# A robust in vivo model for B cell precursor acute lymphoblastic leukemia

Terry J. Fry[1] and Peter D. Aplan[1,2]

[1]Pediatric Oncology Branch and [2]Genetics Branch, Center for Cancer Research, National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA.

**B cell precursor acute lymphoblastic leukemia (BCP ALL) is the most common malignancy in children. While treatments have improved remarkably over the past four decades, resistant disease and late effects that result from cytotoxic chemotherapy remain serious problems for individuals with BCP ALL. Improved genetic tools have led to the discovery of numerous somatic mutations associated with BCP ALL, leading to a framework for the genetic classification of BCP ALL. In this issue of the *JCI*, Duque-Afonso et al. develop an accurate in vivo model for BCP ALL that recapitulates the key features of human disease, including acquired mutations in genes encoding PAX5 and components of the JAK/STAT pathway. The authors further show, as proof of principle, that this model can be used to evaluate the efficacy of drugs designed to target specific acquired mutations in patients with BCP ALL.**

## The genetic basis for B cell precursor acute lymphoblastic leukemia

Acute leukemia has long been recognized as the most common malignancy in childhood (1). The incidence of acute lymphoblastic leukemia (ALL) is about three cases per 100,000 children per year in the United States, with an age peak between two and five years (2). The incidence and age peak of ALL are similar in most industrialized countries (3). Early studies of cell surface ALL markers subclassified the disease into T ALL (15%–20%), B ALL (1%–2%), and non-T, non-B ALL (also referred to as null-cell ALL) (80%) (4, 5). Subsequently, the discovery that most non-T, non-B ALL samples stained for a common ALL antigen (CALLA, which is now known as CD10) led to the realization that most non-T, non-B ALL cases were in fact leukemias of B cell precursors (6, 7). For diagnostic and treatment purposes, pro-B, pre-pro B, and pre-B ALL are generally grouped together as B cell precursor ALL (BCP ALL) (8, 9).

The treatment of children with BCP ALL is one of the most remarkable success stories in modern medicine and has served as a paradigm for the use of multiagent chemotherapy in the context of large, multiinstitutional clinical trials (10). In the 1950s, survival rates of children with BCP ALL were negligible; however, recent rates are approaching 90% in current trials (8). The causes for the increased survival rate are manifold; however, one important factor is the use of risk-adapted or risk-directed therapy (8, 10). This directed approach requires stratification of patients for therapeutic purposes based on prognostic factors. Individuals with a poorer prognosis receive more aggressive (and often more toxic) therapy, while individuals with a better prognosis receive less aggressive therapy that is aimed to minimize toxicity, including late effects. In order for this strategy to be effective, the prognostic factors must be robust. The most useful prognostic variables for all individuals with BCP ALL are age, white blood count (WBC) at diagnosis, and mutational status of the leukemic blasts (10).

▶ Related Article: p. 3667

**Conflict of interest:** The authors have declared that no conflict of interest exists.
**Reference information:** *J Clin Invest*. 2015;125(9):3427–3429. doi:10.1172/JCI83799.

Traditionally, mutations in leukemic cells have been evaluated with cytogenetic tools. Conventional cytogenetic analysis, which is now routinely augmented by molecular tools such as FISH of interphase cells, can detect abnormalities in the cells of most children with BCP ALL (11, 12). These abnormalities typically take the form of abnormal numbers of chromosomes (ploidy) or balanced chromosomal translocations. Hyperdiploidy, commonly in the form of trisomies of chromosomes 4, 10, and 17, carries a favorable prognosis (11), while hypodiploidy (<44 chromosomes) confers a worse prognosis, and the results of these analyses are used to help select risk-adapted therapy (13). Structural chromosomal abnormalities also affect prognosis. For example, the balanced translocation t(4;11)(q21;q23) leads to production of an oncogenic MLL-AF4 chimeric protein, which confers a poor prognosis, resulting in more aggressive therapy for children with this translocation (14).

The advent of high-density nucleotide arrays and massively parallel ("next-generation") sequencing techniques has revolutionized the field of cancer gene discovery (15, 16). Application of these techniques has identified recurrent mutations, such as single nucleotide variants, small insertions or deletions (indels), and larger deletions that are cytogenetically undetectable in BCP ALL (17). These mutations involve genes that encode factors critical for normal B cell differentiation (such as PAX5 and IKZF1), as well as those that drive proliferation (such as IL-7R, and NRAS). A working hypothesis based on co-occurrence of mutations in BCP ALL patients suggests that an initial lesion (such as an MLL-AF4 fusion) leads to increased stem cell self-renewal, followed by a collaborating lesion (such as *PAX5* deletion) that results in developmental arrest, and a mutation in a key signaling pathway (such as JAK1/2/3) that results in leukemic transformation (Figure 1 and ref. 17).

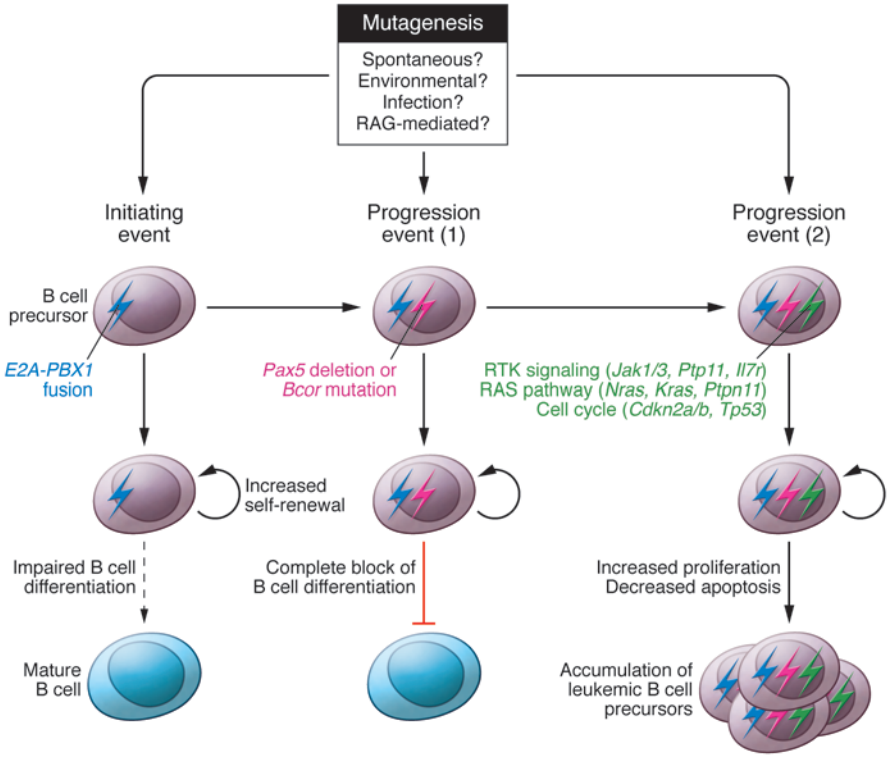


**Figure 1. Collaborative mutations lead to BCP ALL.** In the context of the E2A-PBX1 model, the initiating lesion (an E2A-PBX1 fusion) is engineered into the mouse germline and results in increased self-renewal and impaired differentiation of B cell precursors. Subsequent mutations – that either occur spontaneously or are engineered into the mouse germline – lead to a complete block to B cell differentiation. Further mutations lead to increased proliferation or decreased apoptosis, resulting in an accumulation of leukemic B cell precursors. These additional mutations involve several overlapping pathways, including those involved in receptor tyrosine kinase (RTK) signaling, RAS signaling, and cell cycle progression. Mutations in several genes, including *Jak1*, *Ptp11*, *Il17r*, *Nras*, *Kras*, *Ptpn11*, *Cdkn2a/b*, and *Tp53*, were identified in the E2A-PBX1 model and were associated with disease progression. It should be noted that this order of mutations, while applicable for E2A-PBX1 mice with engineered mutations, is not necessarily invariant. For instance, an RTK mutation may precede a block to differentiation under certain circumstances. The general framework of this model is based on sequence data from leukemic patients (17, 28).

## An in vivo model for BCP ALL

A corollary of Koch's postulates is that in vivo models of disease are important for establishing disease causation (18). In addition, in vivo models are invaluable for studying disease processes as well as for producing important preclinical platforms for the development of more effective and less toxic therapies. A general approach that has been useful in modeling leukemia in vivo is the use of genetic engineering techniques to express a fusion protein in hematopoietic cells of mice (19). Unfortunately, this approach has been largely unsuccessful in modeling BCP ALL in vivo. While several investigators have generated mice that express an ETV6-RUNX1 fusion (the most common translocation in BCP ALL patients), these animals do not develop BCP ALL (19). Another common translocation in BCP ALL patients is the t(1;19)(*E2A-PBX1*), which leads to a chimeric fusion protein that joins the amino terminal portion of E2A with the carboxy terminal portion of PBX1 (20). Mice that express an E2A-PBX1 fusion developed ALL; unexpectedly, they developed a T lineage ALL rather than B lineage ALL (21). However, crossing the E2A-PBX1–expressing mice onto a CD3e-deficient background delayed the onset of T ALL, and a fraction of these mice developed BCP ALL (22).

In this issue, Duque-Afonso and colleagues took a different approach to modeling E2A-PBX1 leukemia (23). As the *E2A-PBX1* transgene is strongly oncogenic in T cells, rather than working with T cell–deficient mice to avoid T cell leukemias, Duque-Afonso et al. engineered a mouse model in which expression of the E2A-PBX1 fusion was limited to the B cell lineage. Specifically, the authors used B cell–restricted promoters (*Cd19* or *Mb1*) to drive Cre expression, resulting in activation of the *E2A-PBX1* transgene only within cells of the B lineage. This approach resulted in development of BCP ALL in over 50% of the E2A-PBX1–expressing mice by 9 months of age. Furthermore, as predicted by the hypothesis put forward by Hunger and Mullighan (Figure 1 and ref. 17), competitive repopulation experiments showed a 50-fold enhancement in self-renewal of B cell precursors that was induced by expression of the *E2A-PBX1* transgene in the $CD19^+$ compartment. Interestingly, Duque-Afonso and colleagues used three promoters that were active at different stages of B cell development to drive Cre expression and found that the frequency of leukemia development increased with earlier expression of the *E2A-PBX1* transgene.

Duque-Afonso et al. predicted that mice that developed BCP ALL would also have undergone spontaneous mutations in genes that encode factors important for B cell transformation; therefore, whole-exome sequencing (WES) of isolated ALL cells was performed to identify candidates (23). Using copy number variation (CNV) analysis of the WES, the authors identified a homozygous deletion of *Pax5* in one of six mice. Moreover, extension of these findings to a larger cohort of mice revealed that 30% (13 of 43) of the E2A-PBX1–expressing animals had heterozygous or homozygous deletions of *Pax5*, accompanied by reduced expression of PAX5. This outcome is strikingly similar to clinical observations, as approximately 45% of patients with E2A-PBX1 fusions also have deletions of one or both copies of *PAX5* (24). Duque-Afonso and colleague then crossed the conditional E2A-PBX1 mice with mice that were haplosufficient for *Pax5* and showed a decreased latency of BCP ALL onset and increased penetrance of the leukemic phenotype. Prior to the onset of leukemia, the E2A-PBX1–expressing $Pax5^{+/-}$ mice exhibited a block to differentiation at the pro-B to pre-B transition, as predicted by the model depicted in Figure 1.

The E2A-PBX1 leukemias generated in this model could be divided into pre-$BCR^-$

and pre-BCR$^+$ leukemias. BCL6 expression is reported to increase in cells that have an active pre-BCR (25), and in the E2A-PBX1–expressing mice, high levels of BCL6 expression correlated perfectly with the presence of an active pre-BCR, with one exception. The exceptional mouse had a nonsense mutation of the X-linked gene *Bcor* (for BCL6 corepressor), leading to complete absence of the full-length *Bcor* gene product. Together, these observations suggest the intriguing possibility that a *Bcor* truncation mutation can block BCL6-mediated maturation of pre-BCR$^-$ to pre-BCR$^+$ cells.

Finally, as predicted by the model (Figure 1), WES, followed by targeted Sanger sequencing, identified spontaneous mutations in genes encoding members of both the JAK/STAT pathway and the RAS/MAPK pathway. Specifically, 20 of 51 (39%) mice had acquired mutations in *Jak1*, *Jak3*, *Ptpn11*, or *Il7r*, and 10 of 51 (20%) had acquired mutations in RAS/MAPK pathway genes. Some of these mutations were shown to increase phosphorylated STAT5 (p-STAT), p-AKT, or p-ERK1/2, consistent with constitutive activation of JAK/STAT or RAS/MAPK pathways.

## Conclusions and perspective

Duque-Afonso et al. have produced several robust in vivo models for BCP ALL, with conditional E2A-PBX1 activation resulting in a penetrance of approximately 50% at one year of age (23). The combination of conditional E2A-PBX1 activation and PAX5 inactivation increased the penetrance to 100% by 6 months of age. Remarkably, this mouse model conforms well to the working hypothesis based on sequencing studies in BCP ALL patients (17), with mutations identified in genes affecting self-renewal (*E2A-PBX1*), B cell differentiation (*Pax5*, *Bcor*), and cytokine signaling (*Jak1/3*). Importantly, CNS infiltration was present in the E2A-PBX1 models, indicating that they could be useful for studying CNS leukemia.

The models generated by Duque-Afonso and colleagues will certainly be of interest to the ALL research community, as they allow BCP ALL to be modeled in mice with normal immune systems. Given the intense interest in immune therapy for BCP ALL (26, 27) the use of nonimmunodeficient mice will be invaluable to properly interpret the in vivo effects of these therapies. Finally, the mice can also be used as preclinical platforms to evaluate small-molecule therapy. Indeed, Duque-Afonso et al. presented preliminary evidence that the JAK inhibitor ruxolitinib can improve survival in mice with leukemias driven by E2A-PBX1 and mutant *Jak1*.

## Acknowledgments

This work was funded by the Intramural Research Program of the NCI, NIH (ZIA BC 011295, ZIA BC 011565, ZIA SC 010378, and ZIA BC 010982, to P.D. Aplan and T.J. Fry).

Address correspondence to: Peter D. Aplan, Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 37, Room 6002, 37 Convent Drive, Bethesda, Maryland 20892, USA. Phone: 301.435.5005; E-mail: aplanp@mail.nih.gov.

1. Cooke JV. The occurrence of leukemia. *Blood*. 1954;9(4):340–347.
2. Jemal A, et al. Cancer statistics, 2008. *CA Cancer J Clin*. 2008;58(2):71–96.
3. Hrusak O, et al. Acute lymphoblastic leukemia incidence during socioeconomic transition: selective increase in children from 1 to 4 years. *Leukemia*. 2002;16(4):720–725.
4. Foon KA, Schroff RW, Gale RP. Surface markers on leukemia and lymphoma cells: recent advances. *Blood*. 1982;60(1):1–19.
5. Humphrey GB, Lankford J. Acute leukemia: the use of surface markers in classification. *Semin Oncol*. 1976;3(3):243–251.
6. Pui CH, Behm FG, Crist WM. Clinical and biologic relevance of immunologic marker studies in childhood acute lymphoblastic leukemia. *Blood*. 1993;82(2):343–362.
7. Vogler LB, Crist WM, Bockman DE, Pearl ER, Lawton AR, Cooper MD. Pre-B-cell leukemia. A new phenotype of childhood lymphoblastic leukemia. *N Engl J Med*. 1978;298(16):872–878.
8. Hunger SP, et al. Children's Oncology Group's 2013 blueprint for research: acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2013;60(6):957–963.
9. Mullighan CG, Hunger SP. Therapy of pediatric ALL: from Bowie to Obama. *Blood*. 2013;122(15):2531–2532.
10. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet*. 2008;371(9617):1030–1043.
11. Heerema NA, et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). *J Clin Oncol*. 2000;18(9):1876–1887.
12. Lu XY, et al. The utility of spectral karyotyping in the cytogenetic analysis of newly diagnosed pediatric acute lymphoblastic leukemia. *Leukemia*. 2002;16(11):2222–2227.
13. Sutcliffe MJ, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. *Leukemia*. 2005;19(5):734–740.
14. Behm FG, et al. Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. *Blood*. 1996;87(7):2870–2877.
15. Golub TR, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*. 1999;286(5439):531–537.
16. Ley TJ, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature*. 2008;456(7218):66–72.
17. Hunger SP, Mullighan CG. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. *Blood*. 2015;125(26):3977–3987.
18. Robinson CR. Koch's postulates and the modern era in virus research. *Can Med Assoc J*. 1958;79(5):387–389.
19. Jacoby E, Chien CD, Fry TJ. Murine models of acute leukemia: important tools in current pediatric leukemia research. *Front Oncol*. 2014;4:95.
20. Nourse J, et al. Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. *Cell*. 1990;60(4):535–545.
21. Dedera DA, et al. Chimeric homeobox gene E2A-PBX1 induces proliferation, apoptosis, and malignant lymphomas in transgenic mice. *Cell*. 1993;74(5):833–843.
22. Bijl J, Sauvageau M, Thompson A, Sauvageau G. High incidence of proviral integrations in the Hoxa locus in a new model of E2a-PBX1-induced B-cell leukemia. *Genes Dev*. 2005;19(2):224–233.
23. Duque-Afonso J, et al. Comparative genomics reveals multistep pathogenesis of E2A-PBX1 acute lymphoblastic leukemia. *J Clin Invest*. 2015;125(9):3667–3680.
24. Mullighan CG, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470–480.
25. Geng H, et al. Self-enforcing feedback activation between BCL6 and pre-B cell receptor signaling defines a distinct subtype of acute lymphoblastic leukemia. *Cancer Cell*. 2015;27(3):409–425.
26. Lee DW, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet*. 2015;385(9967):517–528.
27. Maude SL, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371(16):1507–1517.
28. Kuiper RP, et al. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia*. 2007;21(6):1258–1266.