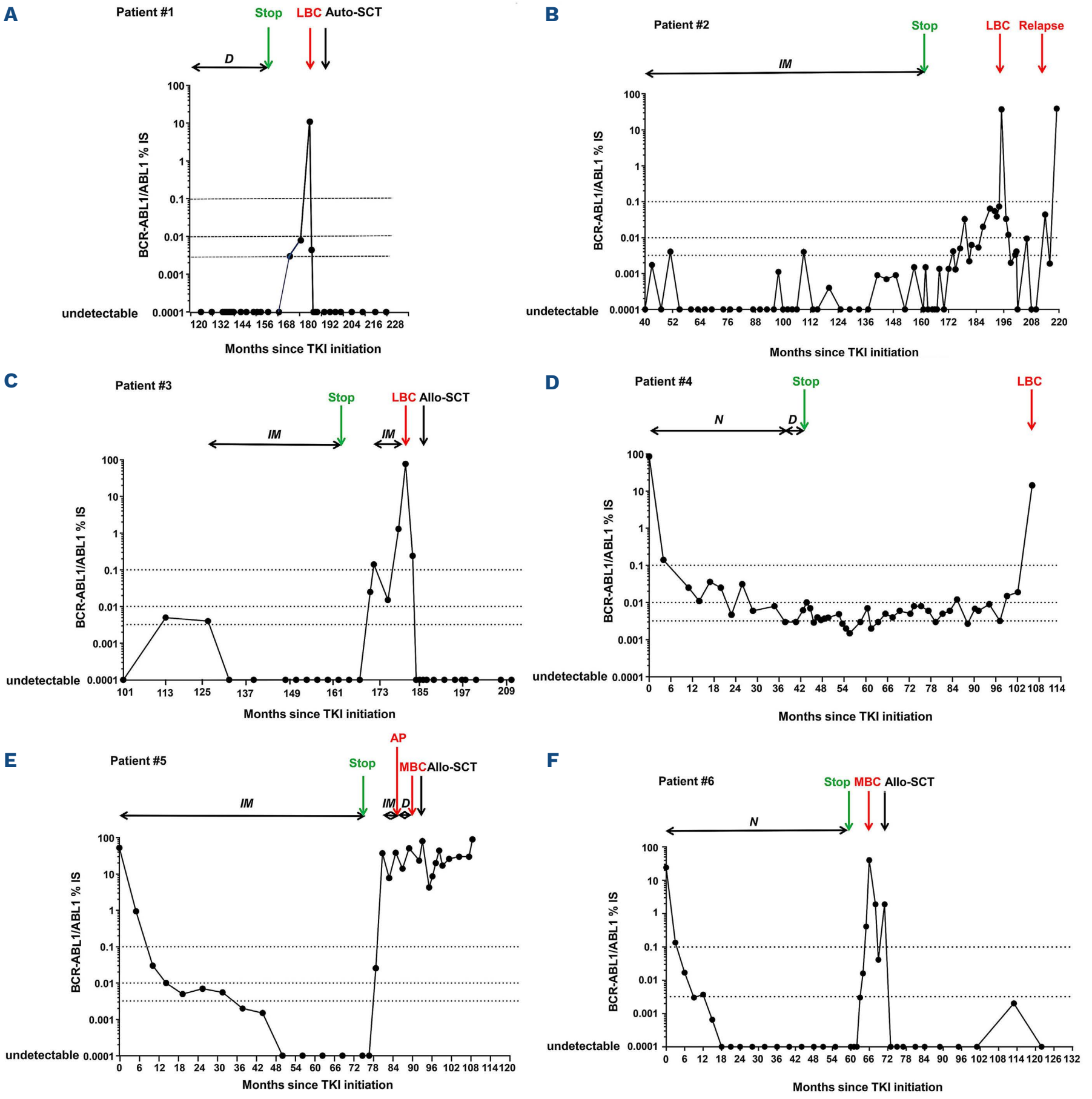

Figure 1. Kinetics of transcript aligned on international scale (*BCR::ABL1/ABL1*^{IS}). (A) Patient 1, (B) patient 2, (C) patient 3, (D) patient 4 (E) patient 4 and (F) patient 6. Months (M) corresponding to the dates from which transcript was aligned on international scale, treatment discontinuation, blast crisis, and last molecular follow-up from chronic phase chronic myeloid leukemia (CP-CML) diagnosis are indicated on the x axis. IM: Imatinib; D: Dasatinib; N: Nilotinib; IFN: interferon; Ara-C: Cytarabine; Allo-SCT: allogeneic stem cell transplantation; auto-SCT: autologous stem cell transplantation; AP: accelerated phase; LBC: lymphoid blast crisis; MBC: myeloid blast crisis; MMR: major molecular response; DMR: deep molecular response (i.e., MR4 and MR4.5).

Table 2. Patient characteristics at onset of blast crisis.

Pt	Age at BC, yr	BC onset since diag, mth	BC phenotype	Cytogenetics at BC	NGS Mutated gene, (VAF % of mut.)	CNV	ABL1 TKD mut.	Treatment of BC	Allo/Auto SCT	Follow-up since BC (Mo.)	Outcome
1	53	198	Lymphoid	46,XY,t(9;22)(q34;q11)[2]/idem,-Y[20]	***SETD2*** (1% and 1.3%) ***KMT2A*** (5.2%)	*IKZF1* (e2-8)(x2), deletions	No	Vincristine + DXM + Nilotinib then IV MTX + Ara-C	Auto-SCT	41	Alive in CR
2	72	196	Lymphoid	47,XX,del(3)(p11),t(9;22) (q34;q11), +der22 t(9;22)(q34;q11)[6]/46,XX[9]	No mutation	*RhoA, CRBN, MYD88, SETD2, CDKN2A*(*e1*) *CDKN2B(e2)IKZF1 (e2-8*)((x2) deletions *ABL1* amplification	T315I E255K Y253H	Vincristine + DXM + Nilotinib	/	25	Alive in relapse
3	48	181	Lymphoid	46-49,XX,+X,-13,-14,der(22)t(9;22)(q34;q11)X2,3-5mar[cp20]/46,XX[1]	No mutation	*ABL1, RUNX1, U2AF1* amplifications	No	Vincristine + DXM + Dasatinib then Nilotinib alone	Allo-SCT	29	Alive in CR
4	68	106	Lymphoid	46,XX,t(9;22)(q34;q11)[11]/47, idem,+X[7]/46,XX[10]	***SETD2*** (40.7%)	*SETD2* (e16-21) *IKZF1* (e2-8)(x2) deletions	No	Vincristine + DXM + Ponatinib then Dasatinib	/	1	Alive in CR
5	58	90	Myeloid	46,XY,t(2;3)(p21;q26); t(9;22)(q34;q11)[20]	***ASXL2*** (50%) ***EP300*** (51.8% and 45.5%)	*XBP1* deletion	F317L T315I E255V	Idarubicin + Ara-C + Ponatinib	Allo-SCT	16	Death
6	63	66	Myeloid	46,XX,inv(3)(q21;q26), t(9;22)(q34;q11)[2]/46,XX[19]	***ASXL1*** (37%)	No	No	Daunorubicin+ Ara-C + Ponatinib	Allo-SCT	55	Alive in CR

Ara-C: Cytarabine; BC: blast crisis; CNV: copy number variation, e: exon; CR: complete remission; diag.: diagnosis; DXM: dexamethasone; mth: months; MTX: methotrexate; NGS: next - eneration sequencing; Pt: patient; auto-SCT: autologous stem cell transplantation; allo-SCT: allogeneic SCT; TKD: tyrosine kinase domain; VAF: variant allele frequency; Yr: years.

chemotherapy+nilotinib. A stroke during induction led to dose interruption. Relapse was observed at 18 months from induction with *BCR::ABL1* at 0.04% IS in the blood and 9.5% IS in the marrow. She received four injections of vincristine/dexamethasone+ponatinib but acute pancreatitis onset led to a switch to dasatinib. Blasts and transcripts progressed particularly from the T315I clone (VAF: 100%) at latest follow-up. Patient 5 was refractory to induction and received hydroxyurea+6-mercaptopurine+ponatinib, resulting in a partial response, a sibling donor allo-SCT was performed after conditioning with clofarabine+cytarabine+cyclophosphamide+busulfan+ATG. The patient progressed after allo-SCT and received three DLI+dasatinib with no success. Haplo-SCT with his daughter was further proposed. Unfortunately, thrombotic microangiopathy and multiple infections resulted in death. At latest follow-up the three other patients are in sustained DMR >12 months after transplant (M37, M25, M49) (Figure 1A, C and F). After 1.5 months follow-up, patient 4 is currently in cytological remission and recovering from induction chemotherapy with dexamethasone+vincristine+ponatinib and has been switched to dasatinib due to liver toxicity.

BC currently is an exceptional event during the course of CML with 0.7-4.5% of CP-CML patients on IM front-line treatment progressing to BC3 especially during early years.[4] These progressions occur in patients with secondary resistance or suboptimal response or failure to TKI,[1] and are myeloid in 75%, or lymphoid in 25% of the cases.[11,13] The prognosis of BC remains poor[4,5,13] despite intensification procedures. According to the large number of patients enrolled, France is currently a pioneer in TFR clinical studies (≥600 patients). Based on these studies we estimate the risk of BC in TFR as being very low, below 0.1%. Until now, only two cases have been reported.[6,7] We hereby report six cases occurring after 41 (range, 6-124) months median sustained MR4.5 prior to cessation and for four of these six cases after a median of 40 months of cessation, while the two remaining patients went into BC following TKI resumption after 6 and 11 months of TFR. Interestingly, four of six BC were lymphoid which is not the current pattern of BC seen in TKI first-line treatment (75% myeloid[8,13]), and all of the cases with one exception (patient 5) occurred suddenly as seen in BC cases observed in patients in cytogenetic response on imatinib or IFN-α[9,17] and in Al Fayez *et al.* in TFR.[7] The same clone as identified by identical *ASXL-1* mutation (patient 6) or a derived sub-clone identified by identical *ASXL-2* and *EP300* mutations, with clonal evolution (patient 5) were detected again at BC. This suggests that stem cells, had survived the various TKI challenges and although undetectable, had remained unstable and capable of promoting disease transformation. Clearly, the malignant cells or an aggressive subfraction of them had not been eradicated. In addition, these two patients harbored a myeloid phenotype. *ABL-1* mutations may be observed in up to 80% of BC cases and while occurring in late-CP patients, are associated with a greater likelihood of progression. Other mutations or CNV are historically known to be associated with progression,[10] particularly genes involved in myeloid (~25% *TP53* mutated or deleted[11]) or lymphoid phenotypes (50% *p16* deleted). Recently, other genes have been identified as being involved at CP diagnosis as well as at BC diagnosis, such as *RUNX-1, IKZF1, ASXL1, DNMT3A, SETBP1, WT1, TET2, IDH1, NRAS, KRAS, CBL*.[12-14] In this article, mutation(s) and/or CNV were identified in all the BC patients, which is a common finding, however, in two of six patients some mutations/deletions in two genes, *EP300* (patients 3 [LBC] and 5 [MBC]) and *SETD2* genes (patients 1 and 4, [both LBC] were found to have recurred. These two genes are involved in epigenetic regulations and are rarely reported in *de novo* lymphoblastic B-ALL (~3.86-10% of cases[15,16]) or in CML-BC (<5%[13]). Whether or not these represent BC-TFR-related markers requires further investigation. Complex copy number alterations were found in two of six patients, comparable to that of Ochi *et al.*[13] TKI probably exert sustained therapeutic pressure on residual leukemia stem cells and progeny, thus preventing overt genetic instability for being induced in *BCR::ABL1*$^+$ stem cells.[8]

These six cases underline the necessity for sustained long-lasting molecular follow-up for patients in TFR.

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Disclosures

FEN is a consultant for Incyte Biosciences, Sun Pharma Ltd, and Kartos Inc; is a speaker for Novartis, Incyte Biosciences and a board member for Novartis, Pfizer and Incyte Biosciences. SD is a speaker for Novartis and Incyte Biosciences. JMC is a speaker for Novartis and Incyte Biosciences. IS is a speaker for Incyte Biosciences. PCM is a speaker for Novartis, BMS and Pfizer. LL is a speaker for Novartis, Incyte Biosciences, BMS, Pfizer and Amgen. FXM is a consultant for Novartis and a speaker for Incyte Biosciences, BMS, Novartis and Pfizer. SH, PFG, YLB, FB, PC, CLJ, HM, KC, and LR have no conflicts of interest to disclose.

Contributions

SD, FEN compiled the data and wrote the article. FB, PC, PCM, CLJ, LL, HM, LR, FXM, DR and FEN enrolled the patients and conducted the follow-up. SD, SH, JMC, PFG, YLB and IS performed molecular follow-up. SH performed NGS and CNV analysis. All the authors contributed to the collection of data, have proofread the manuscript and agree on its content.

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Data-sharing statement

Anonymous clinical and molecular data were collected and entered in password-protected Excel worksheets and files and exchanged between the first and last author securely. They are available to third-party individuals with a password via two separate mails upon request to the corresponding author.

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