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Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse

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T cell acute lymphoblastic leukemias (ALLs [T-ALLs]) are aggressive malignancies derived from the transformation of lymphoblastic precursors in the thymus. Despite major therapeutic improvements, a fraction of patients with T-ALL still relapse and experience eventual refractory leukemia (Bailey et al., 2008; Pui et al., 2008). ALLs are characterized by the deregulated expression of oncogenic transcription factors combined with the frequent inactivation of the tumor suppressor CDKN2A/P16/ARF and activation of the NOTCH1 pathway (Ferrando et al., 2002; Weng et al., 2004; De Keersmaecker et al., 2005; Soulier et al., 2005; Aifantis et al., 2008). Recently, high-resolution cytogenetic tools have identified frequent additional cryptic deletions and duplications in T-ALL cells (Clappier et al., 2007; Van Vlierberghe et al., 2008; Gutierrez et al., 2009; Tosello et al., 2009; Kleppe et al., 2010). We used our established xenotransplantation assay of human T-ALL in immunodeficient mice and expanded a more aggressive malignancy, recapitulating the process of relapse in patients. These findings may contribute to the design of novel strategies to prevent or treat relapse.
immunodeficient mice (nonobese diabetic [NOD]/scid or NOD/scid/IL-2Rγ null; Armstrong et al., 2009) to test the hypothesis that xenografted leukemia cells could experience clonal evolution.

RESULTS AND DISCUSSION
Establishment of xenograft leukemias frequently selects minor genetic subclones, reminiscent of relapse in patients

We compared by high-density DNA arrays the genome of a series of 18 primary T-ALL cases at the time of diagnosis (diagnosis leukemia) and after primary engraftment in immunodeficient mice (xenograft leukemia; Fig. 1 A). A very good overall stability of the T-ALL genome was observed in all cases, with a pattern of copy number alterations being largely consistent between diagnosis and xenograft leukemia samples (Fig. S1 and Table S2). However, some changes were also observed (Fig. 1 and Table S2). Strikingly, these new genomic abnormalities in xenograft cells mainly targeted genes known as human oncogenes or tumor suppressor genes like PTEN, MYC, MYB, CDKN2A, DLEU7, or WT1. Sequencing T-ALL cancer genes NOTCH1, FBXW7, and PTEN also showed changes in the mutation pattern of xenograft versus diagnosis cells (Fig. 1 D and Table S3). Altogether, genomic changes were detected in 12 out of 18 T-ALL pairs (67%). In some cases, the stepwise sequence of genomic events could be reconstructed and demonstrated that xenograft cells had derived not from the diagnosis cells but from a common prediagnosis ancestor cell (Fig. S2, A and B). To investigate whether the additional genomic abnormalities found in xenograft leukemia cells occurred de novo in the mouse or had preexisted in the patient, we performed highly sensitive lesion-specific genomic PCR backtracking to test for their presence in rare cells at diagnosis. In the four analyzed cases, the genomic lesions were indeed detected at low levels in the diagnosis sample, demonstrating that the engrafted leukemia in the mouse had arisen from a minor genetic subclone present in the patient’s leukemia (Fig. 1 F and Fig. S2 C). These results, along with the fact that the additional lesions target cancer genes, suggest that clonal selection related to the activation of prototypical human oncogenic pathways occurs during leukemia reinitiation and expansion in immunodeficient mice.

Intraclonal heterogeneity at diagnosis and clonal evolution at relapse have been reported in human ALL (Zuna et al., 2004; Choi et al., 2007; Mullighan et al., 2008; Yang et al., 2008; Tosello et al., 2009; Anderson et al., 2011). Genomic analysis of relapsing ALLs has suggested that the relapse frequently does not simply result from mutations of specific drug-resistance genes but rather from additional oncogenic abnormalities that contribute to the emergence of a leukemic subclone (Mullighan et al., 2008). Reminiscent of this process, our results suggest that development of human leukemia in the immunodeficient mouse does not simply result from specific adaptation to the mouse environment but rather from the oncogenic selection and expansion of a preexisting leukemic subclone. Therefore, we hypothesized that leukemia reinitiation in the mouse could mirror the leukemia relapse process in patients. We investigated a series of T-ALL patients who had further relapsed; mice were injected with the patient diagnosis sample, and the resulting xenograft, diagnosis, and relapse DNA triads were compared (n = 8 triads). In all triads but one, relapse and xenograft samples harbored a range of new genomic lesions compared with diagnosis cells (Figs. 1 G and 2 and Tables S1 and S3). Oncogenetic trees showed that the xenograft and the relapse leukemia cells had derived not from the bulk diagnosis cells but from a common prediagnosis ancestor cell in seven of eight T-ALL triads, as did the diagnosis cells, which is consistent with a branched rather than linear clonal evolution (Fig. 2). In five out of eight cases (XLE_61, XLE_92, XLE_95, XLE_96, and XLE_98), the xenograft and relapse clones were located in the same branch, whereas in two cases, the xenograft clone was closer to the bulk diagnosis clone (XLE_93 and XLE_97), and in one case the three clones were equally distinct (XLE_94). It is noteworthy that distinct genetic lesions targeting the same gene could be found in several samples from the same patient (like CDKN2A/P16/ARF deletions, MYB duplications, and NOTCH1 mutations), demonstrating that they had independently arisen more than once (Fig. 2). Altogether, these results are consistent with a neo-Darwinian genetic diversification of leukemia, in accordance with the model of Anderson et al. (2011), that is especially uncovered by relapse or xenograft.

Additional genomic lesions of xenograft leukemias can be drivers of increased leukemia-initiating activity

At this point, we reasoned that if some additional genomic abnormalities observed in the engrafted subclones were responsible for their preferential engraftment, this advantage should be conserved when primary xenograft cells are reinjected into secondary recipients. Indeed, xenografted cells reestablished leukemia more rapidly than diagnosis cells in secondary versus primary xenografts experiments (Fig. S3). Although increased proliferation could be involved in such accelerated secondary engraftment, clonal selection may also have enriched the xenograft leukemia in leukemic stem cells, as suggested by limiting-dilution analyses (Fig. S3 C). Interestingly, Boiko et al. (2010) have suggested that the more cancers are advanced, the more efficiently they engraft, which is consistent with a progressive enrichment in cancer stem cells and the loss of hierarchical structure as tumors progress.

We then sought to model the selective advantage and gain of malignancy likely conferred by the acquisition of an additional genomic lesion in leukemia cells. The tumor suppressor gene PTEN was a good candidate here as (i) it is involved in both proliferation and leukemia stem cell properties (Yilmaz et al., 2006), (ii) it has been associated with cancer progression and relapse including in T-ALL (Palomero et al., 2007; Gutierrez et al., 2009), and (iii) we found it newly mutated or deleted in xenograft leukemia cells from several patients (Fig. 1 F and Tables S2 and S3). Using lentiviral transduction, we genetically modified the diagnosis primary leukemia cells (Gerby et al., 2010) of a patient who had further relapsed and harbored a PTEN deletion in both his relapse and xenografted cells but
Figure 1. Genomic changes associated with clonal selection in xenografted leukemias. (A) Strategy of sample analysis. (B) Array CGH plots showing focal deletions in a T-ALL case, either in xenografted cells only (WT1 locus at 11p13) or present in both diagnosis and xenografted cells (11q14.2 locus). (C) FISH analysis of TCR-β (red probe) and MYC (green probe) loci in diagnosis and xenografted leukemia cells. Bar, 12 µm. (D) DNA sequence chromatograms showing NOTCH1 and PTEN mutations in xenograft samples. Arrows indicate the mutated nucleotides. (E) List of cancer-associated genes targeted by genomic changes in xenograft leukemias. (F) Representative lesion-specific genomic PCR backtracking in a T-ALL case: focal PTEN deletion as detected by array CGH in the xenograft but not the diagnosis bulk leukemia cells, sequence of the deletion breakpoints junction, and amplification curves from specific quantitative PCR experiments of the undiluted diagnosis sample (indicated by an arrow) compared with 10-fold dilutions of the xenograft sample from 10% to 0.01%. The horizontal green line indicates the threshold for PCR linearity. The horizontal purple line is the cursor of the Genomic Workbench software, centralized on the deletion. Each quantitative PCR point was performed in duplicate. (G) Array CGH plots in a T-ALL triad case showing an 11q deletion in both the xenograft and relapse but not in the diagnosis samples. XLE, unique identification numbers for T-ALL cases.
not in the bulk diagnosis cells. Competitive engraftment experiments clearly demonstrated the selective advantage of PTEN knockdown leukemia cells over control diagnosis leukemia cells (Fig. 3, A–C). Moreover, kinetics and limiting-dilution analyses in noncompetitive engraftment experiments showed an increased leukemia initiating activity caused by PTEN knockdown in primary leukemia cells (Fig. 3 D). These results demonstrate that the additional genomic lesions in xenograft leukemia can be drivers of the increased malignancy and related clonal selection of the human leukemia cells in mice. Importantly, this original experimental strategy faithfully reproduces the process of tumor progression from diagnosis toward relapse.

**Xenograft samples strongly express cell cycle and mitosis genes and display in vitro diminished sensitivity to drugs, reminiscent of leukemias at relapse**

To gain insight into the functional changes of the xenograft cells relative to diagnosis cells, we profiled by large-scale expression analysis 9 paired xenograft and diagnosis T-ALL samples along with 93 other human T-ALL samples. First, unsupervised analysis showed that the diagnosis and xenograft samples from the same patient clustered next to one another for all pairs, demonstrating the excellent overall relevance of the xenograft model (Fig. 4 A). We then performed a paired supervised analysis that sorted out a list of up-regulated genes that individually differentiated the xenografts from the corresponding diagnosis leukemias (Fig. 4 B; the list of genes is in the supplemental material). Strikingly, this list was dramatically enriched in a cluster of genes clearly linked to cell cycle and mitosis (see Gene Ontology annotations in Table S1), suggesting that an increased proliferation contributes to the selective advantage of the engrafted cells. The xenograft signature also included a few other unrelated genes, including CD133 (PROMD1/Prominin-1), which is a marker of hematopoietic stem cells and of cancer stem cells (Singh et al., 2004). Interestingly, the xenograft signature was strongly expressed in all of the nine xenograft samples relative to the paired diagnosis samples, including in three cases without identified additional genomic changes in the xenograft sample (cases XLE_40, XLE_52, and XLE_69; Fig. 4 B). Cryptic genomic lesions or epigenetic mechanisms could be involved in these cases, also converging on cell cycle and proliferation.

Several studies have found cell cycle, mitosis, and proliferation signatures in relapsing ALL patients (Bhojwani et al., 2006; Kirschner-Schwabe et al., 2006; Staal et al., 2010). We analyzed the relevance of the xenograft signature in relapse samples from patients. We performed gene set enrichment analyses using the expression data from two independent series of paired diagnosis and relapse ALLs (Bhojwani et al., 2006; Staal et al., 2010). Strikingly, the xenograft signature was significantly enriched in the relapse cells relative to the diagnosis cells in both series (Fig. 4 C). These data indicate that xenograft and relapse leukemias share a unique profile of increased proliferation and highlight the relevance of the xenograft leukemia system to model patient relapse.

Although the majority of ALL patients at diagnosis respond well to treatment and reach complete remission, patients who relapse frequently experience drug resistance and eventual refractory leukemia (Klumper et al., 1995; Choi et al., 2007; Bailey et al., 2008). Additional oncogenic abnormalities, like PTEN inactivation, could be involved in acquired resistance to treatment (Palmero et al., 2007). Because we found evidence of clonal selection and gain of malignancy in xenografted leukemia cells, we assessed whether this oncogenic progression was also associated with an acquired diminished...
sensitivity to drugs. We therefore assayed the sensitivity of xenografted leukemia samples to glucocorticoids, which are essential components of ALL treatment, and to γ-secretase inhibitors, which synergistically target T-ALL cells as the result of oncogenic activation of the NOTCH1 pathway (Weng et al., 2004; Real et al., 2009). In vitro cultures of leukemia samples on the Notch ligand–expressing stromal cells MS5-DL1 (Armstrong et al., 2009) first confirmed that xenografted cells were much more proliferative than diagnosis cells (Fig. 4 D). Moreover, although xenograft cells were still inhibited in a dose-dependent manner, high doses of glucocorticoids were necessary to efficiently reduce the cell number, and the addition of γ-secretase inhibitors was required to increase apoptosis (Fig. 4 D). These data suggest that increased proliferation and inhibition of apoptosis result in an overall lesser sensitivity of xenograft samples to treatment, as observed in patients at relapse. This is strengthened by our finding that xenograft cells, like relapsing cells, often have additional genomic lesions targeting cancer genes that are related to cell cycle and proliferation rather than specific drug-resistance genes. Such mechanisms could contribute to the poor response to treatment of relapsing leukemia. It is noteworthy that additional mutations in PTEN, MYC, or NOTCH1, as found in this study in xenograft leukemias, may also modulate the NOTCH pathway and thus the sensitivity of the leukemia cells to γ-secretase inhibitors (Weng et al., 2004; Palomero et al., 2006, 2007).

Despite major therapeutic improvement in ALL, relapsing patients still have a dismal prognosis, highlighting the need for more efficient strategies to treat these aggressive tumors. Our data indicate that the development of human T-ALL in
Figure 4. Increased proliferation and overall diminished drug sensitivity in xenografted leukemias. (A) Paired diagnosis and xenograft leukemias were analyzed by unsupervised large-scale gene expression profiling. 9 pairs of diagnosis and xenograft T-ALL samples along with a series of 73
Mice. Immune-deficient NOD.CB17-Prkdc(scid) (abbreviated NOD/scid [NS]) and NOD.Cg-Prkdc(scid/Ij2gvlmlWjfla/Sj) (abbreviated NOD/scid/IL-2Rγ null [NSG]) were both obtained from the Jackson Laboratory. Mice were housed in pathogen-free animal facilities at the Institut de Radiobiologie Cellulaire et Moléculaire (Commissariat à l’Énergie Atomique, Fontenay-aux-Roses, France). All experimental procedures were performed in compliance with the French Ministry of Agriculture regulations (animal facility registration number A290322) for animal experimentation.

Sample preparation and DNA and RNA extraction. White blood cells from patient samples were isolated by Ficoll centrifugation and cryopreserved using standard procedures. After thawing, cells were spun down, and DNA was extracted with the DNA blood mini kit (Qiagen). RNA extraction was performed with the miRNeasy (Qiagen). Nucleic acid quantity and quality was assessed using a spectrophotometer (ND-1000; NanoDrop).

Genome-wide DNA array analysis. Genomic DNAs from paired diagnosis and xenograft samples (n = 25 patients) and from corresponding relapse leukemia samples (in 7 of these patients) were analyzed by high-density array comparative genomic hybridization (aCGH) technologies using the 1 × 1M Microarray SurePrint G3 Catalog (Agilent Technologies) or by single nucleotide polymorphism (SNP) array technologies using the Genome-Wide Human SNP Array 6.0 (Affymetrix) according to the manufacturers’ recommendations (total n = 42 and n = 14 DNA samples, respectively; each sample originating from the same patient was analyzed by the same technology). All Microarray SNP/CGH array data files are available from ArrayExpress under accession no. E-MTAB-593. Analyses were performed using the Genomic Workbench software (Agilent Technologies) with the help of the ADM-2 algorithm for array CGH data. Genome Suite 6.5 software (Partek), and the Hidden Markov Model and Segmentation algorithms for SNP-based copy number and genotyping analyses. The final retained abnormalities were validated by two investigators (E. Clappier and J. Soulier) by visual analysis considering the size and Log2 ratios of the abnormalities with respect to the individual background noise of each array at each particular chromosomal location, as reported previously (Clappier et al., 2007; Kleppe et al., 2010). Final validated data are provided in Tables S2 and S3. The University of California, Santa Cruz Genome Browser was used for locating genes on the human genome. Polymorphic copy number variations were excluded using the Database of the Genomic Variants tracks in the University of California, Santa Cruz Genome Browser or in the Genomic Workbench software.

primary T-ALL cases and 20 T cell lines were analyzed; the 9 pairs are boxed vertically in black. (B) Gene clustering of the 59 unique genes of the xenograft signature, as defined by reference to the paired primary samples ("overexpressed in T-ALL xenografts gene set"). Representative genes are shown on the right of the heat map. (C) Gene set enrichment analysis (GSEA) of the xenograft gene set in the relapse versus diagnosis samples of two independent ALL series (Bhojwani et al., 2006; Staal et al., 2010). (D) Proliferation, apoptosis, and dose-dependent inhibition of paired diagnosis and xenograft leukemia samples treated with dexamethasone (dexam), α-secretase inhibitor (GSI), or both, compared with vehicle only (DMSO; -). Proliferation was assessed as the number of Ki67+ cells, apoptosis as the percentage of annexin V+ cells, and dose-dependent inhibition as the cell number fold change (treated/vehicle). Error bars represent means ± SD of triplicate experiments.
Fluorescent in situ hybridization (FISH) analysis. The unbalanced translocation TCRB-MYC suspected on the array CGH profile in the xenograft sample of case XLE_22 was confirmed by FISH analysis using the bacterial artificial chromosome probes RP11-440A11 (green) at the centromeric part of the MYC locus on 8q24 and RP11-78G15 (red) at the telomeric part of the TCRB locus on 7q34 by demonstrating a fusion in the xenograft but not diagnosis nuclei. Probes were obtained from AmpliTech.

**NOTCH1, FBXW7, PTEN, and WT1 mutations screening.** Mutational screening of the NOTCH1, FBXW7, and WT1 genes was performed. NOTCH1 mutations were searched for in the N- and C-terminal regions of the heterodimerization domain (exons 26 and 27, respectively), the transcriptional activation domain, and the PEST domains (distant part of exon 34); extracellular juxtamembrane expansion mutations (exon 28) were also investigated. For FBXW7, mutations in the WD40 domain were examined by sequencing exons 9 and 10. PTEN mutations were assessed in exon 7, and WT1 mutations were assessed in exon 7.

Quantitative genomic PCR for specific backtracking of abnormalities. Lesion-specific quantitative genomic PCR assays were designed based on the breakpoint or mutation sequences of four genomic abnormalities identified in diagnosis and xenograft cell lines. Genomic DNA was digested with restriction endonucleases, and primers were designed to amplify both flanking primers outward from the genomic locations of CGH array probes defining the minimal regions of deletion at ~500-bp intervals to beyond the next nondeleted probes telomeric and centromeric to the deletion. A combination of forward and reverse PCR primers was used to amplify the region of genomic deletion, and the genomic deletion breakpoints were determined by direct sequencing of PCR products. A specific real-time PCR assay using TaqMan probes or SYBR Green reagents (Applied Biosystems) was designed for each lesion analyzed. Quantification of the genomic lesion in diagnosis sample cells was performed using 10-fold dilutions of the xenograft DNA harboring the lesion. Experiments and interpretation of results were performed according to guidelines for minimal residual disease assessment.

**Large-scale gene expression analyses.** 9 pairs of xenograft and diagnosis T-ALL samples along with a series of 93 T-ALL samples (73 primary T-ALL and 20 T cell lines) were profiled using large-scale gene expression analysis on the GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). RNA extraction, labeling, and microarray hybridization were processed in the same manner for all samples in the same set of experiments. Microarray data files are available from ArrayExpress under accession no. E-MTAB-604. After robust multichip average normalization, unsupervised hierarchical clustering was performed using Dchip tools as previously reported on another set of T-ALLs (Soulier et al., 2005). Probe sets for the unsupervised clustering shown in Fig. 4A were selected on expression variability and reliability among samples after robust multichip average normalization on the following criteria: SD > 1.5 and gene expression >6 in at least 10% of cases and nonredundant genes, resulting in 880 probe sets. To avoid spurious clustering caused by contaminating BM cells, probe sets with high expression in normal BM samples were identified using unsupervised hierarchical classification and excluded, as well as genes from chromosome Y, resulting in a final list of 749 probe sets. The Cell cycle and Mitosis cluster identified from this analysis largely overlaps with the Cluster 6 previously defined (Soulier et al., 2005). Comparison between the paired xenografts and diagnosis samples was performed using the SAM procedure (target proportion of false discoveries, 0.005; 500 permutations; percentile for determining falsely called genes, 90), resulting in 100 probe sets “overexpressed T-ALL xenograft” (58 unique genes; see list in supplemental material). Biological pathways which were statistically linked with this gene set were tested using Gene Ontology and the Broad institute tools. Gene set enrichment analysis of this gene set in relapse versus diagnosis leukemias was performed using two series of paired ALL. These series include n = 35 ALL pairs (32 B cell progenitor ALLs and 3 T-ALLs; Bhoyan et al., 2006) and n = 41 pairs (27 B cell progenitor ALLs and 14 T-ALLs; Staal et al., 2010). Means of probe set expression were ranked using paired Student’s t tests, and 1,000 permutations were performed to calculate nominal probabilities as indicated.

**PTEN gene silencing in primary T-ALL cells.** The pSuper construct containing PTEN short hairpin RNA (shRNA) was generated by cloning the following human PTEN-specific RNA interference target sequence into the pSuper plasmid: 5’-AGACAAAGCCAAACCGATAC-3’. The H1-shRNA-AP/PTEN fragment was then subcloned into the pTRIP/AU3-MND-GFP where the green fluorescent protein coding sequence is under the control of the MND promoter as previously done for a control shCTL directed against the human hepatitis B virus. A lentiviral vector encoding the red fluorescent protein Cherry was subsequently derived from this plasmid. Human T-ALL samples were transduced with unconcentrated G/IL-7SUx envelope pseudotyped vectors as previously reported (Gerby et al., 2010). In brief, human T-ALL cells were co-cultured for 48 h with the mouse stromal cells expressing the Notch ligand DLL1 MS5-DL1 in reconstituted α-MEM supplemented with 10% FCS and 10% human AB serum, in the presence of stem cell factor, rFlt3-L, insulin, and rIL-7 as reported previously (Gerby et al., 2010). Leukemia cells were then spinoculated for 2 h in the presence of plastic-bound human DLL1, 2.5 µg/ml protease, and G/IL-7SUx pseudotyped lentivirus vectors, and transduction was confirmed by immunofluorescence. Cells were again co-cultured with MS5-DL1 cells plus cytokines during 1 wk for maximal reporter fluorescent protein expression. Transduced leukemia cells were then sorted (Flow cytometer Influx; BD) based on the CD45+, CD7+, and Cherry+ phenotype before transplantation into immunodeficient mice.

**In vitro drug sensitivity assay.** As xenograft leukemia cells were co-cultured for 1 wk with MS5-DL1 cells, as described previously (Gerby et al., 2010), in the presence of 5 and 50 nM dexamethasone (Dexa; D-4902; Sigma-Aldrich) and/or 5 µM γ-secretase inhibitor (N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester [DAPT]; EMD) or with the DMSO vehicle alone. Cell cycle activation was quantified using Ki67 labeling, and apoptosis was measured by annexinV–FITC staining (BD) on harvested leukemic cells and analyzed by FACS.

**Online supplemental material.** Fig. S1 shows examples of identical genomic lesions in diagnosis and xenograft leukemia samples. Fig. S2 shows that diagnosis and xenograft leukemias can derive from a common prediagnosis ancestor clone and also shows specific backtracking data in diagnosis samples of genomic abnormalities identified in xenograft samples. Fig. S3 shows that xenografted leukemias have increased leukemia-mutating activity relative to diagnosis leukemias. Fig. S4 shows a model for clonal selection and gain of malignancy in patient and xenograft. Table S1 lists the enriched Gene Ontology terms in the overexpressed in T-ALL xenografts gene set. Table S2, included as an Excel file, is the list of copy number aberrations in T-ALL samples. Table S3, included as an Excel file, is the list of NOTCH1, FBXW7, PTEN, and WT1 mutations in T-ALL samples. The overexpressed in T-ALL xenografts gene set is also listed in the supplemental material. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20110105/DC1.

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REFERENCES

Aafjes, J. N. et al. 2011. Deletion of the protein tyrosine phosphatase gene PTPN2 in T-cell acute lymphoblastic leukaemia. Nat. Genet. 43:530–535. doi:10.1038/ng.857.

Kumpe, E., R. Pieters, A.J. Veerman, D.R. Huismans, A.H. Loonen, K. Hählen, G.J. Kaspers, E.R. van Wering, R. Hartmann, and G. Henze. 1995. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukaemia. Blood. 86:3861–3868.

Mullighan, C.G., L.A. Phillips, X. Su, J. Ma, C.B. Miller, S.A. Shurtleff, and J.R. Downing. 2008. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 322:1377–1380. doi:10.1126/science.1164266.

Palomero, T., W.K. Lim, D.T. Odom, M.L. Sulis, P.J. Real, A. Margolin, K.C. Barnes, J. O’Neil, D. Neuberg, A.P. Weng, et al. 2006. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukaemic cell growth. Proc. Natl. Acad. Sci. USA. 103:18261–18266. doi:10.1073/pnas.0606108103.

Palomero, T., M.L. Sulis, M. Cortina, P.J. Real, K. Barnes, M. Ciofani, E. Caparros, J. Butke, K. Brown, S.L. Perkins, et al. 2007. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukaemia. Nat. Med. 13:205–210. doi:10.1038/nm1636.

Bailey, L.C., P.D. Butler, G.P. Yang, B. Joshua, M.J. Kaplan, et al. 2010. Human brain tumour initiating cells express neural crest nerve growth factor receptor CD271. Nature. 466:133–137. doi:10.1038/nature09160.

Klumper, E., R. Pieters, A.J. Veerman, D.R. Huismans, A.H. Loonen, K. Hählen, G.J. Kaspers, E.R. van Wering, R. Hartmann, and G. Henze. 1995. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukaemia. Blood. 86:3861–3868.

Mullighan, C.G., L.A. Phillips, X. Su, J. Ma, C.B. Miller, S.A. Shurtleff, and J.R. Downing. 2008. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 322:1377–1380. doi:10.1126/science.1164266.

Palomero, T., W.K. Lim, D.T. Odom, M.L. Sulis, P.J. Real, A. Margolin, K.C. Barnes, J. O’Neil, D. Neuberg, A.P. Weng, et al. 2006. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukaemic cell growth. Proc. Natl. Acad. Sci. USA. 103:18261–18266. doi:10.1073/pnas.0606108103.

Palomero, T., M.L. Sulis, M. Cortina, P.J. Real, K. Barnes, M. Ciofani, E. Caparros, J. Butke, K. Brown, S.L. Perkins, et al. 2007. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukaemia. Nat. Med. 13:205–210. doi:10.1038/nm1636.

Singer, S.K., C. Hawkins, I.D. Clarke, J.A. Squire, J. Bayani, T. Hide, R.M. Henkelman, M.D. Cusumano, and P.B. Dirks. 2004. Identification of human brain tumour initiating cells. Nature. 432:396–401. doi:10.1038/nature03128.

Staal, F.J., D. de Riddler, T. Szczepanski, T. Schonewille, T. Palomero, et al. 2009. WT1 number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukaemia. Blood. 114:1038–1045. doi:10.1182/blood-2008-12-192039.

Yilmaz, O.H., R. Valdez, B.K. Theisen, W. Guo, D.O. Ferguson, H. Wu, and J.R. Downing. 2008. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukaemic cell growth. Proc. Natl. Acad. Sci. USA. 103:18261–18266. doi:10.1073/pnas.0606108103.

Zuna, J.A.M. Ford, M. Peham, N. Patel, V. Saha, C. Eckert, J. Kochling, R. Panzer-Grümayer, J.Tika, and M. Greaves. 2004. TEL deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukaemia. Clin. Cancer Res. 10:5355–5360. doi:10.1158/1078-0432.CCR-04-0584.