EBF1-PDGFRB fusion in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL)

DOI:
10.1182/blood-2015-09-670166

Document Version
Accepted author manuscript

Blood First Edition Paper, prepublished online February 12, 2016; DOI 10.1182/blood-2015-09-670166

EBF1-PDGFRB fusion in paediatric B-cell precursor acute lymphoblastic leukaemia (BCP-ALL): genetic profile and clinical implications

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Short title: *EBF1-PDGFRB* fusion in BCP-ALL

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Word counts

Abstract = 200 words
Text =1190
Figure/table =2
References =20

Key points

- *EBF1-PDGFRB* fusion accounts for ~0.5% BCP-ALL and 2.7% B-other subtype
- *EBF1-PDGFRB* positive patients are MRD positive/slow early responders who respond to imatinib

Abstract

The *EBF1-PDGFRB* gene fusion accounts for <1% B-cell precursor acute lymphoblastic leukaemia and occurs within the Philadelphia-like ALL subtype. We report 15 *EBF1-PDGFRB* positive patients from UK childhood ALL treatment trials (ALL97/99, ALL2003, UKALL2011). The fusion arose from interstitial deletion of 5q33 (n=11), balanced rearrangement (n=2) or complex rearrangement (n=2). There was a predominance of females (n=11), median age of 12 years and median white cell count of 48.8 x10^9/l. Among 12 patients who achieved complete remission on earlier trials (ALL97/99 and ALL2003), 10 were minimal residual disease (MRD) positive at the end of induction and 7 relapsed 18-59 months after diagnosis. The majority (9/12) remain alive 6-9 years post diagnosis. There are reports of *EBF1-PDGFRB* positive patients, refractory to conventional chemotherapy, who achieve complete response when treated with the tyrosine kinase inhibitor, imatinib. These findings have prompted screening for *EBF1-PDGFRB* in patients entered to the current trial, UKALL2011: who fail induction, fail to achieve remission by day 29 or remain MRD positive (>0.5%) at week 14. Two UKALL2011 patients, positive for *EBF1-PDGFRB,* received imatinib: one died 6 months after a matched unrelated bone marrow transplant, due to undefined encephalopathy, while the other remains in remission 10 months post diagnosis.

Introduction

Chromosomal abnormalities are the hallmark of B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) and have prognostic relevance.[1] Integration of minimal residual disease (MRD), cytogenetics, age and white blood cell count (WBC) into risk stratification for treatment of childhood BCP-ALL has contributed significantly to improved survival rates.[2] Approximately 25% childhood BCP-ALL harbour none of the established chromosomal abnormalities, termed B-other. A novel subgroup of B-other BCP-ALL has been described, known as Philadelphia-like (Ph-like) or *BCR-ABL1*-like ALL.[3,4] Although they lack the *BCR-ABL1* fusion, these patients have gene expression profiles and high relapse risk similar to *BCR-ABL1* positive ALL. A subset of Ph-like patients harbour tyrosine kinase activating gene fusions,[5] notably *EBF1-PDGFRB* accounting for ~8%,[6] of which a proportion, refractory to conventional therapy, achieved complete response when treated with the tyrosine kinase inhibitor (TKI), imatinib.[6-8] Here we present genetic and clinical data from 15 *EBF1-PDGFRB* positive patients treated on UK childhood ALL treatment trials.

Methods

Patients were BCP-ALL and registered on UK treatment trials (Supplementary Figures 1 and 2): MRC ALL97/99 (1997-2002) (1-18 years), UKALL2003 (2003-2011) (1-24 years), UKALL2011 (2012-present) (1-24 years) (www.isrctn.com/ISRCTN64515327) and relapse trial, ALLR3 (2003-2013), with ethical approval and consent in accordance with the Declaration of Helsinki.[9-11] Demographic, clinical and treatment details were collected by the Clinical Trial Service Unit, Oxford, UK. Cytogenetic analysis was performed in regional cytogenetics laboratories and collated by the Leukaemia Research Cytogenetics Group. *EBF1-PDGFRB* was determined by fluorescence *in situ* hybridization (FISH), using a commercial *PDGFRB* break-apart probe (Cytocell, UK) (Supplementary Figure 3). Involvement of *EBF1* was confirmed using in-house, break-apart probes (Supplementary Figure 3). Copy number changes were identified by Multiplex Ligation-dependent Probe Amplification (MLPA) (SALSA MLPA kit P335 *IKZF1*, MRC Holland, The Netherlands) (n=11) (Supplementary Figure 4)[12,13] and SNP6.0 (n=9) (AROS Applied Biotechnology, Aarhus, Denmark, and Genotyping Console, Affymetrix, USA), mapped to human reference sequence GRCh37. The presence of EBF1-PDGFRB fusion transcripts was validated by RT-PCR (n=9) (Supplementary Figure 5).

Results and Discussion

Patient data are provided in Table 1 and Supplementary Table 1 (their origin identified in Supplementary Figures 1 and 2). For 11/15 patients (nos. 1, 2, 4, 7-14), FISH for *EBF1* and *PDGFRB* showed signal patterns consistent with deletion of 5q33, with breakpoints within *EBF1* and *PDGFRB* (Figure 1, Supplementary Figure 3), as previously reported.[5,7,8] Deletion of *EBF1* exon 16 was seen by MLPA in 8 patients tested and confirmed by SNP6.0 in 6 of them (Supplementary Figure 4, Supplementary Table 1). In cases with available RNA, EBF1-PDGFRB transcripts were confirmed by RT-PCR (n=9, nos. 6, 7, 9-15). *EBF1* exon 15 was fused to *PDFGRB* exon 11, confirmed by Sanger sequencing (n=4), except for patient 13 with an alternative breakpoint (Supplementary Figure 5).

Two cases showed signal patterns consistent with balanced rearrangements by both *PDGFRB* and *EBF1* FISH (nos. 5 and 6) (Figure 1, Supplementary Figure 3). Expression of the EBF1-PDGFRB transcript was confirmed by RT-PCR in patient 6 (Supplementary Figure 5). This patient showed cytogenetic evidence of additional material on the long arm of chromosome 5 (5q). With no copy number abnormalities of chromosome 5 detected by SNP6.0 (data not shown), the karyotype was suggestive of a translocation, t(5;5)(q33.1;q33.3) (Figure 1). However, poor metaphase quality both in this case and in patient 5 precluded confirmation of this subtle abnormality.

Despite showing an apparently balanced rearrangement of *PDGFRB* and *EBF1* by FISH (Supplementary Figure 3), SNP6.0 of patient 3 revealed a series of non-consecutive deletions along 5q, indicating that complex rearrangements may have resulted in the disruption of these genes (Figure 1).

In patient 15, the EBF1-PDGFRB transcript was detected by RT-PCR (Supplementary Figure 5), although FISH showed a balanced abnormal signal pattern for *PDGFRB* and unbalanced abnormal pattern for *EBF1* (Supplementary Figure 3). Due to lack of material, the karyotypic nature of the rearrangement was not determined.

Among the genes tested by MLPA, those often deleted in BCP-ALL were also deleted in this cohort:[3-6,12] *PAX5* (n=5), *IKZF1* (n=3) and *CDKN2A/B* (n=3) (Supplementary Table 1).

In contrast to ALL overall, there was a female predominance (11:4 males), median age was 12 years (range 4-18, >10 years, n=9) and median WBC was 48.8 $x10^9$/l (range 3.4-345 $x10^9$/l, >50$\times10^9$/l, n=9). From an unselected cohort of 287 B-other patients from UKALL2003 (Supplementary Figure 1) 2.7% (n=8) were *EBF1-PDGFRB* positive, indicating an incidence of ~0.5% in BCP-ALL overall, based on the mutual exclusivity of this translocation and its occurrence restricted to the B-other group.

Among 13 patients from the early trials (ALL97/99, n=3, ALL2003, n=10), 12 achieved complete remission (CR), whereas one failed to remit and died after 2 months. Ten remitters had slow response (all ALL2003) and were minimal residual disease (MRD) positive at the end of induction, including 8 with extremely high MRD levels (>10%). Consequently, 8/13 received augmented post induction therapy (regimen C)[10] and 3 had bone marrow transplants in first CR. Seven patients relapsed 18-59 months after diagnosis. The majority (9/13) remain alive at 6-9 years, while 4 died (non-remitter, n=1; infection in remission, n=1; relapse, n=2). Recently, the Dutch Childhood Oncology Group reported 4 *EBF1-PDGFRB* positive patients with one relapse and three remaining in first CR at 6-19 years.[14] Clinical outcomes for both the latter study and ours correlate with the Children's Oncology Group, who showed that although Ph-like patients had poor initial response to treatment, their survival rate improved when treated with MRD-based risk-directed therapy.[15]

Influenced by these findings, patients entered to the current UK trial, UKALL2011, who belong to the B-other group, show induction failure, fail to achieve CR by day 29 or remain MRD positive (>0.5%) at week 14, are screened for the *EBF1-PDGFRB* fusion (Supplementary Figure 2). Thus far, 2 patients have tested positive for *EBF1-PDGFRB*, including a 5 year old female (no. 14) who was MRD positive at day 35 (30%), and 8 year old female (no. 15) with poor response to induction therapy. Detection of *EBF1-PDGFRB* in patient 14 prompted her withdrawal from the trial and off-label addition of

imatinib to an EsPhALL chemotherapy regimen.[16] After 5 weeks of Protocol 1B consolidation therapy with imatinib her MRD levels fell to 6% and she became MRD negative after 3 post-induction treatment blocks (HR1, HR2 and HR3). Given her persistent MRD, she subsequently received a matched unrelated bone marrow transplant, but unfortunately died 6 months later due to undefined encephalopathy. Patient 15 had high levels of MRD post-induction (30%). She was treated with imatinib in addition to Augmented BFM consolidation[10] and became MRD negative at week 14. She continues in CR at 10 months post-diagnosis and is currently receiving maintenance therapy and continuous imatinib.

This study reports the largest cohort of *EBF1-PDGFRB* patients to date. It demonstrates the range of genetic mechanisms by which the fusion may occur. *EBF1-PDGFRB* fusion is associated with female sex, older age and a varied outcome on UK treatment trials. Although these patients had high levels of MRD and tendency to relapse, there was also evidence of durable remission, especially with intensive chemotherapy. Evidence from this study and others[6-8] suggests that these patients respond effectively to imatinib. It is interesting to speculate whether treatment with TKI may avoid the need for intensive chemotherapy to achieve a cure.

Author Contributions

C.S., A.V.M. and C.J.H designed the study; C.S., S.L.R., A.E., J. Murray, S.R., and C.W. carried out the cytogenetic and molecular testing; C.S, L.C., C.J.H and A.V.M analysed and interpreted data. J.Moppett, M.C., O.T., C.A.P., V.S., N.G., and A.V. provided clinical and follow-up data; C.J.H. and A.V.M. provided financial and administrative support and all authors wrote and provided final approval of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Acknowledgments

The authors wish to thank member laboratories of the UK Cancer Cytogenetic Group for providing cytogenetic data and material. Primary childhood leukemia samples used in this study were provided by the Leukaemia and Lymphoma Research Childhood Leukaemia Cell Bank. This work was supported by Bloodwise (formerly Leukaemia and Lymphoma Research, UK).

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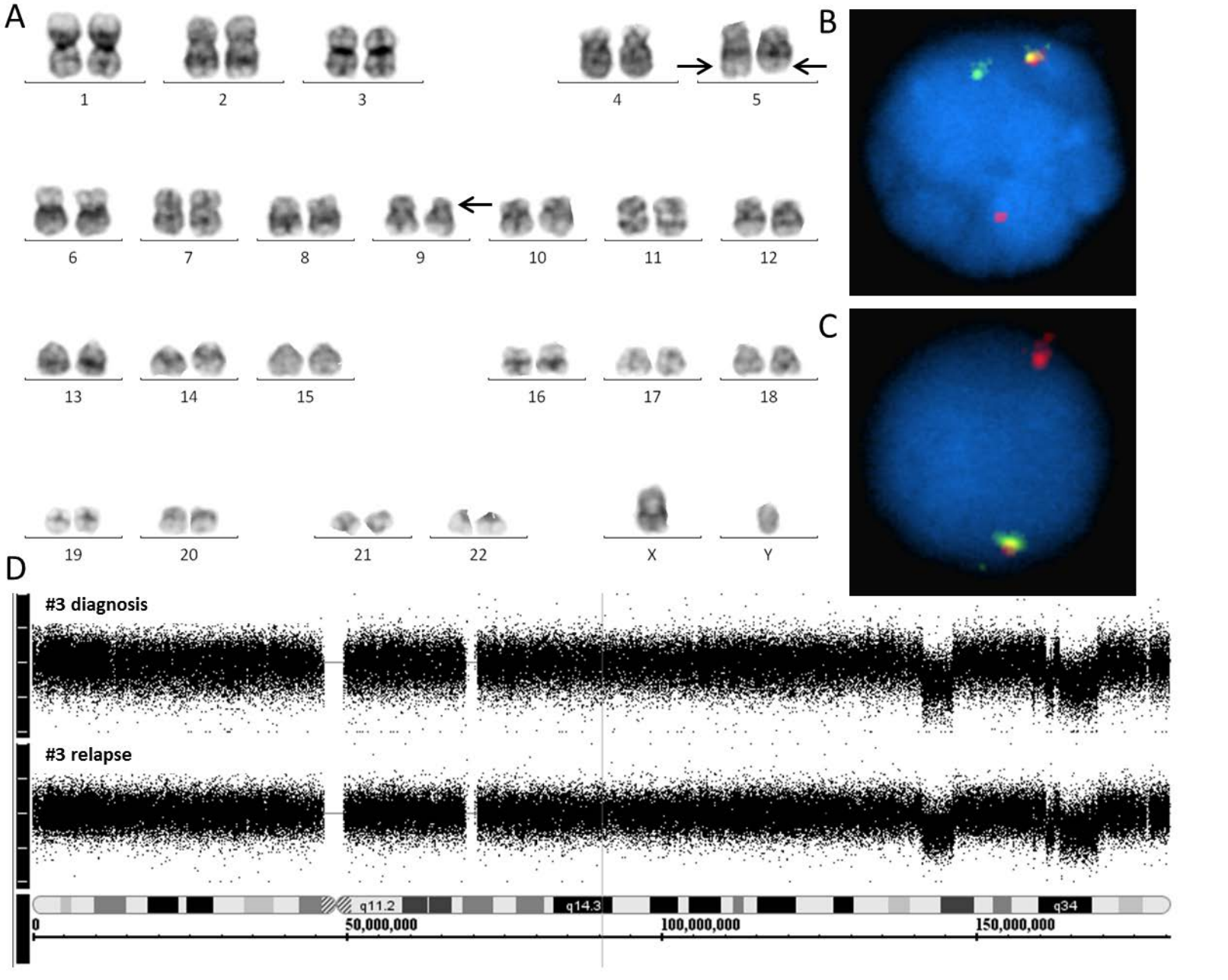
Figure legend

Figure 1 A) Karyogram from the diagnostic bone marrow of patient 6 showing that both copies of chromosome 5 are abnormal, suggesting the presence of a balanced translocation, t(5;5)(q33.1;q33.3) (upper arrows). His karyogram also shows a deletion of the short arm of chromosome 9 (lower arrow). B) FISH using the *EBF1* break-apart probe (described in Supplementary Figure 3B) showing a balanced rearrangement (patient 6) and C) showing an unbalanced rearrangement consistent with the 5q33 deletion (patient 7). D) SNP6.0 profile of chromosome 5 in patient 3, showing a series of deletions along the long arm of chromosome 5 consistent with complex rearrangements, such as chromothripsis. This profile was conserved between diagnosis and relapse.

Patient no.	Trial	Age (years)	Sex	WBC x10^9/l	Complete remission	Slow early responder*	Post induction MRD level	Treatment Allocation†	First remission transplant	Relapse	Months to relapse	Relapse therapy	Status
1	ALL97	6	F	212	Yes	Yes**	ND	Standard therapy		BM & CNS	31	Allogenic transplant	Died from relapse (5 years)
2	ALL97/99	12	F	17	Yes	Yes	ND	Regimen B -> C		No			Alive (7.6 years)
3	ALL97/99	12	F	5	Yes	Yes	ND	Regimen B -> C		BM	39	ALLR3, Intermediate risk, Mitoxantrone, MUD	Alive (7.4 years)
4	UKALL2003	13	F	60	Yes	Yes	>10%	Regimen B -> C	Allogeneic	BM & Bone	27	NK	Alive (9.3 years)
5	UKALL2003	4	F	49	Yes	No	>0.1%	Regimen B -> C		No			Alive (7.5 years)
6	UKALL2003	11	M	70	No	Yes	>10%	Regimen B -> C	MUD	No			Alive (7.3 years)
7	UKALL2003	19	M	8	Yes	Yes	>10%	Regimen B		CNS	40	Allogenic transplant	Alive (6.8 years)
8	UKALL2003	8	M	12	Yes	No	>10%	Regimen A		Skin	59	ALLR3, Standard risk, Mitoxantrone	Alive (6 years)
9	UKALL2003	18	F	3	Yes	Yes	>10%	Regimen B -> C	Allogeneic	No			Alive (5.2 years)
10	UKALL2003	14	M	26	Yes	Yes	>10%	Regimen B -> C		BM	18	ALLR3, High risk , Clofarabine	Died from relapse (1.8 years)
11	UKALL2003	8	F	34	Yes	Yes	>10%	Regimen A -> C		BM	50	Allogenic transplant	Alive (3.8 years)
12	UKALL2003	16	F	68	Yes	NK	>0.01%	Regimen B		No			Died in remission from infection (4 months)

13	UKALL2003	16	F	102	No	Yes	>10%	NA				Primary refractory disease - treated on ALLR3 as high risk with clofarabine	Died from infection with active disease (2 months)
14	UKALL2011	5	F	152	Yes	Yes	>10 %	Imatinib + EsPhALL	Allogeneic (MRD 0.003 % pre BMT)	No			Died in remission 6 months post SCT from undefined encephalopathy
15	UKALL2011	8	F	345	Yes	Yes	> 10 %	Regimen B -> Regimen C + Imatinib (at EOI)	No	No			Alive in CR at 10 months on maintenance therapy

Table 1 Clinical and survival data for *EBF1-PDGFRB* positive patients. * Slow early responder, >25% blasts at first assessment (day 8 for regimen B and day 15 for regimen A);** day 8 - 77% blasts. †Full details of the treatment for ALL97/99 and UKALL2003 regimens have been published.[9,10] Briefly, in ALL99 and UKALL2003, patients were assigned to regimen A or B based on whether they were National Cancer Institute (NCI) standard (<10 years old and WCC <50 × 10^9/L) or high risk (≥10 years old or WCC >50 × 10^9/L), respectively and were randomised to regimen C based on MRD stratification. MUD= matched unrelated donor BM= bone marrow, CNS= central nervous system.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Prepublished online February 12, 2016;
doi:10.1182/blood-2015-09-670166

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.