Distinctive genotypes in infants with T-cell acute lymphoblastic leukaemia

Marcela B. Mansur,¹,² Frederik W. van Delft,¹ Susan M. Colman,¹ Caroline L. Furness,¹ Jane Gibson,³ Mariana Emerenciano,² Helena Kempski,⁴ Emmanuelle Clappier,⁵ Hélène Cave,⁵ Jean Soulier,⁶ Maria S. Pombo-de-Oliveira,² Mel Greaves¹ and Anthony M. Ford¹

¹Centre for Evolution and Cancer, The Institute of Cancer Research, London, UK, ²Paediatric Haematology-Oncology Program, Research Centre, Instituto Nacional de Cáncer, Rio de Janeiro, Brazil, ³Centre for Biological Sciences, University of Southampton, Southampton, ⁴Paediatric Malignancy Cytogenetics Unit, Institute of Child Health & Great Ormond Street Hospital, London, UK, ⁵Centre for Cancer Genetics, Robert Debré Hospital, APHP, and ⁶Haematology Laboratory, Saint-Louis Louis Hospital, APHP, Paris, France

Received 16 April 2015; accepted for publication 25 June 2015

Correspondence: Dr Anthony M. Ford and Dr Marcela B. Mansur, Centre for Evolution and Cancer, The Institute of Cancer Research (ICR) - London, Brookes Lawley Building, 15 Cotswold Road, Sutton - Surrey SM2 5NG, UK
E-mails: tony.ford@icr.ac.uk and mmansur@inca.gov.br

Acute lymphoblastic leukaemia (ALL) in children is a diverse cancer characterized by associations between age at presentation, leukaemic subtype and recurrent genetic alterations (Pui et al., 2004). ALL in infants is a rare subset often associated with KMT2A (also known as MLL) rearrangements (KMT2A-r), a high leucocyte count at diagnosis, an immature or pro-B-cell lineage immunophenotype (CD10⁺) and a prenatal origin in utero (Ford et al., 1993; Biondi et al., 2000). T-ALL is prevalent in older children. Though there is molecular evidence that it can originate in utero (Ford et al., 1997; Eguchi-Ishimae et al., 2008), it is a very rare disease in infants (Biondi et al., 2000; Emerenciano et al., 2013). In contrast to infant pro-B-lineage ALL with KMT2A-AFF1 fusion, in which an in utero origin has been clearly demonstrated (Ford et al., 1993; Gale et al., 1997), the developmental timing for T-ALL is poorly defined.

In a previous study of T-ALL, we evaluated fifteen cases in early childhood (age ≤24 months) for mutations that are prevalent in infant ALL (pro-B) or T-ALL; NOTCH1 mutations, although found less frequently than described for older T-ALL, paediatric cases, were the most frequent alterations among these younger patients, followed by the KMT2A-r (Emerenciano et al., 2006; Mansur et al., 2010).

The availability of a unique series of 13 infant T-ALL cases (iT-ALL, ≤12 months) along with 12 remission samples allowed us to determine the molecular profile of iT-ALL [copy number alterations (CNAs)/gains and losses (loss of heterozygosity, LOH)] using high-density Genome-Wide single nucleotide

Summary

Infant T-cell acute lymphoblastic leukaemia (iT-ALL) is a very rare and poorly defined entity with a poor prognosis. We assembled a unique series of 13 infants with T-ALL, which allowed us to identify genotypic abnormalities and to investigate prenatal origins. Matched samples (diagnosis/remission) were analysed by single nucleotide polymorphism-array to identify genomic losses and gains. In three cases, we identified a recurrent somatic deletion on chromosome 3. These losses result in the complete deletion of MLF1 and have not previously been described in T-ALL. We observed two cases with an 11p13 deletion (LMO2-related), one of which also harboured a deletion of RB1. Another case presented a large 11q14-11q23 deletion that included ATM and only five patients (38%) showed deletions of CDKN2A/B. Four cases showed NOTCH1 mutations; in one case FBXW7 was the sole mutation and three cases showed alterations in PTEN. KMT2A rearrangements (KMT2A-r) were detected in three out of 13 cases. For three patients, mutations and copy number alterations (including deletion of PTEN) could be backtracked to birth using neonatal blood spot DNA, demonstrating an in utero origin. Overall, our data indicates that iT-ALL has a diverse but distinct profile of genotypic abnormalities when compared to T-ALL in older children and adults.

Keywords: infant, T-cell acute lymphoblastic leukaemia, genomic profile, CNAs and in utero origin.
polymorphism (SNP) array accompanied by next generation sequencing (NGS). We sought to investigate the possible prenatal onset of genetic abnormalities in iT-ALL using a ‘backtracking’ approach with neonatal blood spots (Guthrie cards).

Materials and methods

Patient samples

Seven Brazilian (BR1-BR7), one English (UK1) and five French (FR1-FR5) iT-ALLs were included in this study (Supporting Information). Material from diagnostic bone marrow (BM) and/or peripheral blood (PB) was available from all patients and remission samples (non-leukaemic) were collected for all but one patient (BR4, who did not achieve remission). Guthrie cards were obtained from four patients for use in our backtracking approach to trace prenatally acquired mutations.

Leukaemia characterization

In all cases, diagnosis of leukaemia was established by the morphology of lymphoid cells and immunophenotyping by flow cytometry using a previously established panel of monoclonal antibodies (Mansur et al., 2009). The immunological classification of T-ALL was performed according to the European Group for the Immunological Characterization of Leukaemias (EGIL) criteria (Bene et al., 1995).

T-ALL molecular screening

Diagnostic DNA samples from all iT-ALL cases were analysed for the following gene abnormalities: NOTCH1, FBXW7, PTEN, IL7R, KRAS, NRAS, STIL-TAL1 + , TLX3 + and KMT2A-r (Weng et al., 2004; Mansur et al., 2009, 2012; Zenatti et al., 2011; Emerenciano et al., 2013). T-cell receptor gene rearrangements (TR-r; gamma/TRG, delta/TRD and beta/TRB) were assessed using conditions recommended by the BIOMED-2 Consortium (van Dongen et al., 2003; Lange et al., 2012). Clonality was assessed by GeneScan® profiling (Applied Biosystems®, Waltham, MA, USA) followed by cloning of the products and Sanger sequencing. Sequences were analysed using the Ig BLAST (www.ncbi.nlm.nih.gov/igblast/) and the ImMunoGeneTics database (www.imgt.org).

Molecular analyses

Fluorescence in situ hybridization (FISH), CNA analyses, NGS and backtracking of neonatal blood spots were all performed as described in Data S1.

Results

Characterization of infant cases

Thirteen iT-ALL cases were investigated. The median age at diagnosis was 9 months, there was no predominance of gender, and a high leucocyte count (≥50 x 10⁹/L) was observed in 12 out of 13 cases (Table S1). Immunophenotype analyses performed on all 13 diagnostic cases revealed that six patients presented T-IV profile, five cases T-III and for the other two cases, one presented T-I and the other a T-II profile. The T-I profile case (BR4) also expressed two classical myeloid markers CD13 and CD33, which, according to previously published criteria (Coustan-Smith et al., 2009), suggests an Early T-cell Progenitor (ETP) leukaemia.

Molecular analysis

The main results from the targeted molecular analyses carried out on the iT-ALL samples are shown in Table I.

The 13 diagnostic infant samples were screened for the known recurrent mutations in T-ALL, including NOTCH1, FBXW7, PTEN and IL7R (detailed mutation data is shown in Table SII), as well as KRAS, NRAS mutations, STIL-TAL1 fusion and the presence of TLX3. Results showed four cases were mutated for NOTCH1, two being mutated in the HD domain only (BR1 and BR4), one in the PEST only (BR6) and one (FR3) with mutations in both HD and PEST. Patient BR1 presented a combined NOTCH1/FBXW7 mutation. One case (FR5) presented a sole FBXW7 mutation. Three cases presented PTEN alterations (FR1 and FR3 as mutations and BR6 as CNA/deletion) and all patients were IL7R, KRAS and NRAS wild type (WT). KMT2A-r was confirmed in three cases (two KMT2A-MLLT1 and one KMT2A-MLLT4) and, distinct from childhood T-ALL, we observed no infants with either STIL-TAL1+ or TLX3+. TR-r analyses were performed and all but one case (BR4) showed clonal rearrangements (Table I).

SNP-array and FISH data

All diagnostic samples were analysed by SNP-array to identify genomic losses (LOH) and gains (Table SIII), although one sample (BR1) had a low contrast quality control (CQC, Table SIV). The DNA from this sample was extracted from diagnostic BM slides, from which we were able to identify two alterations: TR monoclonal rearrangements and CDKN2A homozygous deletion. Both results were confirmed using polymerase chain reaction (PCR) and quantitative PCR (Q-PCR) approaches.

Among the CNAs identified by SNP-array, we highlight genes considered as ‘drivers’ of the leukaemic process i.e. those genes already causally implicated in the process of oncogenesis (Table SIII). A recurrent 3q25-32 deletion was observed in three out of 13 cases (BR4, BR6 and BR7; Fig 1A) that encompassed MLF1 (myeloid leukaemia factor 1), a negative regulator of cell cycle progression which functions upstream of the tumour suppressor TP53 (Yoneda-Kato et al., 2005). For these 3 cases the deletion range varied between 528,000 bp and 610,000 bp but each deletion...
Table I. Clinical-molecular characterization of infant T-cell acute lymphoblastic leukaemia cases.

| Patient ID | Age (months) | Gender | EGIL | TR-r | NOTCH1 | FBXW7 | PTEN | IL7R | KRA/RAS | STIL/TLI | KMT2A-r | Outcome |
|------------|--------------|--------|------|------|--------|-------|------|------|---------|----------|----------|----------|
| BR1        | 12           | Male   | T-IV | TRG & D | HD Mut | Mut   | WT   | WT   | WT      | WT       | WT       | Deceased |
| BR2        | 8            | Male   | T-IV | TRG, D & B | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| BR3        | 6            | Female | T-IV | TRG, D & B | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| BR4*       | 7            | Female | T-IV | TRG & D | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| BR5*       | 11           | Male   | T-II | TRG, D & B | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| BR6*       | 7            | Female | T-III | TRG & D | PEST Mut | WT   | WT/del | WT   | WT      | WT       | WT       | Deceased |
| BR7*       | 8            | Male   | T-III | TRD   | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| UK1        | 9            | Male   | T-IV | TRG   | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| FR1        | 9            | Female | T-III | TRG & D | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| FR2        | 11           | Female | T-IV | TRG, D & B | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| FR3        | 12           | Male   | T-III | TRG & D | HD/PEST Mut | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| FR4        | 11           | Female | T-IV | TRG, D & B | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |
| FR5*       | 9            | Female | T-IV | TRG & D | WT   | WT   | WT   | WT   | WT      | WT       | WT       | Deceased |

Table II. Main genomic findings observed in our series of infant T-cell acute lymphoblastic leukaemia cases.

| Patient ID | Main CNAs | Gene Alterations |
|------------|-----------|------------------|
| BR1        | CDKN2A/B del | NOTCH1 and FBXW7 mutations |
| BR2        | IKZF1 del ETV6 del FLT3 del | KMT2A-MLLT1 |
| BR3        | 11p13del/LMO2 over | AIM1, SLC35D1, PIK3CB, DTHD1, TIE1, SH3BP2, MLLT4, MZF1, EP300, TLK2, NOL8, PID1D1, RPL3, TCTN2, CHFR, KAT6B, TNK2, DLX6, BPTF, CNGB1, TUSC1 and PDLIM5 |
| BR4        | MLF1 del | AIM1, SLC35D1, PIK3CB, DTHD1, TIE1, SH3BP2, MLLT4, MZF1, EP300, TLK2, NOL8, PID1D1, RPL3, TCTN2, CHFR, KAT6B, TNK2, DLX6, BPTF, CNGB1, TUSC1 and PDLIM5 |
| BR5        | 11p13del/LMO2 over | RBL1 del |
| BR6        | MLF1 del PTEN del | KMT2A-MLLT1 NOTCH1 mutation |
| BR7        | MLF1 del | KMT2A-MLLT4 |
| UK1        | MLLT4 del KMT2A del | KMT2A-MLLT4 |
| FR1        | CDKN2A/B del | KMT2A-MLLT1 PTEN mutation |
| FR2        | CDKN2A/B del | KMT2A-MLLT1 PTEN mutation |
| FR3        | CDKN2A/B del | NOTCH1 and PTEN mutations |
| FR4        | 11q14-q23del/ATM and EED | NOTCH1 and PTEN mutations |
| FR5        | CDKN2A/B del | FBXW7 mutation |

ID: identification; CNAs, copy number alterations; del, deletion; over, overexpression. Encompassed the entire MLF1 gene. MLF1 deletion was confirmed by FISH on case BR6, using a combination of MLF1 and CDKN2A in-house probes (Fig 1B). For the same patient, we also used FISH to confirm both KMT2A-r and PTEN deletion (Fig 1B). Using a Sp6-0-array approach, we could not detect deletion of MLF1 in over 90 European and Brazilian cases of childhood and adolescent T-ALL (unpublished data).

SNP-array analyses also revealed one case (UK1) to harbour small deletions in KMT2A (11q23) and MLLT4 genes (6q27) (Fig 1C) and, because MLLT4 is recognized as a classical KMT2A translocation partner gene, we used FISH to search for a potential KMT2A-r. Consequently, we first detected a KMT2A deletion using the LSI MLL (KMT2A) Dual Colour, Break Apart probe (Fig 1D) and then, using in-house FISH probes for both KMT2A and MLLT4, we confirmed the occurrence of KMT2A-r (Fig 1D).

In common with non-infant paediatric T-ALL, two of our infant cases revealed an 11p13del (BR3 and BR5; Fig S1), a deletion first described in T-ALL at a frequency of 4% that also involves region 11p12 (Van Vlierberghe et al, 2006). Two other studies on childhood T-ALL have identified the same deletion (Mullighan et al, 2008; Szczepanski et al, 2011). A large 11q14-q23 deletion (Fig S1) including ‘driver’ genes (ATM, EED) was observed in one case (FR4) while in another, (BR5), we observed a 13q14-2 deletion that involved the RBL1 gene (Fig S2).

Further analysis of our iT-ALL cohort revealed a lower frequency of CDKN2A deletions than found in paediatric T-ALL. These deletions occur in 70% of T-ALL (Mullighan et al, 2008), but in our study only 38% of iT-ALL cases harboured this deletion (Fig 2).
Copy number assays – Q-PCR and promoter methylation status of CDKN2A

In order to confirm the CDKN2A SNP-array data (Fig 2A) on the five patient samples showing 9p21.3 deletions, we performed real-time Q-PCR assays on all 13 diagnostic samples using three different copy number probes located across the gene. In Fig 2B we highlight in particular the SNP analysis for case BR3, for which the array data presented lower CQC compared with the other 12 cases. In this experiment, we used two normal control DNAs (2 CDKN2A copies), one heterozygous deleted control (1 CDKN2A copy) and DNA from the cell line RPMI-8402 as a homozygous deleted control, (0 CDKN2A copies). Evaluation of the CDKN2A status in the other iT-ALL cases is shown in Fig 2C and Fig S3.

As the majority of our cases did not present a visible CDKN2A gene deletion, we explored a potential alternative pathway for CDKN2A inactivation, i.e. occurrence of CDKN2A promoter methylation. Accordingly, we performed methylation-specific PCR on all 13 iT-ALL patient samples and on two control cell lines: RAJI and HL60 (Supporting Information). Conventional Sanger sequencing of the PCR amplicons established the correct genomic location within
the CDKN2A exon 1 CpG island and confirmed bisulfite modification of the unmethylated cytosine to uracil. As expected, the DNA from the RAJI cell line produced a strong band with methylated primers, while DNA from the HL60 cell line generated a strong band with unmethylated primers. None of the patients revealed a methylated profile for CDKN2A (Fig S4), suggesting that methylation was not an alternative mode of CDKN2A inactivation in these cases.

**NGS data**

Due to the paucity of available DNA from most infants we were unable to perform NGS on all cases and consequently prioritized those cases for which Guthrie Cards were available.

Patient BR4 presented an ETP-profile with no typical T-ALL molecular alterations. We performed whole exome sequencing (WES) on DNA isolated from diagnostic material, however this patient did not achieve remission and therefore no germline material was available for matched analyses. In order to uncover somatic alterations acquired by the leukemic clone, we filtered out mutations listed in the dbSNP and/or 1000genomes databases (http://www.ncbi.nlm.nih.gov/snp/; http://www.1000genomes.org/) and identified a total of 832 single nucleotide variations (SNVs) and 872 insertions or deletions (indels) at diagnosis.

In the BR4 diagnostic sample, after respectively filtering the data by read depth (between 20–250x), coding areas only and deleterious/possibly damaging at protein level (VEP-Ensembl; http://www.ensembl.org/Homo_sapiens/Tools/VEP), we detected 176 SNVs and 272 indels. We decided to focus particularly on 22 affected genes, which have an established causal role in oncogenesis and could therefore be considered as ‘drivers’ of leukemia. For SNVs we chose AIM1, SLC35D1, PIK3CB, DTDH1, TIE1, SH3BP2, MLLT4, MZF1, EP300, TLK2, NOL8, PIDD1, RPL3, TCTN2 and CHFR and for indels we chose; KAT6B, TNK2, DLX6, BPTF, CNGB1, TUSC1 and PDLIM5 (Fig 3A and Table SV). Due to the paucity of available patient material, we simply confirmed selected heterozygous point mutations and indels by Sanger
sequencing in 14 out of the 22 chosen genes, i.e. AIM1, PIK3CB, DTHD1, TIE1, SH3BP2, MLLT4, MZF1, EP300, TLK2, PIDD1, RPL3, KAT6B, BPTF and PDLIM5 (data not shown).

For patient BR6 we used a whole genome sequencing (WGS) approach to precisely determine the CNAs breakpoints with the aim of using this data for subsequent blood spot backtracking analyses. We filtered the WGS data by somatic alterations only, somatic P ≤ 0.05 and exonic areas only and identified 145 mutations (SNVs and indels). Here, we particularly highlight 15 genes that were affected by novel mutations, i.e. mutations not been previously described in reference databases. The genes were: CCDC162P, ADAMTS7, IGSF3, LOC100506990, SPATA31C2, NOS2, ANKR2D2A0ASP, HNRPCL1, TMEM51-AS1, LOC388692, KCTD18, LOC100506060, MLLT10P1, OLG1 and NOTCH1 (Fig 3B and Table SVI). Table II summarises the main genomic abnormalities observed in our rare series of iT-ALL.

**Backtracking aberrations to an origin in utero**

We also sought to investigate the early onset of genetic abnormalities by backtracking to birth the aberrations already present at diagnosis. We obtained archived Guthrie cards of four of the patients (BR4, BR5, BR6 and BR7). Potential clonal markers among these four patients included: a rearranged TRD (BR7); a KMT2A-r, a PTEN deletion and a NOTCH1 indel (all in BR6); and 11p13 and RB1 deletions (both in BR5). The fourth case (BR4) harboured 22 mutations affecting cancer-associated genes and a MLF1 deletion as its only CNA.

We previously determined MLL1 as the partner for KMT2A-r in patient BR6 and subsequently the breakpoint sequence of this rearrangement (Emerenciano et al, 2013), which allowed us to design patient-specific primers to interrogate this rearrangement in the Guthrie card DNAs. From ten individual Guthrie card DNAs examined for BR6, one was KMT2A-MLL1+ (Fig 4A). To investigate the
prenatally as being patient-specific with adequate specificity. In total, we analysed 1200 cloned sequences from the ten blood spot DNAs and identified a single clone with the same NOTCH1-PEST deletion that was present at diagnosis (c.7280delG). Unexpectedly, this clone also harboured a new mutation found 36 base pairs upstream from c.7280delG, (i.e. c.7244_7246delCAC, Fig 4B). This 3 base-pair CAC deletion was also detected alone in 15/1200 Guthrie card clones. Although this deletion was not initially discovered in the bulk DNA analysis of diagnostic material, after cloning the NOTCH1-PEST amplicon from BR6 diagnostic DNA, we found 1/100 clones with both c.7244_7246delCAC and c.7280delG (Fig 4B). Furthermore, as expected for a heterozygous mutation, we observed 50/100 clone sequences with the c.7280delG only. These results suggest that NOTCH1 c.7280delG and c.7244_7246delCAC both occurred prenatally and that the latter potentially occurred (independently) in a cell that did not represent the major clone at diagnosis.

We used WGS to determine the precise breakpoint for the PTEN deletion detected by SNP-array in the BR6 diagnostic sample. The breakpoint in this diagnostic material was cloned using specific primers designed from the WGS coordinates (Fig 4C). Subsequently, we used a semi-nested approach to interrogate the ten DNAs from the Guthrie cards of this patient. One positive DNA (GC5) was identified and Sanger sequenced, confirming that the PTEN deletion in the diagnostic sample was present at birth (Fig 4C). To our knowledge this is the first observation of a gene deletion being identified in a neonatal blood spot.

We next tested the blood spot DNAs from patient BR4 by conventional PCR for the 14 WES mutations validated in the diagnostic DNA. Unfortunately, this patient did not achieve remission and died shortly after diagnosis. All 14 mutations were found in the four Guthrie card DNAs tested (Fig 4A), thus confirming their presence before birth. Nevertheless, we cannot confirm whether these were acquired as somatic mutations in utero solely in haematopoietic cells or were indeed germline mutations.

For patient BR5 we were unable to clone the deletion breakpoints for 11p13 (Fig S1) or for a large heterozygous deletion on chromosome 13 downstream of exon 1 in the tumour suppressor gene RB1 (Fig S2A). However, using Q-PCR copy number analysis (Figs S2B and C) we were able to detect the loss of intron 17 of RB1 in diagnostic and BR5 Guthrie card DNA while intron 1 was undeleted in both. The heterozygous deletion was variably detected in all Guthrie DNAs tested, but not in remission DNA, again suggesting a potential in utero origin for this aberration.

Finally, the TRD rearrangement identified in patient BR7 contained a very small V(N)J junction which, although found to be present in its neonatal blood spot, could not be confirmed as being patient-specific with adequate specificity.

**Discussion**

A novel finding in this unique series of iT-ALL was the loss of 3q25-32 resulting in the complete deletion of MLF1, not previously described in T-ALL nor in acute leukaemias as a deletion. In addition, we have interrogated over 90 European and Brazilian cases of childhood and adolescent T-ALL and were not able to detect this deletion (unpublished data). These data support the notion that deletion of MLF1 may represent a specific marker of iT-ALL. This gene was originally identified as a partner of NPM1 in the translocation t(3;5)(q25;q34), commonly found in acute myeloid leukaemia (AML) and myelodysplastic syndromes and has been shown to play a key role in the leukaemogenesis of these neoplasias (Bras et al, 2012). MLF1 plays a regulatory role in TP53 activity, stabilizing the protein by suppressing its E3 ubiquitin ligase (RFWD2) (Yoneda-Kato et al, 2005). Based on these findings we suggest that this gene deletion may have an oncogenic function because TP53 degradation is triggered by E3 ubiquitin ligase activity in the absence of MLF1. Hence, we hypothesize that MLF1 could act as a recurrent tumour suppressor gene in iT-ALL, however functional studies will be needed to elucidate its role in leukaemogenesis.

We provide evidence that iT-ALL, in common with infant pro-B ALL (Ford et al, 1993; Gale et al, 1997) and at least some cases of T-ALL in childhood (Ford et al, 1997; Eguchi-Ishimae et al, 2008), can be initiated in utero. Definitive data was obtained on one patient (BR6). The neonatal blood spots archived for BR6 harboured the clonotypic KMT2A-MLLT1 fusion sequence, as well as the NOTCH1 mutation and the PTEN deletion, albeit at low frequencies. Additionally, we found evidence suggesting independent deletions in the PEST domain of NOTCH1. Given the very young age of the patient this is not surprising, nevertheless these data suggest not only a prenatal initiation of iT-ALL but significant clonal evolution prior to birth, i.e. sequential acquisition of several mutations. This might help explain the presentation features of high white cell count but very young age (BR6, 7 months). In contrast, for children who present with ETV6-RUNXI pre-B ALL at an older age, usually with low or modest leukaemic burdens, the fusion gene appears to be the only prenatal ‘driver’ event (Ma et al, 2013). Our series of iT-ALL displayed a lower frequency of all the major recurrent gene alterations and CNAs found in childhood T-ALL (Mullighan et al, 2007; Andersson et al, 2015). *PTEN* mutations and/or deletions appeared to be the only abnormality that occurred with the expected frequency (9-20%) (Gutierrez et al, 2009; Mendes et al, 2014). We also observed a lower frequency of alterations affecting *CDKN2A* than has been found in childhood leukaemia, suggesting that its role in iT-ALL leukaemogenesis may be less important than in childhood T-ALL or B lineage ALL (Mullighan et al, 2007). An absence of CNAs was recently reported in KMT2A-ε infant ALL (Andersson et al, 2015).
Previous literature reported a single case with concomitant KMT2A deletion and KMT2A-MLLT4 rearrangement, these abnormalities both being present in the diagnostic sample of a three-year-old child with T-ALL (De Braekeleer et al., 2010). Therefore, patient UK1 is the second reported T-ALL case with these KMT2A alterations combined. Deletion of KMT2A has also been described in cases of precursor B infant ALL with KMT2A-r (Andersson et al., 2015).

A deletion in the classical tumour suppressor gene, RB1, was observed in one (BR5) of our 13 cases. RB1 deletions are described at a frequency of 6-10% for both B-cell precursor ALL and T-ALL in children and adults (Okamoto et al., 2010; Schwab et al., 2013). Deletion at 11p12p13 can act to transcriptionally activate the LMO2 gene, a classic oncogene in T-cell leukaemogenesis (Lecuyer & Hoang, 2004). Deletions affecting locus 11p13 were identified in two of our cases, including BR5, suggesting that this deletion could lead to LMO2 activation and contribute to the development of iT-ALL. Unfortunately, cells were not available from these patients to investigate LMO2 expression. The 11q14-1q23-2del Chr11q/ATM deletions present in patient FR4 have been reported in 30% of chronic lymphocytic leukaemia cases (Edelmann et al., 2012; Skowronskas et al., 2012) and at a lower rate in ALL (Schwab et al., 2013).

Patient BR4 presented an ETP-profile with no typical T-ALL molecular alterations. Hence, we performed WES analyses with a view to uncovering potential ‘driver’ alterations that could account for the emergence of leukaemia. By grouping our 22 highlighted genes according to the hallmarks of cancer (Hanahan & Weinberg, 2011), we uncovered roles including sustaining proliferative signalling, activating invasion and metastasis, resisting cell death and evading growth suppressors. Furthermore, three groups of mutations characteristic of ETP-ALL (RAS signalling, haematopoietic and epigenetic regulators) (Zhang et al., 2012) were also observed in our case. Some of the aberrant genes that we uncovered do not yet have their functions fully elucidated. Given the ease of detection of mutations in the neonatal blood spots of this patient, compared to our other cases, we speculate that they may be germline mutations. It is of interest that an excess of germline variations in KMT2A-r negative infant leukaemia has been described by Valentine et al. (2014), who suggested that such cases may well be enriched for rare coding and deleterious germline variations in cancer-associated genes. The authors postulate that such variations might comprise some proportion of the expected functional imbalance characteristically observed in cancer. This notion aligns with our own KMT2A-r negative case that also did not reveal any of the expected ETP somatic mutations, such as SETD2 or EZH2. Nevertheless EP300, a known ‘driver’ for ETP-ALL (Zhang et al., 2012), was carried as a suspected germline mutation in patient BR4.

In summary, we haveanalysed the genomic abnormalities in a unique series of a rare subtype of paediatric leukaemia – T-ALL in infants. The genotypes or mutational spectra are varied but, overall, different from those of T-ALL in older children and adults. A novel aberration (for acute leukaemia), MLF1 deletion, was present as a recurrent abnormality in three of 13 cases. Finally, we have provided evidence that some of the genetic abnormalities, including a PTEN deletion, were accrued prenatally.

Acknowledgements

The authors would like to thank patients and families that agreed to be involved in the study. Our thanks to the Brazilian Collaborative Study Group of Infant Acute Leukaemia, especially Dr. Terezinha Marques Salles, Dr. Teresa Cristina Cardoso Fonseca, Dr. Isis Quesado Magalhães, Dr. Anna Carolina Dias, Dr. Iamarui Costa, Dr. Jane de Almeida Dobbin and Dr. Renato de Paula Guedes Oliveira for providing patient samples. The authors also acknowledge staff and services provided by INCA and ICR. This research was funded by the Partner Fellowship (#2011/01) awarded to MBM by the European Hematology Association; by the International Award for Research in Leukaemia awarded to MBM by the Lady Tata Memorial Trust; INCA, CNPq and FAPERJ (MSPO); the Kay Kendall Leukaemia Fund (FWvD); the Leukaemia & Lymphoma Research (AMF, CLF and MG) and The Institute of Cancer Research (AMF and SMC). MBM has also been granted a partial scholarship from INCA.

Author contributions

MBM: designed the study, conducted and analysed all the experiments and wrote the paper; FWvD: supervised all SNP procedures and analyses; SMC: assisted in sample preparation and supervised the FISH experiments; CLF and ME: performed FISH and/or molecular investigations; HK, EC, HC, JS: provided clinical samples and immunophenotypic/cytogenetic/clinical data; JG: performed the WGS analyses; MSPO and MG: designed the study and wrote the paper; AMF: designed and supervised the study, generated and analysed experimental data and wrote the paper. All authors critically reviewed and approved the final draft of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Data S1. Materials and methods.
Data S2. Supplementary tables with additional data.
Fig S1. Copy number analysis of chromosome 11 in our iT-ALL series.
Fig S2. Genomic copy number analysis of RB1.
Fig S3. CDKN2A Q-PCR copy number data of the Brazilian infant patients.

Fig S4. Methylation specific PCR (MSP) in our iT-ALL cohort and two control cell lines.

Table S1. Clinical–demographic data of infant T-ALL cases.

Table SII. Mutation screening data of infant T-ALL cases.

Table SIII. SNP-array copy number data of infant T-ALL cases.

Table SIV. Contrast quality control (QC) data from SNP6-0-arrays of the infant T-ALL cases.

Table SV. WES data details for patient BR4.

Table SVI. WGS data details for patient BR6.

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