Does BCR/ABL1 positive acute myeloid leukaemia exist?

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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.

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 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 CDKN1A/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 ones.

**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-Grünewald staining, immuno-cytochemistry and immuno-phenotyping 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 $\times\ 10^9/\text{l}$ | 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) 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-pter & 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 fusion type |
|---------|-------------|-----|----------|---------------------|---------------------|
| 1       | 44          | M   | AML      | 46.XY,t(9;22)(q34q11)[20] | 2F1R1G p210         |
| 2       | 75          | F   | AML      | 46.XX,t(9;22)(q34q11),+18, idic(Ph),+mar[15]/47, idem, +idic(Ph)[5] | 3F1R1G/4F1R1G p210 |
| 3       | 28          | F   | AML      | 46.XX,t(9;22)(q34q11)[1]/45.XX, der(9)(t(9;22), dic(9)(p13:p11-2), add(19)(p13-1)-22, der(22)(t(19;22)(p13q11)[2]/44, idem, -9[17] | 3F1R1G p210         |
| 4       | 35          | F   | AML      | 46.XX,t(9;22)(q34q11)[25] | 2F1R1G p210         |
| 5       | 58          | F   | AML      | 46.XX,t(9;22)(q34q11-2), der(16) | 2F1R1G/ p210         |
| 6       | 36          | F   | Bi-AL    | 45.XX, t(9;22)(q34q11)[18]/47, idem, +2mar[2] | 1F1R1G p210         |
| 7       | 55          | M   | AML      | 46.X-Y, +X, add(3)(p11), der(9)(del)(p12) | 3F1R1G p210         |
| 8       | 52          | F   | Bi-AL    | 46.XX,t(9;22)(q34q11-2)[1]/45, idem, -7[9] | 1F1R1G p210         |
| 9       | 26          | F   | Bi-AL    | 46.XX,t(9;22)(q34q11)[20] | 1F1R1G p210         |
| 10      | 43          | M   | B/ALL    | 46.XY,t(9;22)(q34q11)[20] | 2F1R1G p210         |
| 11      | 17          | M   | B/ALL    | 46.XY,t(9;22)(q34q11)[20] | 2F1R1G p210         |
| 12      | 50          | M   | B/ALL    | 46.XY,t(9;22)(q34q11)[18]/46.XY[2] | 2F1R1G p190         |
| 13      | 52          | M   | B/ALL    | 46.XY,t(9;22)(q34q11)[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.

| Patient | Type   | aCGH ID          | Total CNA | Segmental CNA | Gains | Losses | Cryptic CNA |
|---------|--------|------------------|-----------|---------------|-------|--------|-------------|
|         |        |                  |           |               |       |        | 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/pter, 22p/pter \(b\) | 18q21-23 | NF     | LOH         | N          | LOH   | N          | N          | N          |
| 3       | AML    | 319, 360* (365)  | 45        |                | 19p13, 9q34/pter, 22p/pter \(b\) | 9p12/p24, 20p11/p13 | NF     | LOH         | N          | LOH   | LOH        | LOH        | LOH        |
| 4       | AML    | 315, 316* (362)  | 31        |                | NF    | NF     | LOH         | N          | LOH   | LOH        | LOH        |
| 5       | AML    | 317* (363)       | 37        |                | 1q21/pter \(b\) | 16q21/pter | NF     | N          | LOH         | LOH   | LOH        | LOH        |
| 6       | Bi-AL  | 354, 359*        | 29        |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |
| 7       | AML    | 353* (367)       | 53        |                | Xp/pter | 3p11/p24, 9p13/p24 | NF     | N          | LOH         | LOH   | LOH        | LOH        |
| 8       | Bi-AL  | 352* (366)       | 44        |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |
| 9       | Bi-AL  | 355*             | 18        |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |
| 10      | Bi-AL  | 318 (364)        | 22        |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |
| 11      | Bi-AL  | 267 (297)        | 106       |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |
| 12      | Bi-AL  | 268 (301)        | 31        |                | Xp/pter \(b\) | NF     | LOH         | N          | N     | LOH        | LOH        |
| 13      | Bi-AL  | 269 (305)        | 9         |                | NF    | NF     | LOH         | N          | N     | LOH        | LOH        |

**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.

- \(a\)M 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** – denotes whole chromosome changes; **LOH**, loss of heterozygosity; **HZL**, bi-allelic loss, **N**, normal.

- \(b\)Confirmed by real time polymerase chain reaction (qPCR).
- \(c\)Confirmed by fluorescence in situ hybridization (FISH).
- \(d\)Confirmed by IGH/TRG@ sequencing.
- \(e\)Consistent with deletion (der)(9)t(9;22).
The commonly affected sequences at 19p13/C12 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 3/4). 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_{L,J},D_{1,2},J_{a,b} regions from chr14:105,334,536 to chr14:106,172,371 within IGH complex at 14q32, 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 (IGGL1) at chr22:10,195,941-10,203,094 sub-band 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 7q31.31 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.
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 \( IGH \) (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 \( IGH/V_{\gamma}D_{1.2}J_{\delta6} \) 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), \( NCSI \) at 9q34.1 (neuronal calcium...
sensor 1), PPP1R12B (protein inhibitor) at 1q32/C1, PTPRN2 (tyrosine phosphatase) at 7q36/C1 and oncogene SEPT9 at 17q25/C1 region. Also featured are probes from the regions of 19p13 (PPAN, ICAM1, CCDC94 and PDE4A) and 10q24/C1 (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(+)ALL 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 CDKN1A/B genes (Mullighan et al, 2007; Nacheva et al, 2010). In addition, our

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 022491) 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.

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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; Mártensson et al, 2010). Productive rearrangement of the VH to DΗ 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, KK 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 leukaemia 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 IGLL1 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).
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