bjh research paper

Does *BCR/ABL1* positive acute myeloid leukaemia exist?

Ellie P. Nacheva,[1] Colin D. Grace,[1] Diana Brazma,[2] Katya Gancheva,[1] Julie Howard-Reeves,[2] Lena Rai,[1] Rosemary E. Gale,[3] David C. Linch,[3] Robert K. Hills,[4] Nigel Russell,[5] Alan K. Burnett[4] and Panagiotis D. Kottaridis[2]

[1]UCL Med School, Royal Free Campus, London, [2]Department of Haematology, Royal Free NHS Trust, London, [3]UCL Cancer Institute, London, [4]Department of Haematology, University of Cardiff, Cardiff and [5]Department of Haematology, University of Nottingham, Nottingham, UK

Received 6 November 2012; accepted for publication 25 January 2013

Correspondence: Professor Ellie P. Nacheva, Department of Academic Haematology, UCL Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.
E-mail: e.nacheva@ucl.ac.uk

Summary

The *BCR/ABL1* fusion gene, usually carried by the Philadelphia chromosome (Ph) resulting from t(9;22)(q34;q11) or variants, is pathognomonic for chronic myeloid leukaemia (CML). It is also occasionally found in acute lymphoblastic leukaemia (ALL) mostly in adults and rarely in *de novo* acute myeloid leukaemia (AML). Array Comparative Genomic Hybridization (aCGH) was used to study six Ph(+)AML, three bi-lineage and four Ph(+)ALL searching for specific genomic profiles. Surprisingly, loss of the *IKZF1* and/or *CDKN2A* genes, the hallmark of Ph(+)ALL, were recurrent findings in Ph(+)AML and accompanied cryptic deletions within the immunoglobulin and T cell receptor genes. The latter two losses have been shown to be part of 'hot spot' genome imbalances associated with *BCR/ABL1* positive pre-B lymphoid phenotype in CML and Ph(+)ALL. We applied Significance Analysis of Microarrays (SAM) to data from the 'hot spot' regions to the Ph(+)AML and a further 40 *BCR/ABL1*(+) samples looking for differentiating features. After exclusion of the most dominant markers, SAM identified aberrations unique to *de novo* Ph(+)AML that involved relevant genes. While the biological and clinical significance of this specific genome signature remains to be uncovered, the unique loss within the immunoglobulin genes provides a simple test to enable the differentiation of clinically similar *de novo* Ph(+) AML and myeloid blast crisis of CML.

Keywords: philadelphia chromosome, acute myeloid leukaemia, blast crisis chronic myeloid leukaemia, genomic arrays, immunoglobulin gene loss.

The t(9;22)(q34;q11) or its variants result in the formation of the Philadelphia (Ph) chromosome and the chimeric *BCR/ABL1* fusion gene, encoding a constitutively active tyrosine kinase with oncogenic properties. While this chromosome anomaly is pathognomonic for CML, it is also found in precursor B acute lymphoblastic leukaemia, Ph(+) ALL, especially in adults. Less than 1% of newly diagnosed adults with acute myeloid leukaemia (AML) have the Ph chromosome, Ph(+) AML (Soupir *et al*, 2007) and when a proliferation of *BCR/ABL1* positive blasts is found on presentation, the distinction between CML blast crisis (CML/BC), AML, ALL or bi-lineage acute leukaemia (Bi-AL) may be not clear. Although some features of the bone marrow morphology, immunophenotype and *BCR/ABL1* molecular transcript may suggest a diagnosis, ultimately the distinction between Ph(+)AML and CML presenting in acute phase may be whether chronic phase CML develops after therapy. Indeed, *BCR/ABL1*(+) AML is not included in the World Health Organization classification (Swerdlow *et al*, 2008) as a distinct entity, mostly due to lack of firm criteria to distinguish it from CML myeloid blast crisis (CML/BCM) at presentation.

In a recent multi-institutional retrospective analysis of 16 cases with *de novo* Ph(+) AML, it was shown that the patients share many clinical, pathological, and genetic features with CML/BCM (Soupir *et al*, 2007). It appeared that Ph(+) AML presented less often with splenomegaly, lacked significant blood or marrow basophilia, and had a lower bone marrow myeloid/erythroid ratio. Major additional cytogenetic features characteristic of CML were shown to be less common in Ph(+) AML and response to tyrosine kinase inhibitor (Imatinib) treatment was of limited duration. Nevertheless the authors were unclear whether the clinical, pathological, and cytogenetic differences found between Ph(+) AML and CML/BCM represent true entity-defining characteristics or merely differences reflecting a more rapid clinical presentation of the same disease.

British Journal of Haematology, 2013, **161,** 541–550
First published online 25 March 2013
doi:10.1111/bjh.12301

The advent of array comparative genomic hybridization (aCGH) opened a new possibility to screen an entire genome for cryptic imbalances that may lead to gene dysfunction in cancer cells. This proved to be the case for many haematological disorders including Ph(+)ALL and CML, where deletions of *CDNK1A/B (p16)* and/or *IKZF1* (*Ikaros)* were shown to be unique recurrent features (Mullighan *et al*, 2007; Usvasalo *et al*, 2008; Matteucci *et al*, 2010; Nacheva *et al*, 2010). Here we present a comparative study of 13 samples classified as Ph(+)AML, Ph(+)ALL or Ph(+)Bi-AL leukaemia along with 40 previously analysed samples of Ph(+) ALL, CML chronic and blast phase as well as 10 disease-free controls using high resolution aCGH analysis, posing the question: does Ph(+) AML carry unique, entity defining genome imbalances? We set out to search for genomic features that differentiate clinically similar *BCR/ABL1* positive presentations, the optimal treatments for which are very different even in the era of tyrosine kinase inhibitors (Vardiman, 2009).

Materials and methods

Samples

We studied bone marrow (BM) and/or peripheral blood presentation samples from nine adults with Ph positive acute leukaemia enrolled into the Medical Research Council (MRC) UK AML Trials 10, 11, 12 and 15. The selection criterion for this study was availability of good quality total genomic DNA. Six samples were identified as being of myeloid origin (five undifferentiated and one differentiated type) and three were classified as Bi-AL. The laboratory and clinical features are outlined in Table I. All samples were centrally reviewed by the MRC haematologist using May-Grünwald staining, immuno-cytochemistry and immunophenotyping data when available. None of the patients were treated with tyrosine kinase inhibitors, because they were not part of the MRC treatment protocols for AML. Six patients (Cases 1–6) failed to respond to treatment, including bone marrow transplantation and their overall survival (OS) ranged from 115 to 323 days (Table I). The OS of the remaining three patients (Cases 7–9) ranged between 3·8 and 5·1 years (Table I).

Four Ph(+) samples from the MRC UK ALL12R Trial of pre-B cell origin (CD19+, CD22+) were included in the study as representative of *BCR/ABL1*(+)ALL genomes.

Techniques

The presence of the Ph chromosome and resulting *BCR/ABL1* fusion was identified by conventional karyotyping, fluorescence *in situ* hybridization (FISH) and real time polymerase chain reaction (qPCR) as previously described (Nacheva *et al*, 2010). The immunoglobulin heavy chain (*IGH*) and T cell receptor (*TRG@*) sequencing analyses were carried out following routine protocols (Rai *et al*, 2010).

Genome investigation was conducted in several steps using standard and customized oligonucleotide array platforms (Agilent Technologies UK Limited, Stockport, Cheshire, UK). Firstly, whole genome screening of all samples was performed at uniform resolution by standard 244 K and 1 M array (Agilent Design ID 014693 and 021529) as described previously (Nacheva *et al*, 2010). The Ph(+)ALL cases were only analysed by 244 K array due insufficient material. In brief, the arrays were hybridized following the manufacturer's protocol. 500 ng of genomic test DNA was extracted from either peripheral blood or BM samples. Commercially obtained pooled normal DNA (Promega, UK, Southampton, UK) was used as reference. The arrays were scanned and features extracted using an Agilent DNA Microarray Scanner with Control Software (Agilent, version 9.5). Agilent Genomic Workbench software version 5 (ADM2, threshold 6.0) was used to visualize, detect and analyse aberration patterns from aCGH microarray profiles.

Table I. Clinical data for adults with *BCR/ABL1* positive acute myeloid and biphenotypic leukaemia

Patient	Age (years) Sex	WBC × 10^9/l	FAB type	WHO performance status	CR date	Remission days	Relapse date	OS days/years
1	44 M	81·59	AML Diff.	2	02/03/1994	80	20/05/1994	115
2	75 F	140·69	AML Undiff.	1	14/06/1996	45	29/07/1996	133
3*	28 F	78·29	AML Undiff.	4	Resistant* disease	0	Resistant disease	227
4	35 F	30·19	AML Undiff.	1	11/08/1998	83	02/11/1998	214
5	58 F	174·00	AML Undiff.	0	02/09/1998	93	04/12/1998	153
6	36 F	103·89	Bi-lineage AL	1	11/03/2004	245	11/11/2004	323
7	55 M	85·19	AML Undiff.	3	NA	NA	NA	4·3
8	52 F	63·29	Bi-lineage AL	0	NA	NA	NA	3·8
9	26 F	61·09	Bi-lineage AL	0	NA	NA	NA	5·1

NA, not available; WBC, white blood cell count; FAB, French-American-British classification; WHO, World Health Organization; CR, complete remission; OS, overall survival; M, male; F, female; AML, Acute Myeloid Leukaemia; Diff, differentiated; Undiff, undifferentiated; AL, acute leukaemia.

*Matched unrelated donor bone marrow transplant on 13/12/1998.

Next, aCGH was carried out on six Ph(+)AML samples (Cases 1–5 & 7) and one Ph(+) bi-AL sample (Case 8) using custom 15 K oligonucleotide arrays (Agilent, Design ID 0224931) that cover genome regions, aberrations of which were shown by previous studies to be specifically associated with lymphoid cell origin in *BCR/ABl1* positive cells (Nacheva *et al*, 2010). These results, together with data from 40 Ph(+) samples from a previous study obtained under the same experimental conditions, were explored for differentiating features along with 10 samples from normal healthy donors as negative controls. The 40 samples comprised of 10 Ph(+) B-ALL and 30 CML of which 10 were chronic phase, nine was myeloid, one was mixed type and 10 were lymphoid blast crisis. The chromosome profiles of the other Ph(+) cases matched the karyotype complexity of the Ph(+)AML samples (Table S1). The MultiExperiment Viewer (MeV) implementation of Significance Analysis of Microarrays (SAM) (Tusher *et al*, 2001) was used as previously (Grace & Nacheva, 2012) to search the data from the *BCR/ABL1* positive genomes. Genome addresses are given according to the genome build 36/hg18.

Results

The *BCR/ABL1* fusion was found to result from the classical t(9;22)(q34;q11) in all samples (Table II). Two of the Ph(+) ALL cases had the p190 *BCR/ABL1* fusion, all other samples had the p210 type (Table II). The Ph chromosome was seen as sole chromosome abnormality in two AML samples (Cases 1 and 4, Table II), the rest showed moderately complex karyotypes with up to four additional numerical and/or structural chromosome aberrations. These involved the short arms of chromosome 9 and 19, monosomy of 7, 9 and 22 or marker structures that remained unresolved by G banding. The chromosome changes typical of CML blast phase (i.e. major and minor route aberrations) were not seen in AML although total aberration frequencies were similar (Table II, Table S1).

Array CGH screening using both standard 244 K and 1 M platforms identified genome copy number aberrations (CNA) in all Ph(+) positive samples (Table III). These varied in size, location and recurrence, falling into two main categories: (i) chromosomal/segmental CNA involving regions of 20 Mb or more that can be detected but may not be resolved by conventional karyotyping, and (ii) cryptic aberrations that are beyond the resolution of the G banding analysis. All aberrations additional to the t(9;22)9q34;q11) found by G banding and FISH were also detected by genome array screening (Table II, Molecular karyotypes in Fig 1 and Figs S1 & S2). Whole chromosome aberrations are exemplified by monosomy 7 (Cases 6 and 8) and trisomy X (Case 7). Segmental changes are exemplified by deletions at 9p13-p24 (Cases 3 and 7); cryptic loss at 9q34 consistent with deletions of der (9)t(9;22) (Cases 6, 8 & 9) and gains of 9q34-qter & 22q11 indicative of double Ph chromosome (Case 1 and 2). In three of the six karyotypically abnormal samples of this cohort aCGH revealed an unexpected gain of the short arm of chromosome 19, where both gains and losses were detected

Table II. Summary of G banding, FISH and molecular results for adults with *BCR/ABL1* positive acute leukaemia.

Patient	Age (years) Sex	FAB type	G banding karyotype	*BCR/ABL1* FISH pattern	*BCR/ABL1* fusion type
1	44 M	AML	46,XY,t(9;22)(q34;q11)[20]	2F1R1G	p210
2	75 F	AML	46,XX,t(9;22)(q34;q11), -18, idic(Ph), +mar[15]/47, idem,+idic(Ph)[5]	3F1R1G/4F1R1G	p210
3	28 F	AML	46,XX,t(9;22)(q34;q11)[1]/45,XX, der(9)t(9;22), dic(9;20)(p1?3;p1·2), add(19)(p1?3·1),-22, der(22)?t(19;22)(p?13·1;q11)[2]/44,idem, -9[17]	3F1R1G	p210
4	35 F	AML	46,XX,t(9;22)(q34;q11)[25]	2F1R1G	p210
5	58 F	AML	46,XX,t(9;22)(q34;q11·2),der(16) t(1;16)(q23;q13)[15]/46,XX[15]	2F1R1G	p210
6	36 F	Bi-AL	45,XX,-7,t(9;22)(q34;q11)[18]/47,idem,+2mar[2]	1F1R1G	p210
7	55 M	AML	46,X,-Y,+X,add(3)(p11), der(9)del(9)(p12) t(9;22)(q34;q11), der(22)t(9;22) [11]/46,XY[19]	2F1R1G	p210
8	52 F	Bi-AL	46,XX,t(9;22)(q34;q11·2)[1]/45, idem,-7[9]	1F1R1G	p210
9	26 F	Bi-AL	46,XX,t(9;22)(q34;q11)[20]	1F1R1G	p210
10	43 M	B/ALL	46,XY,t(9;22)(q34;q11)[20]	2F1R1G	p210
11	17 M	B/ALL	46,XY,t(9;22)(q34;q11)[20]	2F1R1G	p210
12	50 M	B/ALL	46,XY,t(9;22)(q34;q11)[18]/46,XY[2]	2F1R1G	p190
13	52 M	B/ALL	46,XY,t(9;22)(q34;q11)[20]	2F1R1G	p190

FAB, French-American-British classification; FISH, fluorescence *in situ* hybridization; M, male; F, female; AML, Acute Myeloid Leukaemia; Bi-AL, biphenotypic acute leukaemia; B/ALL, B cell acute lymphoblastic leukaemia; p210, major BCR breakpoint; p190, minor BCR breakpoint; *BCR/ABL1* D-FISH, dual fusion probe with 2F1R1G signal pattern indicative of *BCR/ABL1* fusion, 3F1R1G/4F1R1G, extra *BCR/ABL1* fusion(s), 1F1R1G, deletion der(9)t(9;22).

Table III. Summary of genome array, FISH, qPCR and sequencing data for adults with *BCR/ABL1* positive acute leukaemia.

				Segmental CNA		Cryptic CNA					
Patient	Type	aCGH ID	Total CNA	Gains	Losses	*IGH* [b, c]	*VPREB1* [a]	*IGLL1*	*TRG@/TARP* [a, b]	*IKZF1* [b]	*CDKN* [b]
1	AML	356* (368)	20	NF	NF	LOH	N	LOH	N	N	N
2	AML	361* (369)	45	4p16, 7p22, 19p13, 9q34/qter[b], 22p/q11[b]	18q21-23	LOH	N	LOH	N	N	N
3	AML	319, 360* (365)	45	19p13, 9q34/qter[b], 22p/q11[b]	9p12/p24, 20p11/p13	LOH	LOH	N	LOH	LOH	LOH
4	AML	315, 316* (362)	31	NF	NF	LOH	N	N	LOH	LOH	LOH
5	AML	317* (363)	37	1q21/q44[b]	16q12/q24	LOH	N	N	LOH	LOH	N
6	Bi-AL	354, 359*	29	NF	7p/q[b], 9q34.1[bd], 22q11.1[bd]	LOH	N	N	LOH	LOH	N
7	AML	353* (367)	53	Xp/q	3p11/p24, 9p13/p24	LOH	LOH	N	LOH	LOH	LOH
8	Bi-AL	352* (366)	44	NF	7p/q[b], 9q34.1[bd], 22q11.1[bd]	LOH	N	LOH	LOH	LOH	LOH
9	Bi-AL	355*	18	NF	9q34.1[bd], 22q11.1[bd]	N	N	LOH	N	N	N
10	B-ALL	318 (364)	22	NF	NF	LOH	N	N	LOH	N	N
11	B-ALL	267 (297)	106	NF	NF	N	HZL	LOH	LOH	LOH	N
12	B-ALL	268 (301)	31	Xp/q[b]	NF	LOH	N	N	LOH	LOH	N
13	B-ALL	269 (305)	9	NF	NF	LOH	N	N	LOH	N	N

Segmental CNA, aberrations involving regions >20 Mb; Cryptic CNA, aberrations smaller than 20 Mb; Gains and Losses list of segmental changes; *IGH*, immunoglobulin heavy chain; *VPREB1* & *IGLL1*, light chains; *TARP*, T cell receptor gamma; aCGH ID, array comparative genomic hybridization identification number, denotes analysis with.

*1M and number in brackets - with customized high resolution (~1 k) oligonucleotide arrays (Agilent custom array ID 021529); Total CNA, includes both copy number variations (CNV) and acquired aberrations (CNA) (details in supplementary data); AML, acute myeloid leukaemia, Bi-AL, bi-phenotypic acute leukaemia; B-ALL, acute lymphoblastic leukaemia; NF - not found; Xp/q and 7p/q – denotes whole chromosome changes; LOH, loss of heterozygosity; HZL,bi-allelic loss, N, normal.

[a]Confirmed by real time polymerase chain reaction (qPCR).

[b]Confirmed by fluorescence *in situ* hybridization (FISH).

[c]Confirmed by *IGH*/*TRG*@ sequencing.

[d]Consistent with deletion (der)(9)t(9;22).

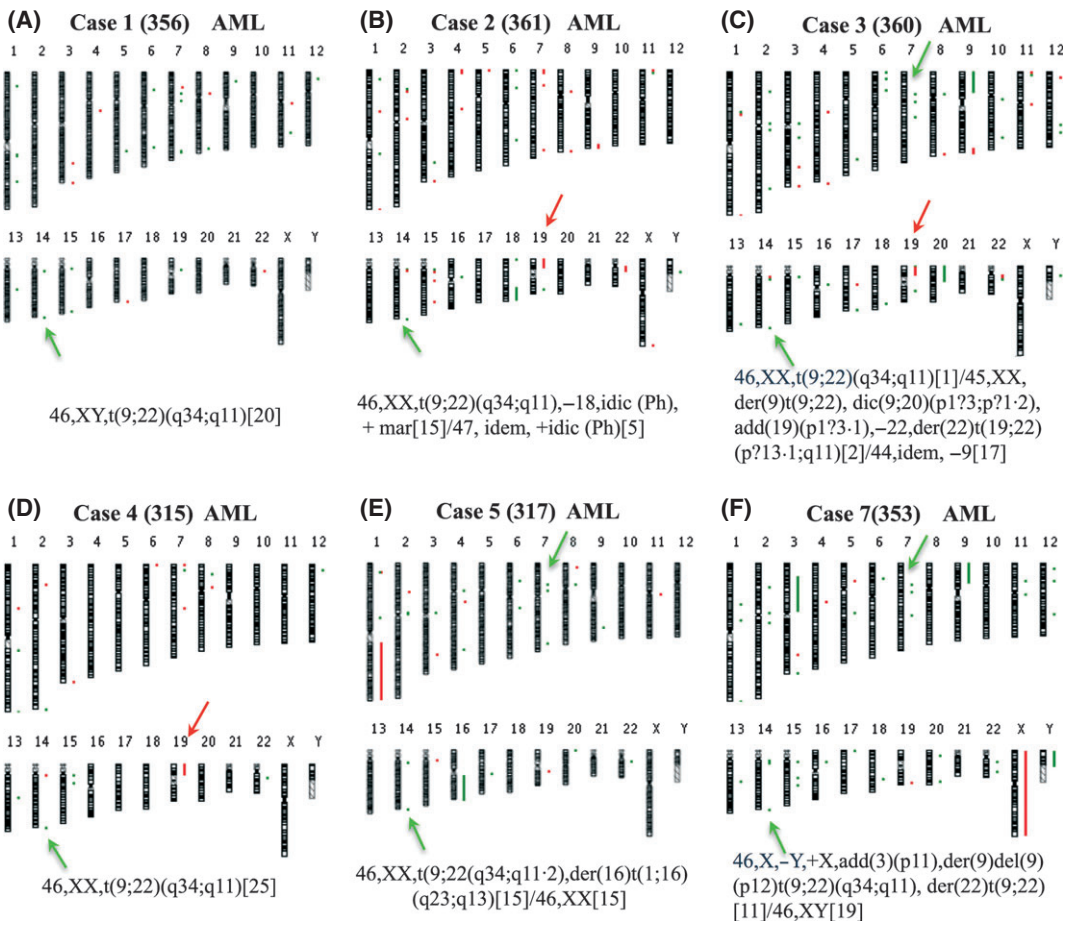

Fig 1. Molecular karyotype of Ph(+) AML: cases 1–5 & 7. Diagrammatic presentation (Cyto report, Genomic Workbench, version 5·0, Agilent) of the genome data for five Ph(+) acute myeloid leukaemia (AML) and one suspected AML samples (A–F, ID numbers as per Tables II & III) with the corresponding G banding results. Bars on the right of each chromosome represent gains (in red) and losses (in green). Copy number aberrations, variable in number and size are present in all samples. Common losses seen in all at 7p14·1 and 14q32·3 are indicated by green arrow, while recurrent gains at 19p are marked by red arrow.

(Figs S3a–c). The commonly affected sequences at 19p13·2 region house relevant genes such as zinc fingers (*RNF126* and *ZDHHC8)*, protein (*MAP2K2* and *PTPRS)* and tyrosine (*TYK2*) kinases.

Cryptic aberrations were revealed by aCGH in all Ph (+) samples (Table III, see cytoreports in Fig 1 and Figs S1 & S2). These varied both in size (from 1 Kb to 20 Mb) and in number (from 9 to 106 per genome) (Table III) but lacked any correlations with the chromosome complement. However, recurrent aberrations typically associated with Ph(+) ALL, such as losses of the *IKZF1* and *CDKN2A* gene regions were seen in 4/6 of the Ph(+)AML samples and at similar frequency in bi- AL (in 2/3). Another uniform feature present in all Ph(+) samples was the concomitant loss within the immunoglobulin (*IGH*) and T cell receptor (*TRG@*) gene complexes, as seen previously in large cohort of CML/BCL samples (Nacheva *et al*, 2010).

We endeavoured to search the genome array data for differentiating aberrant patterns. Indeed, cryptic losses at identical locations were apparent across the Ph(+) AML, Bi-AL and ALL samples (Table III). These included the loss of sequences involving the $V_{3\text{-}6}D_{1\text{-}26}J_{4\text{-}6}$ regions from chr14:105,334,536 to chr14:106,172,371 within *IGH* complex at 14q32·33, seen in all but one Bi-AL (case 9) and one ALL (case 11). However, in the latter two samples deletions were detected in the light chain IG immunoglobulin regions (*IGLL1*) at 22q11·2 (Table III). These chromosome 14 deletions were accompanied by loss within the *TRG@ alternate reading frame protein* (*TARP*) at chr7:38,262,501-38,349,233 sub-band 7p14·1 seen in 4/6 AML, 2/3 Bi-AL and all four ALL samples (Figs 2-4, see molecular karyotypes in Figs S1, S2 & S4). Copy number loss at 7p12·2 involving the *IKZF1* gene was found in 4/6 AML, 2/4 Bi-AL and 2/4 ALL samples. Losses were also detected at 9p21·3 affecting *CDKN2A/2B* in 3/6 Ph(+)AML and 1/3 Bi-AL (Table III). Sequencing data of the *IGH* regions in nine cases with Ph(+)ALL, Ph(+)AML and CML/BCL were in agreement with the recurrent genome losses identified

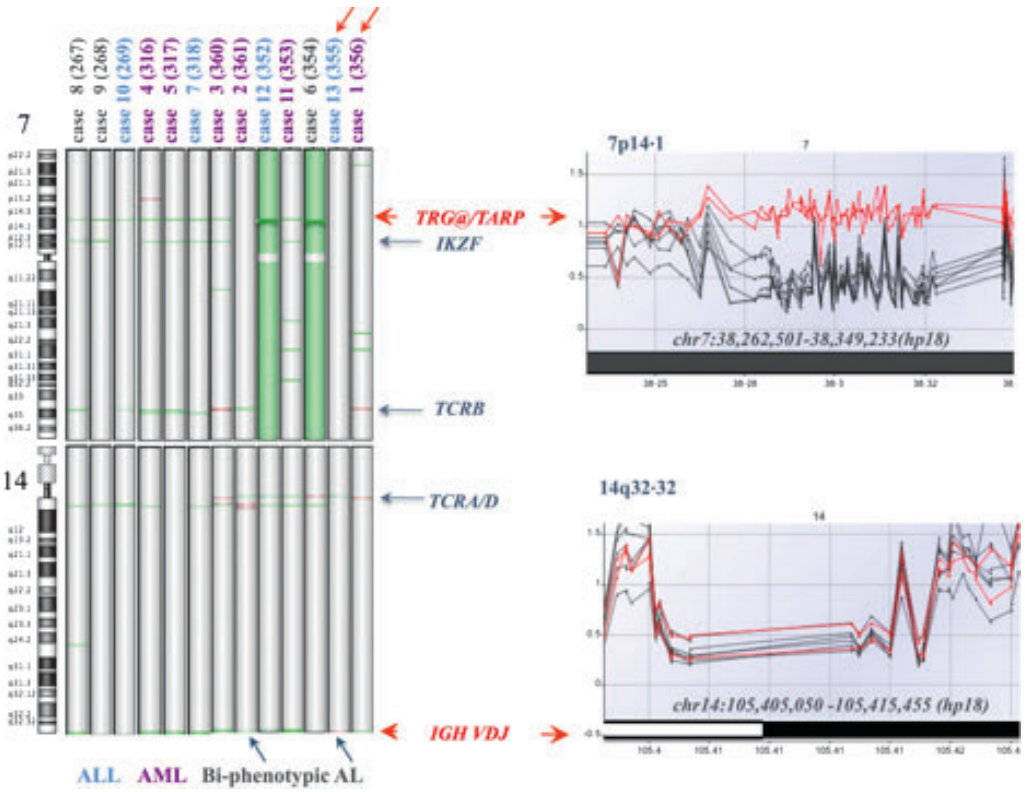

Fig 2. Concomitant deletions at IGH/VDJ and TRG@ (TARP) regions in Ph(+) acute leukemia samples. Diagrams of chromosomes 7 and 14 (Genomic Workbench, Agilent) of Ph(+) AML and ALL samples (ID on the top) are shown with genome loss marked in green and gain - in red. Losses at TRG@ (alternate reading frame protein, TARP) and IGH/VDJ gene regions are indicated by red arrows, while grey arrows show deletions at IKZF1 and imbalances within the TCR alpha/delta and beta loci. Small black arrows at the bottom point at cases 355 and 361, who carry deletions within IGLL1 region (Table II). Graphs on the right (Formatter) display FR values at 1Kb resolution (Agilent, custom arrays ID 0224931), for TRG@ and IGHVDJ regions, in red are FR graph for cases 355 and 356.

by aCGH (Table S2). Also, deletions within the 22q11·2 region affecting sequences at two locations flanking the *BCR* gene were found in AML (Cases 1 & 2), Bi-AL (Cases 8 & 9) and ALL (Case 11). Importantly, both these sequences within 22q11·2 - *VPREB1* located proximal to *BCR* at chr22:20,929,200-20,929,926 and *IGLL1* at chr:22,245,313-495, some 255 kb distal to *BCR,* house the pseudo light chain proteins required for the assembly of a functional *IGHμ* (Figs S5 & S6).

A set of non-randomly affected genome loci ('hot spots'), including sequences within the *IGH* and *TRG@* gene regions were identified in our previous aCGH study of a large cohort of Ph(+) CML (Nacheva *et al*, 2010) and Ph(+) ALL samples (Chanalaris *et al*, 2008). SAM showed that the loss of these loci was restricted to Ph(+) cells with lymphoid phenotype. We used the same custom arrays (Agilent Design ID 0224031) consisting of 15 000 probes covering the 'hot spot' areas at ~1K resolution to assess these genomic regions in the Ph(+) AML samples. A total of 47 *BCR/ABL1* positive samples, comprising of six AML, 1 Bi-AL and 10 each of ALL, CML chronic phase, myeloid, lymphoid and bi-phenotypic blast crisis (positive controls) together with 10 samples from healthy individuals (negative controls) were screened under the same conditions. Among the top 100 most significant loci identified by SAM were the *IGH*/$V_{3\text{-}6}D_{1\text{-}26}J_{4\text{-}6}$ and the *TRG@/TARP* sequences. Samples with these losses formed a cluster of *BCR/ABL1* positive acute leukaemia with lymphoid phenotype, as seen in Fig 3 (areas in green to the left of the heat map). These include all of the Ph(+)AML together with Ph(+)ALL and CML in lymphoid blast transformation. Note that none of the CML chronic phase, myeloid blast crisis or control samples showed this pattern of concomitant *IGH/TRG@* genome loss (Fig 3 and 4).

Given that the losses within *IGH* and *TRG@* are almost universal for the Ph(+) cells of lymphoid origin, we applied SAM to the target array data excluding these regions. Cluster analysis of the top 39 genome loci significantly differentiated a cluster of AML samples (Fig 5, Table S3). The most significant genome loci included relevant genes - *RNF38* at 9p13·2 (protein with a RING motif - a zinc binding domain, found in a number of proteins with a role in diverse cellular processes, including oncogenesis, development, signal transduction and apoptosis), *NCS1* at 9q34·1 (neuronal calcium

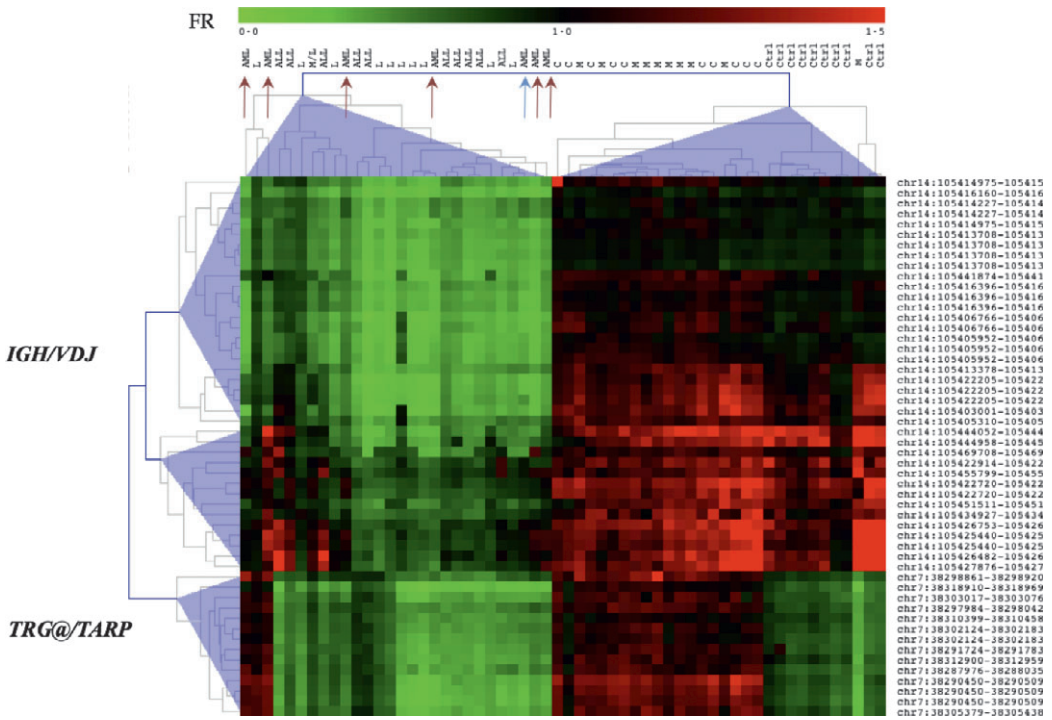

Fig 3. SAM analysis defines the concomitant loss of *IGH/VDJ* and *TRG@* (*TARP*) sequences as significantly associated with lymphoid *BCR/ABL1* positive genomes. Top 52 loci from the *IGH/DJ* and the *TRG@* (*TARP*) regions, the loss of which is shown to be significantly associated lymphoid *BCR/ABL1* (+) genomes (areas in green on the heat map) by SAM investigation of data from custom array (Agilent, custom array ID 0224931) of 47 Ph(+) samples. Red arrows show the six *BCR/ABL1*(+)AML, the blue arrow points to a bi-phenotypic AL, all of which are clustering with the ALL and CML lymphoid BC(L). Note that none of the remaining CML chronic phase (C), CML myeloid blast crisis (M), and 10 controls (ctrl) samples show the concomitant *IGH/TRG@(TARP)* loss.

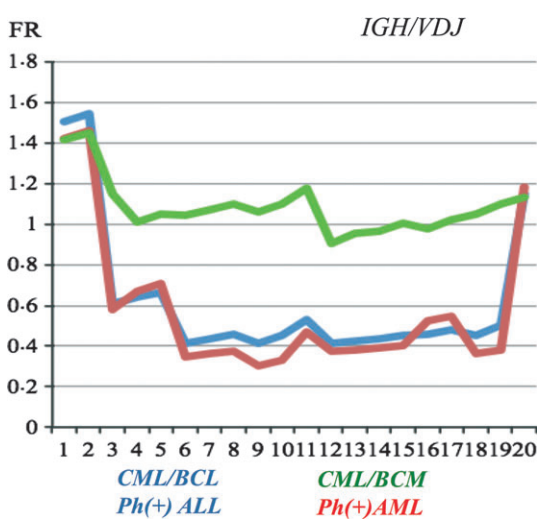

Fig 4. Loss within *IGH/VDJ* region is restricted to *BCR/ABL1* positive genomes of lymphoid origin. Average fluorescence ratio values (FR, vertical axis) for each of the 20 probes (horizontal axis) covering the chr14: 105,405,050 - 105,415,455 (hg18) region from 10 Ph (+) ALL and 10 CML/LBC (combined, graph in blue), 10 CML/MBC (in green) and 6 Ph(+)AML (in red) plotted together demonstrate the unique association of the *IGH/VDJ* loss with lymphoid *BCR/ABL1(+)* genomes.

sensor 1), *PPP1R12B* (protein inhibitor) at 1q32·1, *PTPRN2* (tyrosine phosphatase) at 7q36·3 and oncogene *SEPT9* at 17q25·3 region. Also featured are probes from the regions of 19p13 (*PPAN, ICAM1, CCDC94* and *PDE4A*) and 10q24·1 (*CNNM2*) (Table S3).

Discussion

Typically a multistage disorder, CML progresses from an initial indolent chronic phase to an accelerated phase and, if untreated, to terminal blast crisis with either myeloid or less frequently, lymphoid or mixed immuno-phenotype. Rarely, CML patients may present in blast crisis without a history of preceding chronic phase (Kantarjian *et al*, 2003). In such cases it may be impossible to differentiate CML/BC from Ph (+)ALL and/or *de novo* AML due to the lack of firm criteria at the clinical, haematological and genetic level. Here we report a high-resolution aCGH study of *BCR/ABL1* positive leukaemia where we identified recurrent genome features that allow the distinction of Ph(+)AML from both CML BM and from Ph(+)ALL. To date, we and others have shown that adult Ph(+)ALL and lymphoid blast crisis of CML share common genomic features, in particular, the recurrent but not universal loss of the *IKZF1* and *CDNK1A/B* genes (Mullighan *et al*, 2007; Nacheva *et al*, 2010). In addition, our

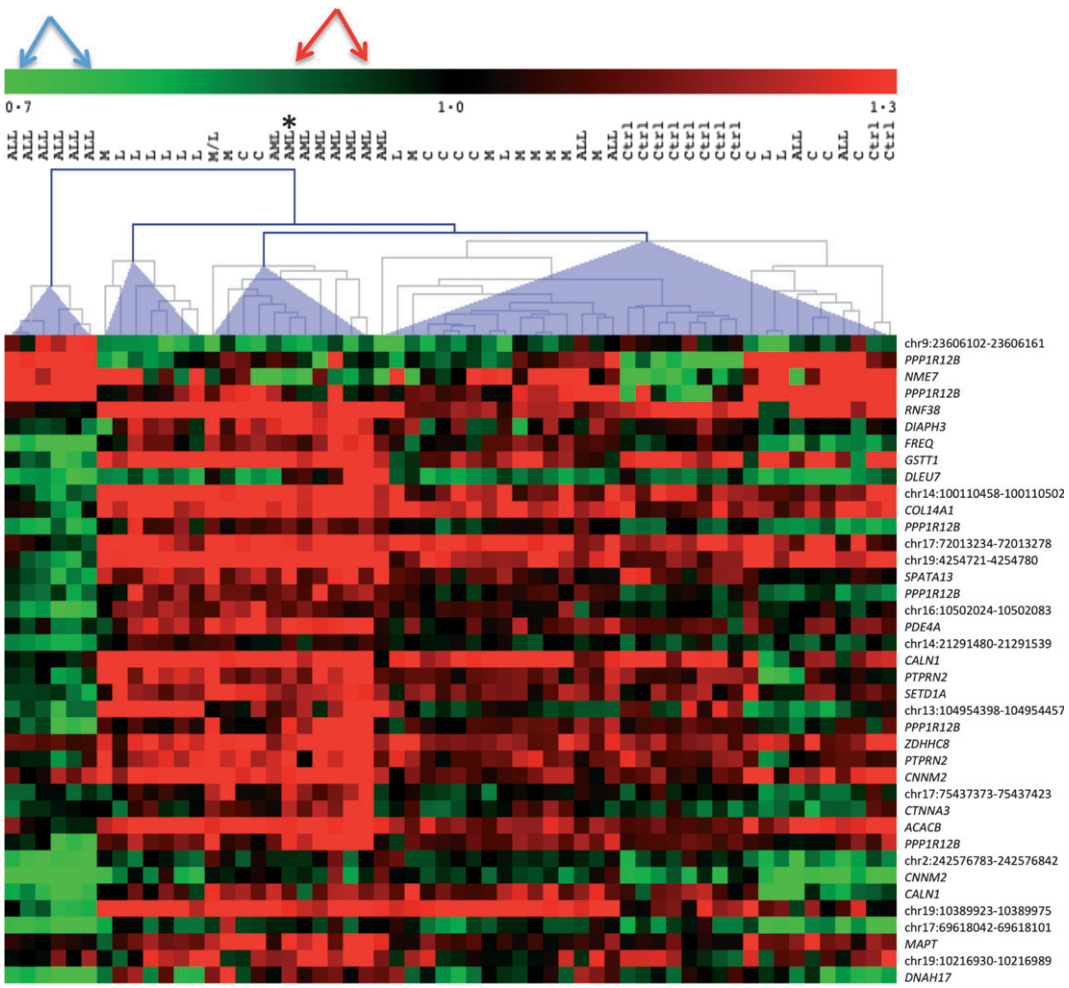

Fig 5. Genome differences in Ph(+)ALL and Ph(+)AML. Cluster analysis of probes (custom array data, Agilent, custom array ID 0224931) judged to be significant for differentiating between Ph(+) ALL and AML in the absence of *IGH* and *TRG@ (TARP)* probes. Note that the AML samples cluster together (red arrows) and six of the 10 ALL samples form a separate group (on the left of the heat map, blue arrows).

laboratory has a substantial collection of Ph(+) B-cell acute leukaemia cases where aCGH identified recurrent concomitant loss of specific sequences within the *IGH* and *TRG@* regions (Chanalaris *et al*, 2008; Nacheva *et al*, 2010). Such illegitimate non-productive *IGH* and *TRG@* gene rearrangements are known to occur in acute leukaemia of both lineages and are used as markers for residual disease (van der Velden *et al*, 2007). Indeed, cryptic deletions within the immunoglobulin genes have been already linked to the malfunction of the RAG1/RAG2 system due to elevated AID expression in *BCR/ABL1* positive acute leukaemia cells (Feldhahn *et al*, 2007).

The combined recurrent losses within the *TRG@* and *IGH* regions appear more common than deletions of *IKZF1* - a gene already closely identified with lymphoid disease. This concomitant loss occurs at genome loci reported to have imbalances in disease-free individuals, referred to as Copy Number Variation (CNV) and considered to be polymorphic markers. It is known that CNVs overlap some 7000 genes in humans, many of them pivotal in pathological pathways (Feuk *et al*, 2006). However, in contrast with a typical CNV that could affect any part of the *IGH* gene, the deletions identified here in the genome of *BCR/ABL1* positive ALL and AML (Table III, Fig 2-3) always involved exactly the same sequences at chr14:105,405,050-105,415,455 and were accompanied almost universally by deletions of *TRG@/TARP*. Since both *IGH* and *TRG@* regions are usually excluded from aCGH analysis such recurrent aberrations may be overlooked. Additionally, deletions at 22q11·2 involving the *VPREB1* and *IGLL1* sequences were found in two of the six Ph(+)AML and one of each Bi-AL and ALL cases. Notably, the losses of *IGH, TRG@*, *VPREB1* and *IGLL1* identified in samples of *de novo BCR/ABL1*(+) AML, Ph(+)ALL and CML lymphoid BC, and confirmed by both qPCR and sequencing, were not detected either in any of the CML chronic phase or myeloid blast crisis, nor in any of AML samples with normal karyotype investigated under the same conditions (data not shown). In these latter cases, both the *IGH*, *TRG@/TARP*

and *VPREB1/IGLL1* regions showed variations similar to those reported in disease-free individuals (Chanalaris *et al*, 2009).

The recurrent ~10 Kb loss at chr14:105,405,050-105,415,455, involves among others the *IGHM*(μ) sequences, which, when coupled to the surrogate two light chain components *VPREB1* and *IGLL1*, forms the pre-B cell receptor, necessary for the normal B cell development (Nahar & Muschen, 2009; Mårtensson *et al*, 2010). Productive rearrangement of the V_H to DJ_H segments is a prerequisite for the expression of the functional μ chain and hence the transition from pro-B to pre-B cells stage. The possible role of a tumour suppressor for the pre-B cell receptor was implicated in B/ALL with and without a Ph(+) chromosome (Den Boer *et al*, 2009) and in therapy-resistant CML (Nowak *et al*, 2010). The function of pro-B cell receptor signalling in 22 cases with Ph(+)ALL was shown to be associated with genome deletions in a set of pre-B cell receptor related genes using the 250 SNP-chip platform (Trageser *et al*, 2009). The high frequency of defects in the pre-B cell receptor can be attributed to the increased genetic instability of *BCR/ABL1* positive ALL, probably owing to the aberrant expression of the mutator enzyme activation-induced deaminase (AID). Indeed the study reported by Klemm *et al*, 2009) provided multiple lines of evidence indicating AID both in generation of point mutations and copy number aberrations in a number of genes that are involved in DNA repair, DNA damage signalling or cell cycle control including immunoglobulin genes. However it is currently unclear whether pre-B cell functioning is required to enable malignant outgrowth in ALL or function to suppress it (Nahar & Muschen, 2009; Gruber *et al*, 2010).

Although the pathogenesis of the unique loss within the immunoglobulin regions is far from clear, it has an important practical benefit – a straightforward way to differentiate two clinically and biologically similar conditions with different treatment requirements – de novo acute myeloid leukaemia and blast phase of CML.

By removing the dominant *IGH* and *TRG@* probes from the high resolution data set, cluster analysis of SAM results highlighted a further list of probes distributed across the genome that could discriminate the Ph(+)AML from any other Ph(+) acute malignancy. The small data set limits the statistical significance of these results but differences in the genome profile found cannot be attributed to karyotype features because the chromosome complexity of the Ph (+)AML and Ph(+)ALL samples interrogated by SAM have similar characteristics (Table S1).

Our results show that Ph(+)AML possesses all the genome characteristics of lymphoid disease: it shares well-documented deletions of *IKZF1* and *CDNK1A/B* as well as the almost universal deletions of the *TRG@* and *IGH* regions shown by us to be specific markers for pre-B cell leukaemia. However, Ph(+)AML does display some differences in the presence of 19p chromosome gains and a unique genomic profile calculated after exclusion of the more dominant markers. These features, supporting the view that Ph(+)AML represents a separate entity that may benefit from alternative treatment protocols, require further investigation to elucidate their biological and clinical significance. A step toward this goal was provided just as this study was under review. In a search for a molecular signature for Ph(+)AML, Konoplev *et al* (2013) screened nine patients with *de novo* Ph(+)AML and five with CML/BCM for mutations in 14 genes, two of which, *ABL1* and *NPM1*, showed recurrent changes. While none of the nine Ph(+)AML patients showed *ABL1* kinase domain aberrations, two had typical *NPM1* exon 12 mutations. In contrast, one of the five CML blast phase (BP) samples had *ABL1* mutations but none displayed *NPM1* changes, thus suggesting that "Ph(+)AML is distinct from CML/BP". These data are consistent with our findings but whereas the *NPM1* gene mutation is a recurrent rather than universal feature, the 100% loss of the immunoglobulin sequences in Ph(+)AML offers a reliable test suitable for routine practice.

In summary, despite its clinical diversity, *BCR/ABL1*(+) AML has a unique genome signature distinctly different from both Ph(+)ALL and CML/BCM. It does exist.

Author contributions

EP Nacheva performed analysis, designed the study and co-wrote the manuscript with P Kottaridis; C Grace carried the bio-informatics analysis; D Brazma performed the array hybridizations; G Gancheva and L Rai did the molecular analysis; J Howard-Reeves helped with the FISH and G banding studies; RE Gale, DC Linch, RK Hills, RN Russell and AK Burnett – carried out the clinical, morphology and immunophenotyping.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Molecular karyotype of bi-phenotypic Ph(+) acute leukemia Case 6, 8 & 9.

Fig S2. Molecular karyotype of Ph(+) ALL Case 10–13.

Fig S3. Imbalances of the short arm of chromosome 19.

Fig S4. SAM analysis of custom array data.

Fig S5. Recurrent genome loss within 22q11.2 includes the regions of *VPREB1* and *IGLLI* gene in ph(+) AML.

Fig S6. Qualification of the TARP and VPREB1 regions by qPCR.

Table S1. Karyotype complexity of the *BCR/ABL1* positive samples.

Table S2. Sequencing data of the *IGH* gene.

Table S3. List of probes significant in differentiating between Ph(+) AML and Ph(+) ALL in the absence of the *IGH* and *TCR* probes as identified by SAM analysis of customised array data (Agilent, array Design ID 0224931).

References

Chanalaris, A., Brazma, D., Rai, L., Howard-Reeves, J., Valganon, M., Grace, C., Foroni, L. & Nacheva, E.P. (2008) Genome Profile Of Ph Positive ALL In Adult Patients. *Haematologica, 13th Congress of the European Hematology Association*, **93**(S1):194 abs 0478.

Chanalaris, A., Grace, C., Gale, R., Linch, D. & Nacheva, E. (2009) Genome profiling of normal karyotype AML with high density array comparative genome hybridization. *Haematologica, 14th Congress of the European Hematology Association*, **94**(Suppl2) 114 abs 0289.

Den Boer, M.L., van Slegtenhorst, M., De Menezes, R.X., Cheok, M.H., Buijs-Gladdines, J.G.C.A.M., Peters, S.T.C.J.M., Van Zutven, L.J.C.M., Beverloo, H.B., Van der Spek, P.J., Escherich, G., Horstmann, M.A., Janka-Schaub, G.E., Kamps, W.A., Evans, W.E. & Pieters, R. (2009) A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *The Lancet Oncology*, **10**, 125–134.

Feldhahn, N., Henke, N., Melchior, K., Duy, C., Soh, B.N., Klein, F., von Levetzow, G., Giebel, B., Li, A., Hofmann, W.-K., Jumaa, H. & Muschen, M. (2007) Activation-induced cytidine deaminase acts as a mutator in BCR-ABL1-transformed acute lymphoblastic leukemia cells. *The Journal of Experimental Medicine*, **204**, 1157–1166.

Feuk, L., Carson, A.R. & Scherer, S.W. (2006) Structural variation in the human genome. *Nature Reviews Genetics*, **7**, 85–97.

Grace, C. & Nacheva, E.P. (2012) Significance Analysis of Microarrays (SAM) Offers Clues to Differences Between the Genomes of Adult Philadelphia Positive ALL and the Lymphoid Blast Transformation of CML. *Cancer Inform*, **11**, 173–183.

Gruber, T.A., Chang, M.S., Sposto, R. & Muschen, M. (2010) Activation-Induced Cytidine Deaminase Accelerates Clonal Evolution in BCR-ABL1-Driven B-Cell Lineage Acute Lymphoblastic Leukemia. *Cancer Research*, **70**, 7411–7420.

Kantarjian, H., O'Brien, S., Cortes, J., Giles, F., Thomas, D., Kornblau, S., Shan, J., Beth Rios, M., Keating, M., Freireich, E. & Talpaz, M. (2003) Sudden onset of the blastic phase of chronic myelogenous leukemia: patterns and implications. *Cancer*, **98**, 81–85.

Klemm, L., Duy, C., Iacobucci, I., Kuchen, S., von Levetzow, G., Feldhahn, N., Henke, N., Li, Z., Hoffmann, T.K., Kim, Y.-M., Hofmann, W.-K., Jumaa, H., Groffen, J., Heisterkamp, N., Martinelli, G., Lieber, M.R., Casellas, R. & Mueschen, M. (2009) The B Cell Mutator AID Promotes B Lymphoid Blast Crisis and Drug Resistance in Chronic Myeloid Leukemia. *Cancer Cell*, **16**, 232–245.

Konoplev, S., Yin, C.C., Kornblau, S.M., Kantarjian, H.M., Konopleva, M., Andreeff, M., Lu, G., Zuo, Z., Luthra, R., Medeiros, L.J. & Bueso-Ramos, C.E. (2013) Molecular characterization of de novo Philadelphia chromosome-positive acute myeloid leukemia. *Leukaemia & Lymphoma*, **54**, 138–144.

Mårtensson, I.-L., Almqvist, N., Grimsholm, O. & Bernardi, A.I. (2010) The pre-B cell receptor checkpoint. *FEBS Letters*, **584**, 2572–2579.

Matteucci, C., Barba, G., Varasano, E., Vitale, A., Mancini, M., Testoni, N., Cuneo, A., Rege-Cambrin, G., Elia, L., La Starza, R., Pierini, V., Brandimarte, L., Vignetti, M., Foa, R. & Mecucci, C., GIMEMA Acute Leukaemia Working Party, Italy (2010) Rescue of genomic information in adult acute lymphoblastic leukaemia (ALL) with normal/failed cytogenetics: a GIMEMA centralized biological study. *British Journal of Haematology*, **149**, 70–78.

Mullighan, C.G., Goorha, S., Radtke, I., Miller, C.B., Coustan-Smith, E., Dalton, J.D., Girtman, K., Mathew, S., Ma, J., Pounds, S.B., Su, X., Pui, C.-H., Relling, M.V., Evans, W.E., Shurtleff, S.A. & Downing, J.R. (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature*, **446**, 758–764.

Nacheva, E.P., Brazma, D., Virgili, A., Howard-Reeves, J., Chanalaris, A., Gancheva, K., Apostolova, M., Valgañon, M., Mazzullo, H. & Grace, C. (2010) Deletions of immunoglobulin heavy chain and T cell receptor gene regions are uniquely associated with lymphoid blast transformation of chronic myeloid leukemia. *BMC Genomics*, **11**, 41.

Nahar, R. & Muschen, M. (2009) Pre-B cell receptor signaling in acute lymphoblastic leukemia. *Cell Cycle*, **8**, 3874–3877.

Nowak, D., Ogawa, S., Muschen, M., Kato, M., Kawamata, N., Meixel, A., Nowak, V., Kim, H.S., Kang, S., Paquette, R., Chang, M.-S., Thoennissen, N.H., Thoenissen, N.H., Mossner, M., Hofmann, W.-K., Kohlmann, A., Weiss, T., Haferlach, T., Haferlach, C. & Koeffler, H.P. (2010) SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations. *Blood*, **115**, 1049–1053.

Rai, L., Casanova, A., Moorman, A.V., Richards, S., Buck, G., Goldstone, A.H., Fielding, A.K. & Foroni, L. (2010) Antigen receptor gene rearrangements reflect on the heterogeneity of adult Acute Lymphoblastic Leukaemia (ALL) with implications of cell-origin of ALL subgroups - a UKALLXII study. *British Journal of Haematology*, **148**, 394–401.

Soupir, C.P., Vergilio, J.-A., Dal Cin, P., Muzikansky, A., Kantarjian, H., Jones, D. & Hasserjian, R.P. (2007) Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. *American Journal of Clinical Pathology*, **127**, 642–650.

Swerdlow, S., Campo, E., Harris, N. & Jaffe, E. (2008) WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. International Agency for Research on Cancer, Lyon, France.

Trageser, D., Iacobucci, I., Nahar, R., Duy, C., von Levetzow, G., Klemm, L., Park, E., Schuh, W., Gruber, T., Herzog, S., Kim, Y.-M., Hofmann, W.-K., Li, A., Storlazzi, C.T., Jäck, H.-M., Groffen, J., Martinelli, G., Heisterkamp, N., Jumaa, H. & Müschen, M. (2009) Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. *The Journal of Experimental Medicine*, **206**, 1739–1753.

Tusher, V.G., Tibshirani, R. & Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5116–5121.

Usvasalo, A., Savola, S., Räty, R., Vettenranta, K., Harila-Saari, A., Koistinen, P., Savolainen, E.-R., Elonen, E., Saarinen-Pihkala, U.M. & Knuutila, S. (2008) CDKN2A deletions in acute lymphoblastic leukemia of adolescents and young adults: an array CGH study. *Leukemia Research*, **32**, 1228–1235.

Vardiman, J.W. (2009) Chronic myelogenous leukemia, BCR-ABL1+. *American Journal of Clinical Pathology*, **132**, 250–260.

van der Velden, V.H.J., Cazzaniga, G., Schrauder, A., Hancock, J., Bader, P., Panzer-Grumayer, E.R., Flohr, T., Sutton, R., Cave, H., Madsen, H.O., Cayuela, J.M., Trka, J., Eckert, C., Foroni, L., Zur Stadt, U., Beldjord, K., Raff, T., van der Schoot, C.E. & van Dongen, J.J.M., European Study Group on MRD detection in ALL (ESG-MRD-AL (2007) Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*, **21**, 604–611.