

CD22 EXON 12 deletion as a pathogenic mechanism of human B-precursor leukemia

Fatih M. Uckun^{a,b,c,1}, Patricia Goodman^d, Hong Ma^{a,b}, Ilker Dibirdik^{b,c}, and Sanjive Qazi^e

^aDivision of Hematology-Oncology, Department of Pediatrics, Children's Center for Cancer and Blood Diseases, University of Southern California Keck School of Medicine, Los Angeles, CA 90027-0367; ^bDevelopmental Therapeutics Program, Institute for Pediatric Clinical Research, Children's Hospital Los Angeles MS#57, Los Angeles, CA 90027-0367; ^cMolecular Oncology and Drug Discovery Program, Parker Hughes Institute, P.O. Box 130366, St. Paul, MN 55113-0004; ^dDepartment of Veterinary Biosciences, University of Minnesota, St. Paul, MN 55108; and ^eDepartment of Biology and Bioinformatics Program, Gustavus Adolphus College, 800 West College Avenue, St. Peter, MN 56082

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Here, we report that primary leukemic cells from infants with newly diagnosed B-precursor leukemia express a truncated and functionally defective CD22 coreceptor protein that is unable to transmit apoptotic signals because it lacks most of the intracellular domain, including the key regulatory signal transduction elements and all of the cytoplasmic tyrosine residues. Expression of this structurally and functionally abnormal CD22 protein is associated with a very aggressive *in vivo* growth of patients' primary leukemia cells causing disseminated overt leukemia in SCID mice. The abnormal CD22 coreceptor is encoded by a profoundly aberrant mRNA arising from a splicing defect that causes the deletion of exon 12 (c.2208–c.2327) (CD22 Δ E12) and results in a truncating frameshift mutation. The splicing defect is associated with multiple homozygous mutations within a 132-bp segment of the intronic sequence between exons 12 and 13. These mutations cause marked changes in the predicted secondary structures of the mutant CD22 pre-mRNA sequences that affect the target motifs for the splicing factors hnRNP-L, PTB, and PCBP that are up-regulated in infant leukemia cells. Forced expression of the mutant CD22 Δ E12 protein in transgenic mice perturbs B-cell development, as evidenced by B-precursor/B-cell hyperplasia, and corrupts the regulation of gene expression, causing reduced expression levels of several genes with a tumor suppressor function. We further show that CD22 Δ E12-associated unique gene expression signature is a discriminating feature of newly diagnosed infant leukemia patients. These striking findings implicate CD22 Δ E12 as a previously undescribed pathogenic mechanism in human B-precursor leukemia.

CD22 is an inhibitory coreceptor of B-cells and B-cell precursors that acts as a negative regulator of multiple signal transduction pathways critical for B-cell homeostasis, survival, activation, and differentiation (1–6). The inhibitory and apoptosis-promoting signaling function of CD22 is dependent on recruitment of the Src homology 2 domain-containing tyrosine phosphatase (SHP)-1 to the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) of its cytoplasmic domain upon phosphorylation by the Src family tyrosine kinase LYN (7–11). Collective genetic evidence from CD22-deficient or SHP-1-deficient mice as well as LYN-deficient mice shows that disruption of the LYN-CD22-SHP1 signaling network can result in development of a B-cell lymphoproliferative state associated with defective apoptosis and maturation as well as systemic autoimmunity (1, 6, 10, 12–17). Likewise, deficiency of the signaling molecule SAP (signaling lymphocyte activation molecule/SLAM-associated protein) that regulates tyrosine phosphorylation and inhibitory immunoreceptor signaling of CD22 can cause a lymphoproliferative syndrome (18). Although a physiologically important role for CD22 has been inferred by these intriguing observations in cellular and animal models, direct genetic evidence for its functional significance in human B-cell ontogeny or its implied tumor suppressor role has been lacking.

B-precursor leukemia (BPL), the largest subset of acute lymphoblastic leukemia (ALL), is the most common form of childhood cancer (19–21). Despite recent improvements in treatment

outcome of childhood BPL, infants with BPL continue to have a disappointingly poor treatment outcome even after intensive chemotherapy and supralethal radiochemotherapy in the context of hematopoietic stem cell transplantation (22–26). Although MLL gene rearrangements have been originally thought to play the key role in the leukemogenesis and poor prognosis of infant BPL, failure of these defects to cause leukemia in transgenic or knock-in mice, absence of universal concordance of BPL in infant monozygotic twins with MLL rearrangements, and clinical biomarker studies in newly diagnosed infant BPL patients have revealed that MLL rearrangements are not sufficient to explain the leukemogenesis or aggressive biology of infant BPL (27–33). These observations support the notion that other as yet undefined molecular abnormalities contribute to the uniquely aggressive biology and poor outcome of infant BPL. Our recent analyses provided evidence that remarkably different pathognomonic transcriptomes dominate the biology of infant versus pediatric BPL (34). The antiapoptotic and promitogenic gene expression profiles of infant BPL cells prompt the hypothesis that a network of multiple constitutively active signaling pathways contribute to their prolonged life span and rapid self-renewal, thereby dictating the aggressive biology and poor treatment outcome of infant BPL (8). An improved understanding of the regulatory defects that exist in leukemic B-cell precursors from infant BPL patients contributing to their hyperproliferative state as well as markedly increased apoptotic threshold may provide the foundation for therapeutic innovation against infant BPL. Because of the potential antiproliferative and apoptosis-promoting physiologic role of CD22 coreceptor in B-cell ontogeny, we set out to evaluate primary leukemic cells from infants with BPL for possible structural and functional CD22 defects. Here, we report that primary leukemic cells from infants with newly diagnosed BPL express a truncated and functionally defective CD22 coreceptor protein that is encoded by a profoundly aberrant mRNA arising from a splicing defect that causes the deletion of exon 12 (c.2208–c.2327) (CD22 Δ E12). Forced expression of the mutant CD22 Δ E12 protein in transgenic mice perturbs B-cell development, as evidenced

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Data deposition: The CD22 gene sequence reported in this paper has been deposited in the GenBank database (accession nos. HQ225617, HQ225618, HQ225619, and HQ225620) and the European Molecular Biology Laboratory database (accession nos. FR687955, FR687956, FR687957, and FR687958). A GEOarchive containing the gene expression profiling data from CD22 Δ E12 transgenic mice was submitted to GEO (accession no. GSE23998). A GEOarchive file containing the gene expression profiling data from ALL patients has been submitted to GEO (accession nos. GSE24000 and GSE24001).

¹To whom correspondence should be addressed. E-mail: fmuckun@chla.usc.edu.

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by B-precursor/B-cell hyperplasia and corrupts the regulation of gene expression, causing reduced expression levels of several genes with a tumor suppressor function. These striking findings implicate CD22 Δ E12 as a previously undescribed pathogenic mechanism in human B-precursor leukemia.

Results and Discussion

Because of the potential antiproliferative and apoptosis-promoting physiologic role of CD22 coreceptor in B-cell ontogeny, we set out to evaluate primary leukemic cells from infants with BPL for possible structural and functional CD22 defects. By using Western blot analyses, we detected a truncated CD22 protein in primary leukemic B-cell precursors from infant patients with newly diagnosed BPL (Fig. 1 *A–C*), that was not present in fetal liver-derived normal B-cell precursors or Burkitt's leukemia/lymphoma cell lines (Fig. 1 *D* and *E*). Furthermore, while a 140 kDa intact CD22 coreceptor protein was detected in all control cell lines and primary leukemic cells from a pediatric BPL patient, no or very low levels of intact CD22 could be detected in the lysates of leukemic cells from some of the infant BPL patients (Fig. 1 *A–E*). To test whether the truncated CD22 coreceptor of infant BPL cells can transmit apoptotic signals, we analyzed the effects of CD22 ligation on infant BPL cells vs. control cell lines using the apoptosis-inducing HB22.7 monoclonal antibody that blocks the ligand binding site of CD22. HB22.7 induced apoptosis in DAUDI and FL8.2⁺ cell lines that express an intact CD22 protein but not in infant BPL cells from PT1 and PT5 expressing a truncated CD22 or PT6 with negligible levels of CD22 (Fig. 1 *F* and *G*). Thus, the truncated CD22 coreceptor that is selectively expressed in infant BPL cells is functionally defective. To determine if the abnormal CD22 protein expression profile

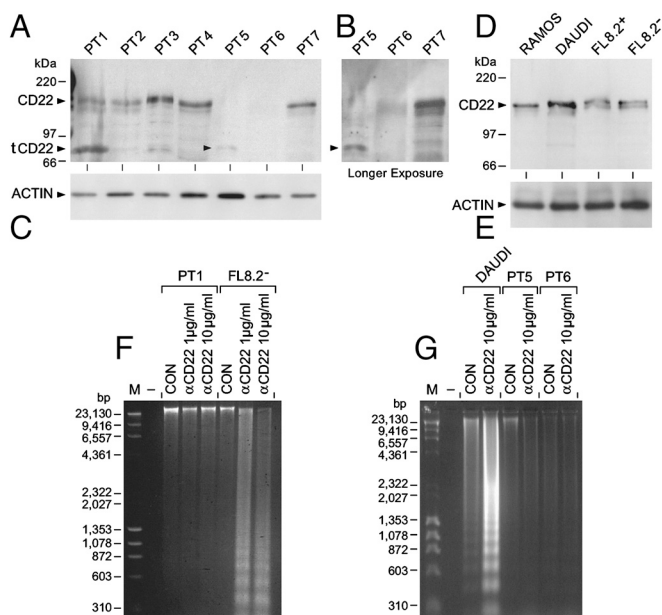
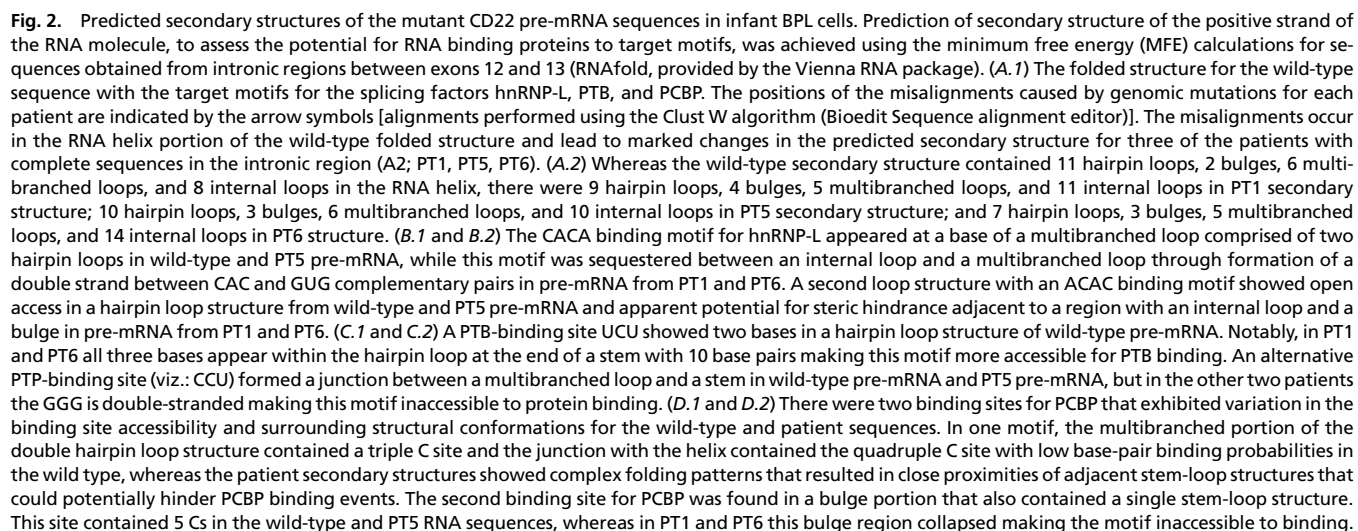


Fig. 1. Infant BPL cells express a truncated CD22 receptor that does not transmit apoptotic signals. Whole cell lysates of primary leukemia cells from six infant BPL patients (PT1–PT6) and a pediatric BPL patient (PT7) (*A–C*) as well as RAMOS, DAUDI, FL8.2⁺, and FL8.2[−] cell lines (*D* and *E*) were subjected to CD22 Western blot analysis using N-20 antibody recognizing the N terminus of the human CD22 molecule as well as antiactin immunoblotting. A truncated CD22 protein was detected in the lysates of leukemic cells from PT1, PT3, and PT5 but not in the lysates of the control cell lines. No significant levels of intact CD22 protein could be detected in the lysates of leukemic cells from PT5 or PT6. Cells were treated with HB22.23 anti-CD22 monoclonal antibody at the indicated concentrations to induce apoptosis via engagement of the CD22 receptor. Examination of supernatants from Triton-X-100 lysates showed apoptotic ladder-like DNA fragmentation in control FL8.2[−] and DAUDI cells but not in primary leukemia cells from PT1, PT5, or PT6 (*F* and *G*).

affects the *in vivo* biological behavior of infant BPL cells we compared the ability of primary leukemic cells from PT1, PT3, PT5, expressing a truncated CD22 and PT6, expressing very low levels of intact CD22 vs. primary leukemic cells from PT2, and PT4, expressing abundant levels of an intact CD22 in the absence of truncated CD22 to cause disseminated leukemia in a SCID mouse xenograft model of infant leukemia. While 38 of 40 SCID mice inoculated with BPL cells exhibiting an abnormal CD22 expression profile developed disseminated leukemia within 60 d, none of the 20 mice inoculated with BPL cells with a normal CD22 expression profile did (Table S1, Fishers Exact test, $P < 0.0001$). These results suggest that the expression of a truncated CD22 protein devoid of proapoptotic function may provide infant BPL cells with an *in vivo* growth and survival advantage. We hypothesized that the inability of the truncated CD22 coreceptor to deliver apoptotic signals is likely caused by genetic lesions affecting its signal transmitting transmembrane and cytoplasmic domains that are encoded by CD22 exons 11–14. To explore the genetic mechanism for the expression of a structurally and functionally defective CD22 coreceptor protein in infant BPL cells, we amplified and sequenced by PCR exons 10–14 in genomic DNA samples from primary leukemia cells of six infants with newly diagnosed BPL. Normal size PCR products were obtained in each of the six infant BPL cases, including those with truncated or near absent CD22 coreceptor protein expression, providing strong evidence against genomic deletions of these CD22 exons encoding the cytoplasmic domain as a cause for the observed expression of a truncated CD22 protein or substantially reduced expression levels of an intact protein (Fig. S1). While searching the sequences of the cloned PCR products from infant BPL patients for possible deviations from the wild-type consensus sequences, we discovered multiple homozygous mutations within a 132-bp segment of the intronic sequence between exons 12 and 13 (NC_000019.9: c.2327 + 104/G^[35,836,727]–c.2328 – 195/G^[35,836,859]) (but not within exon 12 or its splice acceptor/donor sites or in the intronic sequence between exons 11 and 12), including transversions/transitions, deletions, and insertions (Figs. S2 and S3 and Table S2). This “mutational hotspot” region of the CD22 gene or any of the identified mutations has not been highlighted in any of the previous reports of CD22 variations or genome-wide profiling of genetic alterations in ALL (35–39). Genomic mutations discovered in the infant BPL patients were next cross-referenced with the database of single nucleotide polymorphisms (SNP) housed in NCBI’s Entrez system of data mining tools (dbSNP; <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>). We queried the intronic region of interest between exons 12 and 13 of the CD22 gene (forward mRNA strand at NM_001771) and found that it contains five SNPs of unknown clinical or biologic significance, namely rs4805119, rs10406539, rs10413500, rs10413526, and rs4805120. Notably, four of four mutations in PT1, one of two mutations in PT5, and two of six mutations in PT6 were at the exact locations of these previously reported SNPs (Table S2). Five of the six mutations in PT6 and all six mutations in PT3 were unique and showed no concordance with reported SNP sites.

Intronic sequences often dictate the correct splicing of pre-mRNA and pathogenic intronic mutations, including single point mutations, have been linked to aberrant splicing and human disease (35–39). RNA splicing requires a complex interplay of multiple RNA-binding proteins that are equipped with domains to bind sequence motifs on single stranded RNA to ensure accurate determination of exon recognition. In silico interrogation of the wild-type pre-mRNA segment derived from the 132-bp mutation-rich intronic sequence between exons 12 and 13 for accessible splicing factor binding sites resulted in identification of multiple potential binding sites for the RNA-binding proteins hnRNP-E2/PCBP, hnRNP-I/PTB, and hnRNP-L, three members of the heterogeneous nuclear ribonucleoprotein family of splicing factors



hnRNP-E2/PCBP, hnRNP-I/PTB, and hnRNP-L as well as the surrounding structural features in the predicted pre-mRNA molecules (Fig. 2B–D). The observed impact of the intronic mutations of the predicted secondary structures of the patients' CD22 pre-mRNA molecules prompted us to hypothesize that these mutations would likely affect the recognition of 5' splice site of exon 12 by the splicing machinery and perturb proper spliceosome assembly thereby causing aberrant pre-mRNA splicing.

In order to test this hypothesis, we performed RT-PCR assays that specifically amplified a 975-bp region of CD22 mRNA (c.1801–c.2776) encompassing exons 11–14 encoding the entire cytoplasmic domain of CD22 (Fig. S5A). RT-PCR analysis of fetal liver-derived normal B-precursor cell line FL8.2⁺ showed the anticipated 975-bp single PCR product, whereas infant BPL cells yielded a smaller second PCR product of approximately 850-bp size as well (Fig. S5B). Both PCR products hybridized to a CD22 exon 11-specific oligonucleotide probe (Fig. S5C). To pursue this result further we performed EcoRI restriction analysis of cloned CD22 RT-PCR products. FL8.2⁺ cells yielded two fragments of the expected sizes of 600-bp and 350-bp (Fig. S5D). In contrast, EcoRI restriction analysis of cloned CD22 RT-PCR products from primary infant BPL cells yielded abnormal fragment pairs of 500-bp (instead of 600-bp) + 350-bp in the majority of the clones (Fig. S5E and F). These findings indicate that the truncated CD22 coreceptor in infant BPL cells is the product of abnormal CD22 mRNA species. Sequence analysis of the RT-PCR products demonstrated that the smaller approximately 850-bp RT-PCR product in infant BPL cells results from a profoundly aberrant coding sequence due to a splicing defect causing the deletion of exon 12 (c.2208–c.2327) (Fig. S5G and H). This exon skipping (CD22ΔE12) involves an exact splice with no other mutation at the splice junction. CD22ΔE12 was not detected in normal B-precursor cells (Table S3). A minority of PCR clones from adult hairy cell leukemia (HCL) patients and a single clone from a pediatric BPL patient also harbored CD22ΔE12 (Table S3). We propose that the mutations within the downstream intronic sequence flanking exon 12 of CD22 gene contribute to the observed splicing defect in infant BPL cells by altering the genomic sequence environment for the exon 12 splice sites and influencing their recognition by the pre-mRNA splicing machinery. The observation that some infant BPL cases had very low levels of CD22 protein expression also suggests that these mutations may adversely affect pre-mRNA stability and efficiency of transcription in some cases. The deletion of exon 12 in CD22 mRNA results in a truncating frameshift mutation starting at K736 with an insertion of 15 amino acids (RCRVLRLDAETS PGLR) not seen in wild-type CD22 sequence followed by a TGA termination codon (Fig. S5I). Wild-type CD22 has 14 exons, exons 3–9 each encode a single Ig domain, exon 10 encodes the transmembrane domain, whereas the cytoplasmic tail is encoded by exons 11–14 (50). Mutant CD22ΔE12 protein lacks the conserved tyrosines and ITIMs that provide docking sites for the SH2 domains of the tyrosine phosphatase SHP1 (7–11). It also lacks regions homologous to ITAMs, tyrosine-based activation motifs, which are docking sites for SH2 containing proteins (7–11). An YXXM motif recognized by the N-terminal SH2 domain of the p85 subunit of PI3-kinase (7–11) is also located within the deleted cytoplasmic portion of CD22ΔE12. Thus, CD22ΔE12 mRNA encodes a truncated CD22 protein lacking most of the intracellular domain including the key regulatory signal transduction elements and all of the cytoplasmic tyrosine residues, which is in agreement with the results of Western blot analyses and apoptosis assays of infant BPL cells (depicted in Fig. 1). To further examine the functional significance of the exon 12 splicing defect for B-lineage lymphoid cells, we forced the expression of human CD22ΔE12 in transgenic mice under control of the immunoglobulin enhancer E_μ that is activated in early B-cell ontogeny prior to Ig gene rearrangements (Fig. S6). At six weeks of age, hCD22ΔE12 trans-

genic mice showed flow cytometric evidence for B-precursor/B-cell hyperplasia (Fig. 3A). To examine the deregulatory biologic effects of the expression of the defective CD22ΔE12 protein at a molecular level, we compared the gene expression profiles of sple-

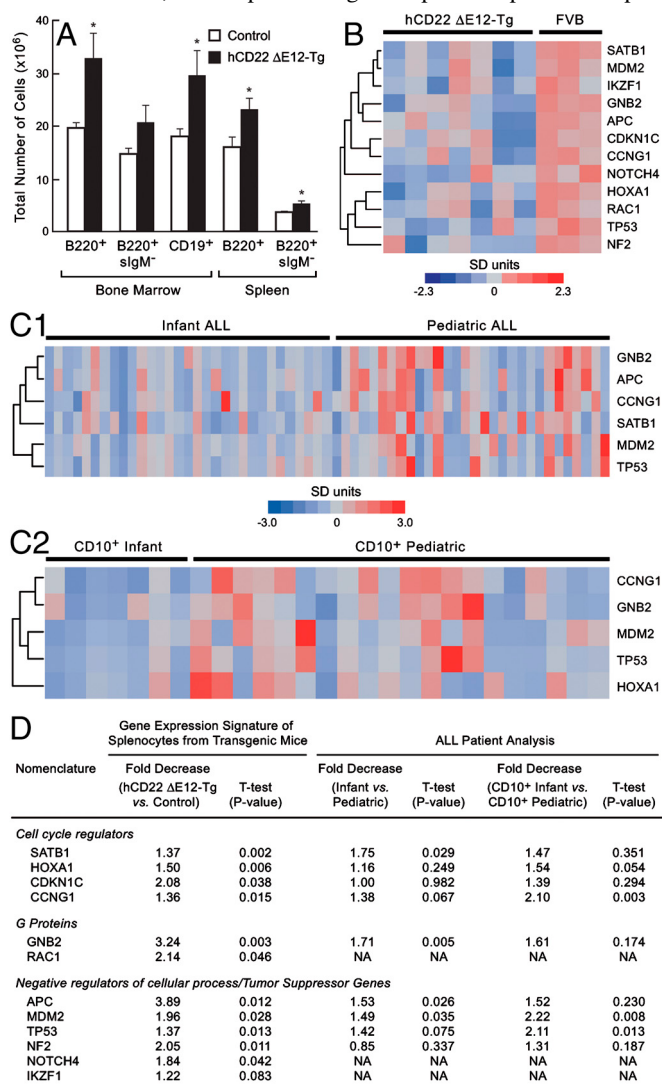


Fig. 3. B-precursor/B-lymphocyte hyperplasia and unique gene expression signature of CD22ΔE12 transgenic mice. (A) The B220⁺ ($23.0 \pm 2.2 \times 10^6$ /spleen vs. $16.2 \pm 1.8 \times 10^6$ /spleen, T -test $P = 0.042$; $32.9 \pm 4.7 \times 10^6$ /bone marrow from 2 femurs vs. $19.9 \pm 0.9 \times 10^6$ /bone marrow from 2 femurs, T -test P -value = 0.026) total B-lineage lymphoid cell numbers as well as B220⁺sigM⁺ B-precursor numbers ($5.3 \pm 0.5 \times 10^6$ /spleen vs. $3.6 \pm 0.3 \times 10^6$ /spleen, T -test P -value = 0.017; $20.8 \pm 3.1 \times 10^6$ /bone marrow from 2 femurs vs. $15.0 \pm 0.8 \times 10^6$ /bone marrow from 2 femurs, T -test P -value = 0.1) in the spleen as well as bone marrow were moderately elevated in hCD22ΔE12 transgenic mice as compared to control FVB mice. Likewise, there were more CD19⁺ B-lineage lymphoid cells in the bone marrow of hCD22ΔE12-Tg mice than in control mice ($29.7 \pm 4.5 \times 10^6$ vs. $18.0 \pm 1.4 \times 10^6$, $P = 0.038$). (B) Gene expression profiles of hCD22ΔE12-Tg mouse splenocytes. Mean centered, standardized values for the most discriminating 12 signature genes (rows) across the 7 hCD22ΔE12-Tg and 3 control FVB mice (columns) were subjected to hierarchical clustering. The heat map represents the color-coded expression value reported as standard deviation units (SD) relative to the average expression levels across 10 mice. (C1) Of the 12 down-regulated signature genes, 9 were represented on the human Clontech array and 6 of these 9 genes were identified as differentially expressed genes for patients with infant ALL vs. pediatric noninfant ALL. (C2) Gene expression profiles were also compared for CD10⁺ infant vs. pediatric ALL patients and showed that 5 of the 9 signature genes were down-regulated in infant patients. (D) Differential expression of the hCD22ΔE12-associated signature genes in transgenic mice and infant ALL patients.

nocytes from hCD22ΔE12 transgenic mice and nontransgenic wild-type control mice. Twelve differentially expressed genes that had standardized values of expression outside the range of the control values in wild-type mice were classified as the most discriminating genes. This CD22ΔE12-associated unique 12-gene signature transcriptome included (i) tumor suppressor genes TP53 (as well as TP53 regulator MDM2), neurofibromatosis 2 (NF2) (as well as NF2 regulator RAC1), and the adenomatous polyposis coli (APC) gene, a tumor suppressor known to regulate the Wnt/β-catenin signaling; (ii) genes for chromatin remodeling/global gene expression regulators with a tumor suppressor function IKZF1/IKAROS and SATB1; as well as (iii) cell cycle regulatory genes CDKN1C, CCNG1, and NOTCH4 (Fig. 4*B* and *D*). These results provide compelling evidence that CD22ΔE12 corrupts the regulation of gene expression and results in reduced expression levels of several genes that have a tumor suppressor function. We next performed gene expression profiling of primary leukemia cells from 31 infants and 30 noninfant children with ALL to determine if any of these signature genes are differentially expressed in infant ALL vs. pediatric ALL. Reduced expression levels of six of the nine CD22ΔE12 signature genes that were represented on the human cDNA arrays, including TP53 and APC as well as MDM2, SATB1, CCNG1, and GNB2 discriminated infant BPL from noninfant BPL (Fig. 3*C.1* and *D*). Comparing the gene expression profiles of CD10 antigen positive infant leukemia cells that do not have MLL gene rearrangements with those of CD10⁺ pediatric ALL cells, we next confirmed that this signature transcriptome was independent of MLL gene rearrangements (Fig. 3*C.2* and *D*). We next used a meta-analysis to interrogate each of the most discriminating signature genes with significant *T*-test statistics (viz., APC, GNB2, MDM2, and SATB1) for its previously reported expression values and associations in 10 B-lineage leukemia studies with 11 comparative analyses and 5 B-lineage lymphoma studies with 15 comparative analyses in the OncomineTM Research Data Base (51). Each of these genes was expressed in malignant cells from patients with B-lineage lymphoid malignancies at significantly lower levels than in normal B-cell controls (Table S4). Among these, the down-regulation of SATB1 was the most pronounced as well as the most enriched aberration suggesting a potentially more critical role in leukemogenesis (Table S4). The key target for SATB1 activity, MYC oncogene, is a master regulator of multiple interacting genes in B-cell ontogeny (52), and its forced expression driven by immunoglobulin enhancers induces aggressive B-lineage lymphoid malignancy in transgenic mice (53). SATB1 represses the expression of MYC in lymphoid cells (54), and its down-regulation in infant pro-B ALL cells with a CD22ΔE12 would be expected to cause an overexpression of MYC. Notably, our recent gene expression profiling studies have indeed revealed that MYC is expressed at 7.8-fold higher levels in CD10⁺ poor-risk pro-B subset of infant ALL patients than in patients with CD10⁺ infant pre-pre-B ALL (*P* = 0.0006) (34). The documented molecular and functional abnormalities involving CD22 in primary leukemic cells from patients with newly diagnosed infant BPL uniquely implicate deficiency of this B-lineage restricted coreceptor protein in the genesis of infant leukemia. The presented collection of experimental data is unprecedented in that it links a genetic defect involving a B-lineage specific regulatory gene to the most aggressive human B-lineage lymphoid malignancy that arises from an uncoupling of proliferation and differentiation of B-precursors during the earliest stages of B-cell ontogeny. A key challenge in the future will be to decipher the precise molecular mechanism by which the genetic CD22ΔE12 defect contributes to the clonal evolution and aggressive biology of infant BPL. The very young age of infant patients who express CD22ΔE12, ranging from 1 month to 6 months, points to a prenatal or very early neonatal origin for this genetic defect. Concordance of infant BPL in monozygotic twins indicates a shared inherited or genetic susceptibility (55). Homozygosity for

a cluster of potentially predisposing SNPs corresponding to the synonymous mutations in the intronic segment between CD22 exons 12 and 13 may confer a genetic risk for development of infant BPL. We are planning to undertake a genome-wide association study to address the important question of whether the presence of these SNPs alone or in clusters confers a genuine genetic risk for development of infant BPL. Dissecting the contribution of CD22ΔE12 to infant BPL leukemogenesis should provide valuable mechanistic insights and help determine if this genetic lesion can be used to gain a therapeutic advantage against infant leukemia. Because the CD22ΔE12-associated signature transcriptome was independent of MLL gene rearrangements (Fig. 3), the well established clinical impact of MLL gene rearrangements in infant ALL indicates that nonoverlapping aberrations in addition to those caused by CD22ΔE12, such as the presence of MLL-AF4 fusion protein, can affect the biology and treatment response of infant ALL as independent risk factors. It will be important to perform a systems analysis of the likely cooperation between CD22ΔE12 and MLL fusion proteins in the leukemogenesis of infant ALL using both appropriately designed knock-in and bistransgenic mouse models.

The expression of CD22 on leukemic B-cell precursors has motivated the development and clinical testing of CD22-directed recombinant fusion toxins and antibody-drug conjugates as therapeutic agents against BPL in children (56–59). The toxin or drug moieties of these biotherapeutic agents are delivered into target leukemia cells together with the targeted CD22 molecules by antibody-mediated endocytosis (56–59). Antibody-mediated internalization of CD22 is dependent on its physical interaction with the clathrin-associated AP-2 adapter complex via tyrosine-based specific internalization motifs within its cytoplasmic domain (59, 60). Tyr⁸⁴³ or Tyr⁸⁶³ and surrounding amino acids in the cytoplasmic tail of CD22 comprise the primary binding site for the AP50 subunit of AP-2 (60, 61). Therefore, indiscriminate use of anti-CD22 fusion toxins and immunoconjugates would not offer an effective treatment opportunity for BPL with CD22ΔE12 lacking the internalization motifs required for their antileukemic action. Our findings provide strong support for the consideration that anti-CD22 fusion toxins and immunoconjugates be employed in a patient-tailored fashion, and in this context, CD22ΔE12 be used as a biomarker for exclusion of BPL patients who are not likely to benefit from these otherwise promising new biotherapeutic agents. Furthermore, antisense oligonucleotides can now be designed as an innovative strategy with therapeutic intent to shift the CD22 splicing pattern by preventing exclusion of exon 12 in the CD22 mRNA by targeting and sterically blocking the splice site boundaries flanking exon 12, similar to the modification of MCL-1 pre-mRNA splicing in basal cell carcinoma cells (62).

Materials and Methods

In Vitro Assays. We used standard assays and procedures, including Western blot analyses, apoptosis assays, gene expression profiling, RT-PCR, and genomic PCR (13, 27, 28, 34) (SI Text).

hCD22ΔE12 Transgenic Mice. The hCD22ΔE12 transgene construct was micro-injected into the male pronucleus of fertilized FVB/N mouse oocytes using standard protocols (SI Text). The oocytes were implanted into the oviducts of pseudopregnant female mice to generate hCD22ΔE12-Tg mice. Tg mice were subjected to detailed characterization to confirm the transgene expression (SI Text). Single-cell suspensions of splenocytes and bone marrow cells obtained from electively sacrificed 6–7 wk old Tg mice and their wild-type controls were immunophenotyped by direct fluorescence staining and flow cytometry using anti-CD19-phycoerythrin (PE), anti-B220/CD45R-PE, and anti-IgM-FITC (SI Text).

SCID Mouse Model of Infant BPL. SCID mice were inoculated intravenously with 1×10^6 primary infant BPL cells. All SCID mice were electively killed at 60 d unless they died or became moribund earlier due to their disseminated leukemia (SI Text).

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