

Immunoglobulin heavy chain locus chromosomal translocations in B-cell precursor acute lymphoblastic leukemia: rare clinical curios or potent genetic drivers?

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Chromosomal translocations involving the immunoglobulin heavy chain (*IGH*) locus define common subgroups of B-cell lymphoma but are rare in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Recent fluorescent in situ hybridization and molecular cloning studies have identified several novel *IGH* translocations involving genes that play important roles in normal hemopoiesis, including the cytokine receptor genes *CRLF2* and *EPOR*, all members of the CCAAT enhancer-binding protein gene family, as well as genes not normally ex-

pressed in hemopoietic cells including inhibitor of DNA binding 4. *IGH* translocation results in deregulated target gene expression because of juxtaposition with *IGH* transcriptional enhancers. However, many genes targeted by *IGH* translocations are also more commonly deregulated in BCP-ALL as a consequence of other genetic or epigenetic mechanisms. For example, interstitial genomic deletions also result in deregulated *CRLF2* expression, whereas *EPOR* expression is deregulated as a consequence of the *ETV6-RUNX1* fusion. The possible clin-

ical importance of many of the various *IGH* translocations in BCP-ALL remains to be determined from prospective studies, but *CRLF2* expression is associated with a poor prognosis. Despite their rarity, *IGH* chromosomal translocations in BCP-ALL therefore define not only new mechanisms of B-cell transformation but also clinically important subgroups of disease and suggest new targeted therapeutic approaches. (Blood. 2010;115:1490-1499)

Introduction

It is nearly 50 years since the cytogenetic description of the first recurrent chromosomal translocation in malignancy, the Philadelphia chromosome in chronic myeloid leukemia (CML).¹ Since then, a plethora of different chromosomal translocations has been identified in nearly all forms of malignancy.² Many recurrent translocations are the primary genetic cause of disease; that is, the production of tumor-specific gene products derived from the fusion of genes normally on 2 different chromosomes, or alternatively, deregulated gene expression, arising as a consequence of chromosomal translocation, play a pivotal role in the emergence of the neoplastic phenotype. Molecular analysis of these translocations was initially conducted via positional cloning strategies, led by cytogenetic observations³ but now increasingly from whole genome and high-throughput transcriptional studies.⁴ These studies, supplemented by sophisticated animal models,⁵ define mechanisms of transformation and suggest new targeted therapeutic approaches. Successful inhibition of the B-cell precursor acute lymphoblastic leukemia (BCP-ALL) fusion protein in CML by imatinib⁶ is a paradigm that urgently needs to be emulated, although whether other malignancies will have such a vulnerable “Achilles heel” as CML remains to be determined.

However, as more is understood about the genetic complexities of cancer, problems emerge concerning the relative importance of the multiple and different abnormalities that exist within different subpopulations of the neoplastic clone, raising questions concerning which to target therapeutically. Clues may be derived either

from the biologic functions of the involved genes or from clinical associations with specific subtypes of disease; but in many instances, these data may not be available. For example, are all chromosomal translocations or deletions and gene mutations of equal importance in the pathogenesis of disease, or are some secondary events, or perhaps even epiphenomena, of little or no biologic significance, reflecting the relative “genetic instability” of cancer cells? In short, which are “drivers” of disease, and which merely “passengers?”⁷ This problem becomes more acute with the availability of whole genome sequences. The genome sequence of a case of acute myeloid leukemia (AML) with minimal myeloid differentiation, lacking any cytogenetic abnormality, compared with that of normal skin fibroblasts derived from the same person, revealed an estimated 750 somatically acquired mutations.^{8,9} Most mutations occurred in nonconserved and non-protein-coding regions of the genome and therefore are presumed to have no role in disease pathogenesis. Of the 64 mutations within conserved regions, only 12 occurred in protein-coding regions, with the remaining 52 in noncoding but highly conserved DNA sequences that presumably have regulatory functions. Of the protein-coding mutations, 7 were predicted to alter protein function and therefore might be considered “drivers.” Four mutations, involving *NPM1*, *NRAS*, *IDH1*, and a conserved region of chromosome 10, were found to be recurrent in a screen of 180 other cases of AML. *IDH1* (isocitrate dehydrogenase) has also been found to be mutated in

Submitted September 25, 2009; accepted December 8, 2009. Prepublished online as *Blood* First Edition paper, December 30, 2009; DOI 10.1182/blood-2009-09-235986.

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primary brain tumors and results in production of the onco-metabolite, 2-hydroxyglutarate, which may contribute to neoplastic transformation by the production of reactive oxygen species.¹⁰

The oncogenic potential of *NPM1*, *NRAS*, and *IDH1* is therefore well established from both their known biologic activities and the presence of mutations in several tumor types. However, whether any of the other apparently sporadic mutations were of pathogenic significance remains unclear and must await more detailed functional analysis, a formidable undertaking if all tumors have similar frequencies of sporadic mutations. The picture emerging from unbiased whole genome approaches to malignancy therefore is one of increasingly genetic complexity, with a few of commonly mutated gene “mountains” and a much larger number of gene “hills” mutated at low frequency.¹¹

In this article, we review recent results on the nature and consequences of immunoglobulin heavy chain (*IGH*) chromosomal translocations in BCP-ALL. Several previously unidentified *IGH* translocations have been reported in BCP-ALL over the past 3 years.¹²⁻¹⁷ All are relatively rare events, occurring individually in less than 1% of all cases and many are probably sporadic. This is in marked contrast to the situation in mature B-cell malignancies, where specific *IGH* translocations are commonly found in most subtypes of disease and underlie disease pathogenesis.¹⁸ One response to the finding of many different and perhaps sporadic *IGH* translocations in BCP-ALL might be to discount these events as rare curios or perhaps epiphenomena, of little biologic or clinical significance to the pathogenesis of most cases of BCP-ALL. In contrast, despite their rarity, we argue that the genes identified from their direct involvement in *IGH* translocations nevertheless define important new mechanisms of B-cell precursor transformation, define new prognostic subgroups of disease, and indicate new therapeutic targets. Moreover, we demonstrate that several of the genes targeted by *IGH* translocations in BCP-ALL are also deregulated as a consequence of other mechanisms in significant subgroups of patients.

General considerations of *IGH* translocations in B-cell malignancies

Chromosomal translocations involving the immunoglobulin loci have long been recognized to play a central role in the pathogenesis of several subtypes of B-cell malignancy.¹⁸ Burkitt lymphoma is characterized by translocations involving *MYC* on chromosome 8q24, follicular lymphoma by involvement of *BCL2* on 18q21, and mantle cell lymphoma by involvement of *CCND1* on 11q13. Each translocation is present in the great majority of cases and, thus, like the Philadelphia chromosome in CML, plays an important role in disease pathogenesis. How the specificity of each type of chromosomal translocation might arise is beyond the scope of this article but presumably represents differences in the higher-order chromatin structures in B cells at different stages of differentiation.¹⁹ *IGH* translocations directly involve either the joining (*J*) or switch (*S*) regions of the *IGH* locus, regions physiologically involved in *VDJ* and class-switching recombination, respectively (Figure 1A). The principal consequence of *IGH* translocations is deregulated transcription of the translocated gene because of the physical juxtaposition of transcriptional enhancers within the *IGH* locus (Figure 1B). In mature B-cell malignancies, the enhancers are composed of the E μ enhancer lying between the *IGHJ* and *IGHMS* segments and the 2 centromeric enhancers lying distal to both *IGH* constant region C α (*IGHCA*) gene segments. In normal B-cell precursors, another

transcriptional enhancer, located between *IGHCD* and *IGHG3*, is also functional and contains many Ikaros transcription factor recognition motifs.²⁰ This may be pathologically significant because approximately 5% of BCP-ALL demonstrate an interstitial deletion within the *IGH* locus on chromosome 14q32 with biallelic deletion of the *IGHJ/IGHCM* and *IGHCD* sequences extending to the *IGHCD-IGHCG3* interval.^{21,22} Interestingly, Ikaros itself is also recurrently deleted in BCP-ALL, but the possible biologic and clinical consequences of these *IGH* deletions remain to be determined.²³ Importantly, none of the *IG* translocations alone is sufficient for the emergence of the neoplastic phenotype; concurrent activation of synergistic oncogenes and loss of tumor suppressor gene functions are necessary.

IGH translocations in BCP-ALL

In contrast to the lymphomas, BCP-ALL lack common recurrent *IG* translocations. Why this should be is unclear as normal B-cell precursors express high levels of recombinase activating genes (RAGs) necessary to induce double-strand DNA breaks associated with *VDJ* recombination, whereas levels are much lower in peripheral B cells; concurrent expression of RAG and activation-induced deaminase (not normally expressed in B-cell precursors) may be necessary for optimal generation of *IGH* translocations.²⁴ However, it is now apparent that there is a plethora of rare *IGH* translocations in this disease. Their identification has been facilitated by the use of *IGH* “breakapart” probes located centromeric of the *IGHC* and within the (telomeric parts of the) *IGHV* regions on interphase fluorescence in situ hybridization (FISH); these probes should normally be physically adjacent, whereas the *IGHV* signal is moved from the der(14) to the derivative partner chromosome by an *IGH* translocation, which results in a “split” signal in interphase cells by most *IGH* translocations. A list of all cloned *IGH* translocations in BCP-ALL is shown in Table 1. These cases have been garnered from cytogenetic samples collected in many different laboratories worldwide, over many years, from patients who have been treated with different chemotherapeutic protocols. Thus, the precise frequency and possible prognostic significance of each individual *IGH* translocation in BCP-ALL remain to be determined from uniformly treated series of patients. Overall, *IGH* translocations are seen in approximately 2% to 3% of all cases of BCP-ALL.^{14,35,36} The *CRLF2/IGH* translocation is the most frequent, followed by *inhibitor of DNA binding 4 (ID4)* and the collective CCAAT enhancer-binding protein (*CEBP*) translocations; the remaining *IGH* translocations appear to be sporadic. For reasons that are unclear, most cases of *IGH* translocation present in adolescents or young adults, outside the peak incidence of childhood BCP-ALL. Whether this reflects a postnatal, rather than a fetal, origin of these malignancies is not known. The spectrum of partner genes is specific to BCP-ALL; with the exception of translocations involving *hTERT* and *BCL2*, these translocations are not seen in malignancies of mature B cells. Importantly, although there are some similarities, there is no obvious common motif to unify all the various translocations functionally.

Involvement of the cytokine receptor *CRLF2* in both *IGH* translocations and interstitial deletion of the *PAR1*

The cytokines that drive proliferation of B-cell precursor subpopulations appear to be at least partially species-specific and may vary at different stages of embryonic and adult development.³⁷ In adult

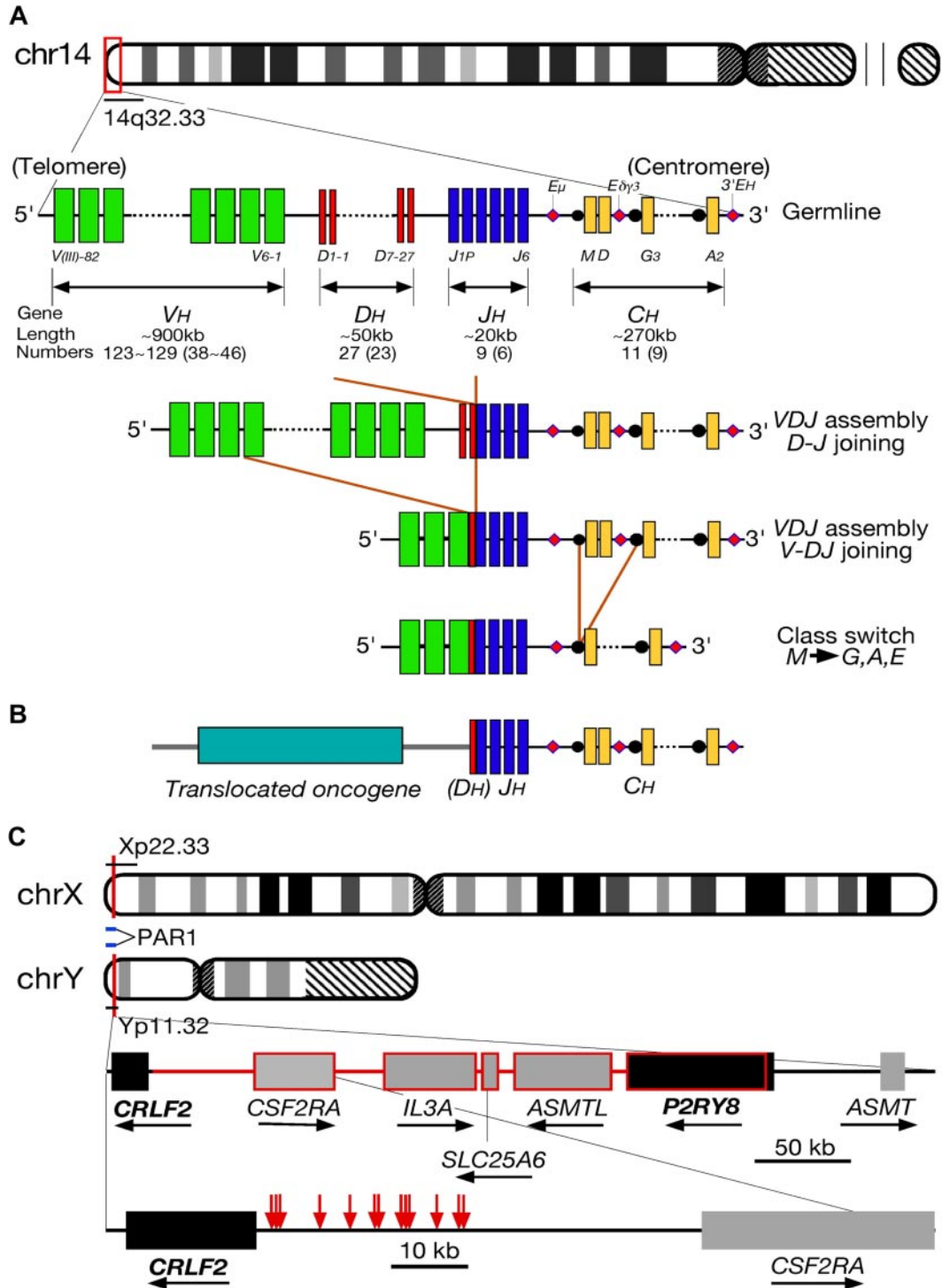


Figure 1. Overview of IGH translocations. (A) Schematic of the IGH locus. The human IGH locus is located adjacent to the telomere of chromosome 14q. The locus is composed of dispersed variable (VH, denoted by green bars), diversity (DH, red), and joining (JH, blue) region segments that undergo somatic recombination in B-cell precursors to produce a functional VDJ unit. These are expressed with constant region gene segments (CH, yellow) with transcription being driven by enhancers (denoted by pink diamonds). (B) A diagram of IGH translocation in BCP-ALL. As a result of an error of D-J or V-DJ recombination, a partner gene from another chromosomal region becomes adjacent to J or DJ region. Expression of the translocated gene is driven by the transcriptional enhancers within the IGH locus. Usually, the entire open reading frame of the translocated gene is maintained without mutation. (C) Schema showing breakpoints of CRLF2/IGH translocation and PAR1 interstitial deletion. Red arrows indicate the positions of break points of the cloned IGH translocations (falling in the region immediately 5' of CRLF2), whereas the portion consisting of lines and frames in red represents the deletion between P2RY8 and CRLF2 resulting in fusion of the 2 genes.

mice, B-cell lymphopoiesis is strictly interleukin-7 (IL-7) dependent. However, the precise role of this cytokine in human B-cell lymphopoiesis is more controversial.³⁸ Another cytokine, which may have similar species-specific roles, is thymic stromal lympho-

poietin (TSLP).³⁹ TSLP was originally identified as a cytokine produced from a derived murine thymic stromal cell line able to support growth of a pre-B-cell line and the development of IgM⁺ B cells from normal mouse fetal liver cells.⁴⁰ Murine TSLP shows

Table 1. Summary of IGH chromosomal translocations in BCP-ALL

Target gene	Location	Functions in normal B cells	Presumed mechanism of transformation	Deregulated expression in BCP-ALL without IGH translocation?	Comments	References
<i>CRLF2</i>	Xp22Yp11	Heterodimerization with IL7RA to mediate TSLP signaling	Homodimerization mediating constitutive STAT5 phosphorylation	Also deregulated by interstitial deletion on XY chromosome. Juxtamembrane <i>CRLF2</i> mutations also seen	Associated with DS, JAK2 mutation, and poor prognosis	17, 25, 26
<i>EPOR</i>	19p13 3 cases	Not expressed in normal B-cell precursors	Constitutive STAT5 phosphorylation	<i>EPOR</i> also a documented target gene of <i>ETV6-RUNX1</i> fusion	<i>EPOR</i> expression also seen in many other malignancies	16
<i>ID4</i>	6p21 14 cases	Not expressed in normal B-cell precursors	Unknown	High-level expression also seen BCP-ALL without <i>IGH</i> translocation		12, 15
<i>CEBPD</i>	8q11 44 cases	Not expressed in normal B-cell precursors	Unknown		Association with DS and t(9;22)	14, 27, 28
<i>CEBPA</i>	19q13	Not expressed in normal B-cell precursors	Unknown		Mutated in AML subject to acquired uniparental disomy in AML	13, 14
<i>CEBPE</i>	14q11	Not expressed in normal B-cell precursors	Unknown		Predisposition to BCP-ALL within <i>CEBPE</i>	14
<i>CEBFB</i>	20q13 3 cases		Unknown			14
<i>CEBPG</i>	19q13 Single case		Unknown			14
<i>IL3</i>	5q31	Cytokine	—		Associated with hyper eosinophilia	29
<i>IGF2BP1</i>	17q21.33 cases	RNA binding protein	Enhanced translation of oncogenic proteins			32
<i>MIR125B</i>	11q24.12 cases	Intergenic microRNA	Down-regulation of p53	Deregulated by interstitial insertion into <i>IGH</i> locus		30; AUPO (2008)
<i>BCL9</i>	1q21 2 cases/1 cell line	Involved in WNT signaling; binds to β -catenin	Deregulated WNT signaling	CEMO-1 cell line harbors this translocation		31; AUPO (2008)
<i>hTERT</i>	5p15.3 Single case	Maintenance of telomeres		Identical translocations also seen in mature B-cell malignancies		Nagel et al (2009), MS under revision
<i>LHX4</i>	1q21 Single case	LIM homeobox domain protein; ? ectopic expression in BCP-ALL	Unknown	Normally involved in control of pituitary development		33
<i>DUX4</i>	10q26 Single case (cell line)	Unknown	Unknown	Cell line only; nature of deregulated transcript uncertain		AUPO (2008)
<i>BCL2</i>	18q21 Single case (cell line)	Suppression of apoptosis		Cell line only; derived from 15-year-old with BCP-ALL with no obvious antecedent lymphoma		34

Some *IGH* translocations, including those involving *CRLF2*, *hTERT*, and *DUX4* involve genes immediately adjacent to the telomeres and cannot therefore be detected by conventional cytogenetics alone. AUPO indicates authors' unpublished observations.

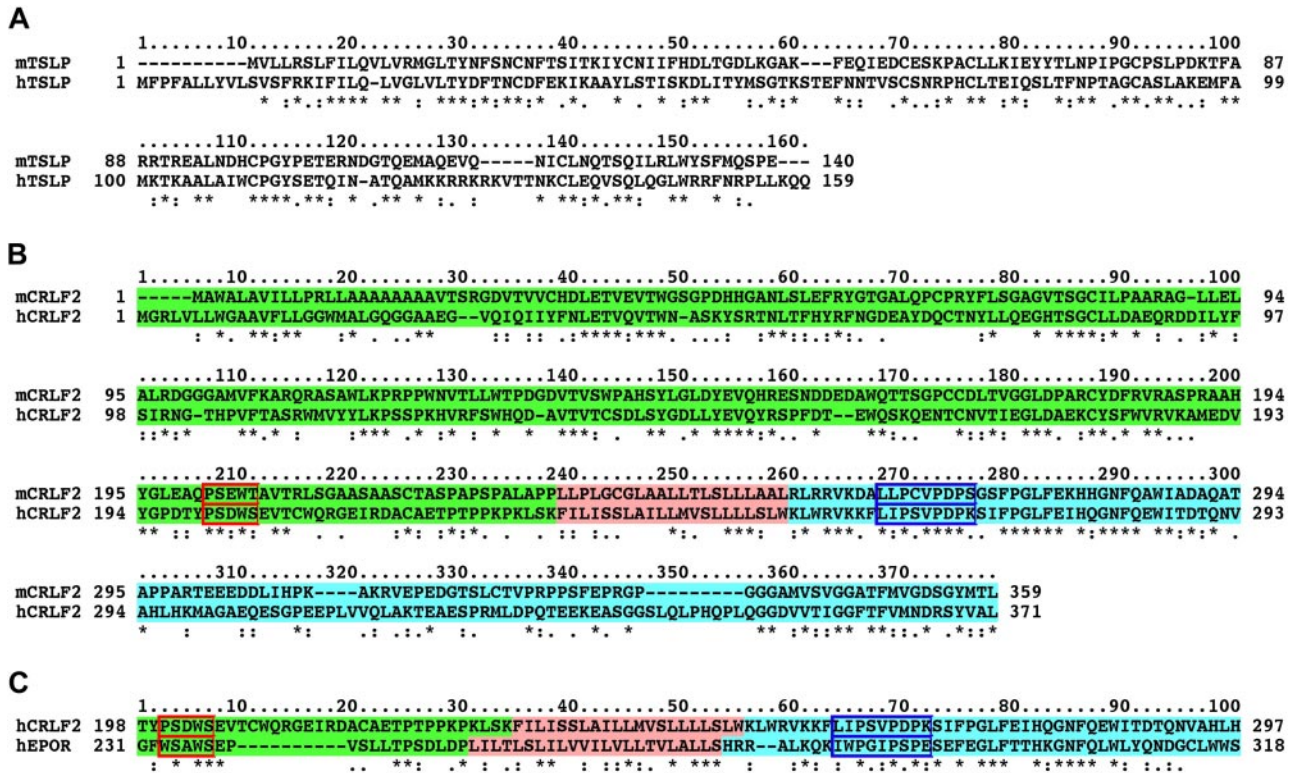


Figure 2. Sequence alignments of TSLP, CRLF2, and EPOR. (A) Lack of homology of mouse and human CRLF2 amino acid sequences. Extracellular domain (green), transmembrane region (pink), and intracellular domain (blue). Blue box represents box 1 necessary for JAK activation; red box, cytokine receptor motif WSXWS sequence. (*) denotes identity; (:) indicates strong homology; and (.) indicates weak homology. (B) Lack of homology of mouse and human TSLP amino acid sequences. (C) Comparison of human CRLF2 and EPOR to show limited homology within box 1 of both proteins.

little sequence homology (43%) to its human counterpart (Figure 2A). Similarly, the specific receptor for TSLP (cytokine receptor-like factor 2 [CRLF2]) differs significantly with only 39% amino acid sequence homology (Figure 2B). CRLF2 interacts with IL-7RA to mediate high-affinity TSLP binding and signaling.³⁹ As might be anticipated from the resulting lack of both IL-7 and TSLP signaling, the murine IL-7RA mutant mouse has a more severe immunodeficient phenotype than the IL-7 mutant. However, surprisingly, given the *in vitro* data, inactivation of murine CRLF2 resulted in no obvious B-cell phenotype in adult mice, although in one model TSLP signaling was necessary for full lymphoid recovery after sublethal irradiation.^{41,42} Human, but not murine, TSLP induces dendritic cell activation and proliferation of some derived myeloid cell lines but has no obvious proliferative effects on lymphocytes; thus far, its possible effects on T- and B-cell lymphopoiesis remain unclear. One study has shown that TSLP may induce proliferation of BCP-ALL cell lines.⁴³ There are no reported mutations of either TSLP or CRLF2 in humans or mice associated with immunodeficiency. Therefore, despite its discovery as a cytokine signaling pathway able to support B-cell growth and differentiation *in vitro*, there has been little evidence for either TSLP or CRLF2 playing a pivotal role in human or murine B-cell development.

However, we and others have shown direct involvement of CRLF2 in BCP-ALL as a consequence, not only of IGH chromosomal translocation but also more commonly as a result of an interstitial genomic deletion.^{17,25,26} CRLF2 maps to the pseudoautosomal regions (pseudoautosomal region 1 [PAR1]) of the short arms of both sex chromosomes; the gene maps just more than 1 Mb from the telomere. This, and the fact that the IGH locus is also at the telomere of chromosome 14q, renders the translocations

cytogenetically cryptic; FISH studies are essential for detection. Both sex chromosomes are involved in translocation with IGH. The precise frequency of the IGH translocation in adolescent and adult patients remains unknown. However, in an unselected series of pediatric cases, the frequency of the translocation was 0.8%. The consequences of CRLF2/IGH translocation are deregulated expression of CRLF2 at the cell surface.^{17,26}

Fascinatingly, deregulated expression of CRLF2 may also be seen after a small interstitial deletion within PAR1. Again, this event is cytogenetically cryptic and has to be detected by FISH, quantitative PCR approaches, or array-based imbalance mapping (ie, array comparative genomic hybridization or single nucleotide polymorphism arrays). Interstitial deletion of PAR1 was more common than the CRLF2/IGH translocation (occurring overall in 4.2% of pediatric cases in our series and in 7% of cases in the St Jude's series) and occurred in younger patients than with the CRLF2/IGH translocation. In all series reported to date, there was a strong association with Down syndrome (DS), with approximately 50% to 60% of DS BCP-ALL exhibiting the deletion. Molecular mapping revealed a recurrent rearrangement involving CRLF2 with a centromeric gene P2RY8. RT-PCR experiments showed expression of a chimeric fusion mRNA composing the first noncoding exon of P2RY8 with the entire coding region of CRLF2 (Figure 1C).^{25,26} DNA sequence analysis of the genomic breakpoints showed high conservation of the deletion breakpoints with flanking recombination signal sequences, indicating that the deletions arise because of aberrant RAG activity, as has been observed with other recurrent deletions in BCP-ALL.²³ As with the CRLF2/IGH translocation, the principal consequence of the deletion was expression of CRLF2 at the cell surface. One copy of the IL-3

receptor gene (*IL3RA*) is also lost as a consequence; whether this contributes to the pathogenesis of disease is not known.

In experimental models, deregulated expression of *CRLF2* alone does not appear to result in B-cell progenitor transformation. Expression of human *CRLF2* in the murine IL-3–dependent pro-B-cell line Ba/F3 did not result in cytokine-independent cell growth, and retroviral transfection of normal mouse fetal liver cells resulted in short-term proliferation.^{17,25} Similarly, knockdown of *CRLF2* mRNA in a derived human BCP-ALL cell line harboring the *CRLF2/IGH* translocation resulted in slowed proliferation but not cell death, emphasizing the role for secondary, cooperating oncogenic events.¹⁷ In 3 cases with *PAR1* deletion in DS BCP-ALL, an activating mutation of *CRLF2* (F232C) occurring within the juxtamembrane region of *CRLF2* has been observed.²⁶ However, the most common associated mutation reported to date is *JAK2*, with approximately one-third of DS BCP-ALL cases with *PAR1* deletion exhibiting concurrent *JAK2* mutations. Like *CRLF2*, *JAK2* mutations do not transform Ba/F3 cells. In contrast, cotransfection of both *CRLF2* and mutated *JAK2* into either Ba/F3 cells or Ba/F3 cells transduced with human *IL-7RA* resulted in marked synergy with cytokine-independent cell growth.^{25,26} Whether *JAK2* is the physiologic and only *CRLF2* kinase remains controversial, and it is possible that mutations or alterations in other kinases might synergize with *CRLF2*.⁴⁴ The necessity of *IL-7RA* for the proliferative effect of deregulated *CRLF2* expression is also unclear; it is possible that both *IGH* translocation and *PAR1* deletion result in *CRLF2* homodimerization, leading directly to *JAK2* and *STAT5* phosphorylation and thus proliferation.

JAK2 mutations are associated with a poor prognosis in BCP-ALL.⁴⁵ However, preliminary data also indicate that *CRLF2* expression is also associated with poor outcome, defining a new poor prognostic subgroup in otherwise good-risk prognosis BCP-ALL^{26,46} (G.C., M. Zimmerman, R. Romey, S. Gesk, J. Harbott, A. Schrauder, A. Moericke, S. Igraely, T.A., M.J.S.D., R.S., M. Schrappe, and M. Stanulla, manuscript under review). Given that *CRLF2* expression can be readily detected by flow cytometry on BCP-ALL blasts, we would recommend the addition of *CRLF2* antibodies to the routine diagnostic immunophenotypic panels. The restricted expression of *CRLF2* in normal human tissues and the association with poor prognostic disease therefore make this an excellent target for immunotherapy, comparable with *HER2* amplification in breast cancer. Whether inhibition of *CRLF2* signaling might have a role in suppressing normal lymphopoiesis, and therefore have a possible role in maintenance therapy for BCP-ALL, remains to be determined.

***EPOR/IGH* translocations and *EPOR* expression in BCP-ALL**

Like *CRLF2*, the erythropoietin receptor (*EPOR*) is recurrently involved in BCP-ALL by 2 distinct mechanisms. First, rare cases may exhibit chromosomal translocation t(14;19)(q32;p13), sometimes as the only detectable cytogenetic abnormality.¹⁶ The translocation results in high-level *EPOR* expression. However, *EPOR* is also a transcriptional target gene for the *ETV6-RUNX1* fusion protein resulting from t(12;21)(p13;q22) that occurs in approximately 25% of pediatric BCP-ALL.⁴⁷ From analysis of the available electronic RNA expression data, *EPOR* mRNA does not appear to be expressed at appreciable levels in normal human B-cell precursors, indicating that *EPOR* expression in BCP-ALL is ectopic.⁴⁸ Sequence analysis of *EPOR* and *CRLF2* showed that both have the box 1 motif required for *JAK* interaction (Figure 2C), and it is likely that deregulated *EPOR*, like *CRLF2*, contributes to B-cell precursor transformation by constitutive *STAT5* phosphorylation.

As *ETV6-RUNX1* induces resistance to transforming growth factor- β –mediated growth suppression rather than proliferation, it is possible that *EPOR* expression confers a survival advantage in this context.⁴⁹ Ectopic *EPOR* expression is also seen in other malignancies, including breast carcinoma.⁵⁰

Other components of the cytokine signaling pathways do not appear to be commonly implicated in the pathogenesis of lymphoid malignancies. The cytokine IL-3 is occasionally involved in *IGH* translocation, results in deregulated IL-3 expression, and is seen specifically in BCP-ALL with associated eosinophilia.⁵¹ This has now been recognized as a specific disease entity by the World Health Organization.²⁹

***ID4/IGH* translocations in BCP-ALL**

Originally reported in a single case, we have subsequently shown chromosomal translocation t(6;14)(p22;q32) to be a recurrent event in a series of 13 cases; all involve the *ID4* gene on chromosome 6p22.3.^{12,15} The precise overall frequency and the possible prognostic and clinical significance of the *ID4* translocation remain unknown, but from our initial series of cases, it may be associated with relatively good prognosis disease. All patients presented with low peripheral blood blast counts (median, $3 \times 10^9/L$; range, 1–11 $\times 10^9/L$) and given the older age of most patients, most appeared to respond well to chemotherapy. However, this needs to be confirmed from prospective studies of uniformly treated cohorts of patients. Unlike both the *CRLF2* and *CEBP* translocation cases, most *ID4/IGH* cases had a complex karyotype with several other structural and/or numerical chromosomal changes. A common karyotypic/FISH feature was deletion of the *CDKN2A* locus on chromosome 9p21 in all cases, suggesting possible synergy with *ID4* dysregulation.

In contrast to the *CEBP/IGH* translocations, only *ID4* of the *ID* gene family is involved in *IGH* translocation in BCP-ALL, suggesting that *ID4* has specific transforming properties. How *ID4* contributes to neoplastic transformation of normal B-cell precursors remains unknown. *ID4* was first identified through its sequence homology to 3 related genes (*ID1-ID3*).⁵² All 4 family members share a highly homologous and conserved helix-loop-helix motif found in many developmentally regulated transcription factors but lack the basic DNA binding domain found in basic-helix-loop-helix (bHLH) family of transcription factors (Figure 3A). *ID* proteins cannot act directly as transcription factors but bind to bHLHs and affect their ability to bind DNA and thus inhibit their functions. In hemopoietic cells, *ID* proteins bind to E-box protein family members, including *E2A*, but also other transcription factors, including *PAX5*, *pRb*, and *ETS*.^{53,54} *ID* proteins therefore act as dominant negative inhibitors of transcription factor activity. However, several other roles, for example, in cell-cycle control independent of transcription factor binding and nuclear localization, have also been described.⁵⁵ Of all 4 *ID* family members, *ID4* has been least studied. In terms of oncogenesis, *ID4* can behave either as an oncogene or as a tumor suppressor gene depending on cellular context. Overexpression of *ID4* in mouse p16Ink4a/Arf^{-/-} astrocytes, and glioblastoma cells drives both cyclin E expression to induce proliferation and Notch activation to induce a neural “stem cell–like” state.⁵⁶ *ID4* is not expressed in human hemopoietic cells.^{48,57} In addition, B-cell precursors from mouse *ID4* knockout mice do not show any obvious proliferative defects but differentiate more rapidly than wild-type counterparts (M.C., M.J.S.D., F. Sablitsky, unpublished observations, 2008). However, in contrast to neural cell malignancies, *ID4* appears to behave as a tumor suppressor gene in mature lymphoid cells, with heavy methylation of the *ID4* promoter seen in all cases of chronic

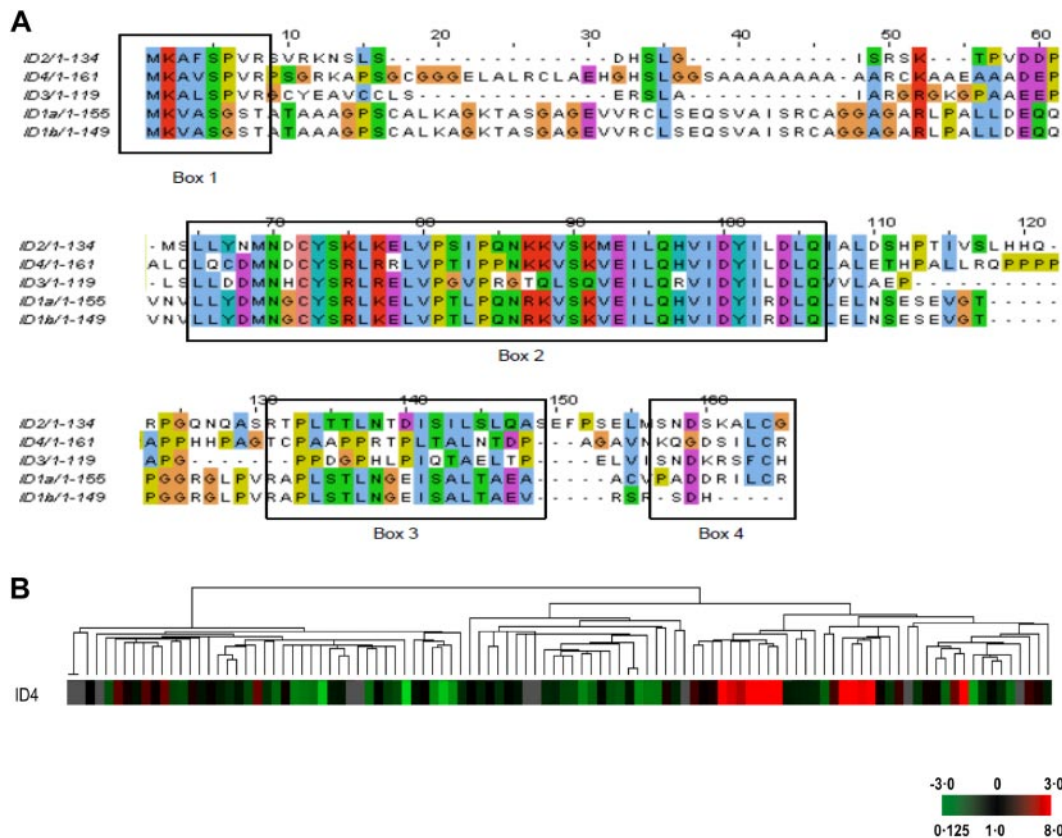


Figure 3. Comparison of ID4 amino acid sequences and RNA expression in BCP-ALL. (A) Protein sequence comparison of the human ID proteins. The region of highest homology between ID family proteins is within the HLH region (box 2), although there are other small areas of homology (boxes 1, 3, and 4). An amino-terminal CDK2-dependent phosphorylation site has been identified (SPVR) in all IDs except ID1 (box 1).⁸⁶ All members except ID3 contain a D-box region (RXXLXXN in box 3) that targets the protein for proteasomal degradation.⁴⁸ Phosphorylation status may have implications for nuclear localization and cell-cycle effect of ID proteins.^{86,87} (B) Dendrogram from unsupervised clustering of gene expression data obtained from 106 cases of pediatric BCP-ALL lacking *ETV6-RUNX1*, *BCR-ABL*, or *MLL-AF4* chromosomal translocations showing clustering of cases with high-level *ID4* expression. (G.C., T.A., R.S., and M.J.S.D., unpublished observations, August 2008). Hierarchical clustering analysis was carried out considering only those clones that had a 2-fold difference in expression from the mean on at least 2 arrays (4421 clones). Each column represents one sample. The expression of *ID4* is displayed as a variation in color as indicated in the lower right corner. Gray represents data that were omitted because they were not well measured.

lymphocytic leukemia.⁵⁸ Loss of ID4 accelerates disease progression in one mouse model of chronic lymphocytic leukemia.⁵⁹ Overexpression of ID4 in transformed lymphoid cell lines results in rapid G₁ cell-cycle arrest⁵⁸ (P.D., T.A., Y.F.L., E.L.K., and M.J.S.D., unpublished observations, September 2009).

Deregulated *ID4* expression in BCP-ALL may also arise from mechanisms other than chromosomal translocation; 11.5% of 297 unselected cases of pediatric BCP-ALL showed a high *ID4* expression as defined by an expression of at least 100-fold higher than the median expression of all samples using quantitative RT-PCR. Moreover, analyzing genome-wide unselected gene expression data using unsupervised clustering algorithm, those samples with a high *ID4* expression clustered together, suggesting distinct biologic subgroup(s) of disease (G.C., T.A., R.S., and M.J.S.D., unpublished observations, August 2008; Figure 3B). What mechanism(s) underlies the expression of *ID4* in these cases is not known. It is also unknown in cases lacking the *ID4/IGH* translocation whether *ID4* is a “driver” or a “passenger.” If *ID4* expression genuinely acts a “driver,” this might make *ID4* a therapeutic target in BCP-ALL, as there is only very low-level or absent expression of *ID4* in most normal adult tissues.^{60,61}

CEBP family/IGH translocations in BCP-ALL

CEBPs compose a family of 5 multifunctional basic leucine zipper (bZIP) transcription factors. The protein family is defined by a conserved carboxy-terminal bZIP domain, composing a leucine

zipper dimerization and basic DNA-binding domains. All but CEBPG exhibit an amino-terminal transcriptional transactivation domain; CEBPG therefore, like ID4, is thought to act as a natural dominant negative inhibitor of CEBP functions. All 5 *CEBP* genes are involved in *IGH* translocation at various frequencies, implicating the conserved bZIP domain in B-cell precursor transformation.¹⁴ The *IGH* translocation involving *CEBPD* is the most common and has been best characterized clinically.²⁷ Data from 44 cases have recently been collated.²⁸ The median age at presentation was 13 years, and the median white blood cell count at presentation was low ($9 \times 10^9/L$). The t(8;14)(q11;q32) was the sole acquired cytogenetic abnormality in 13 of 44 cases, occurring in conjunction with t(9;22)(q34;q11) in 7 cases. The translocation occurred in 12 patients with DS, none of whom had a t(9;22)(q34;q11). The linkage of the *CEBPD/IGH* translocation with DS appears to be specific among the *CEBP/IGH* translocations; to our knowledge, only one other *CEBP* translocation (involving *CEBPE*) has been reported in DS BCP-ALL.¹⁴ Why *CEBPD/IGH* translocations and the PAR1 interstitial deletion should preferentially occur in persons with DS is not yet clear. *CEBPA* translocations are the next most frequent, whereas *CEBPE*, *CEBPB*, and *CEBPG* translocations are probably sporadic. Given the variable age of the patients and the different chemotherapy protocols used, the prognostic significance of the *CEBP/IGH* translocations is not known. However, overall, the rate of early relapse appears to be low; whether this translates into a good survival is not yet clear.

Although rare, both individually and collectively and therefore potentially not of great clinical significance, the *CEBP/IGH* translocations are of great interest biologically. First, it is remarkable from a genetic perspective that all 5 *CEBP* family members are involved in *IGH* chromosomal translocations in the one disease. The closest parallel is the involvement of the cyclin D gene family in various mature B-cell malignancies.^{18,62,63} Second, it is all the more remarkable given that the *CEBP* genes suppress cell growth and induce differentiation under many conditions both in vitro and in vivo. For example, transduction of *CEBPD* in BCR-ABL⁺ CML cell lines resulted in prompt cell-cycle arrest, apoptosis, and differentiation into neutrophils.⁶⁴ This result is of some interest because, in BCP-ALL, the t(8;14)(q11;q32) involving *CEBPD* is often seen specifically in conjunction with the t(9;22)(q34;q11).²⁸ However, the apparent discrepancies are most marked in the case of *CEBPA*. *CEBPA* is normally expressed exclusively in cells of the myeloid and monocytic lineages with no detectable expression in normal B-cell precursors.⁶⁵ Loss of *CEBPA* expression results in enhanced cell proliferation and self-renewal of normal hemopoietic stem cells.⁶⁶ Expression of *CEBPA* in committed lymphoid cells suppresses the lymphoid differentiation program and, moreover, may drive the reprogramming of mature slg⁺ B cells to macrophages, albeit only at low frequency and under strong selective pressures.⁶⁷ *CEBPA* expression in uncommitted hemopoietic stem cells usually leads to up-regulation of PU.1 and down-regulation of PAX5, with consequent suppression of B-cell differentiation and commitment to the myeloid lineage.⁶⁸ However, BCP-ALL expressing any of the *CEBP* gene family appear to have typical BCP-ALL immunophenotypes with no overt evidence for commitment to other lineages. Third, in myeloid cells, *CEBPA* is unequivocally a tumor suppressor gene, with loss of *CEBPA* function observed in AML by several different mechanisms.⁶⁹ Approximately 7% of all cases of AML exhibit either monoallelic or biallelic acquired mutations/deletions of *CEBPA*, and the region is also subject to acquired uniparental disomy. Some familial cases of AML exhibit germline *CEBPA* mutations.⁷⁰ Furthermore, both transcription and translation of *CEBPA* may be suppressed directly or indirectly by various AML-associated chromosomal translocations.⁷¹ Consequently, the deregulated expression of all 5 *CEBP* genes in BCP-ALL is unexpected and constitutes something of “a spanner in the works.”⁶⁵

How deregulated *CEBP* expression contributes to the neoplastic transformation B-cell precursors remains unknown. The involvement of all family members suggests that transformation is mediated via the common dimerization and DNA binding regions with concurrent loss of the growth-suppressive activities of the amino-terminal transactivational domains. There are at least 3 possible mechanisms, all of which require the presence of prior genetic alterations before the *CEBP/IGH* translocation occurs. First, *CEBP/IGH* translocation may occur in a committed B-cell precursor where the myeloid differentiation pathway has already been “blocked.” There is some evidence for this in a case of BCP-ALL with t(14;14)(q11;q32) involving *CEBPE* where the same *IGH DJ* rearrangement was seen in both a *VDJ* rearrangement and at the chromosomal translocation breakpoint sequence, suggesting that the translocation occurred in a clone that had already undergone transformation.¹⁴ Second, there may be selective translation of short *CEBP* protein isoforms or alternatively posttranslational modifications that allow proliferation.^{65,72} Alternatively, despite the lack of B-cell phenotype in most *CEBP* mutant mice, the *CEBP* genes may have unsuspected functions in normal human B-cell precursors; recent genome-wide association studies have shown that single nucleotide polymorphisms within *CEBPE* may predispose to BCP-ALL.⁷³

The involvement of the *CEBP* gene family and *ID4* in BCP-ALL was unanticipated from previous biologic studies; if *ID4* and *CEBP* deregulation contribute to the neoplastic transformation of normal B-cell precursors, it seems probable they must all act in a strictly cell context–dependent fashion.

***MIR125B1/IGH* translocation and insertion**

MicroRNA genes play pivotal roles in normal and malignant hemopoiesis. One gene, *MIR125B1* on chromosome 11q23.3, has been implicated in the pathogenesis of various forms of myelodysplasia evolving to AML exhibiting t(2;11)(p21;q24) and more rarely in BCP-ALL with t(11;14)(q24;q32)^{30,74} (T.A., R.S., and M.J.S.D., unpublished observations, August 2008); both translocations result in deregulated expression of *MIR125B1*. In human myeloid cells, overexpression of this microRNA resulted in blocked myeloid differentiation⁷⁴; the effects of deregulated expression in B-cell precursors are so far unknown. Recent studies, however, have shown that *MIR125B1* binds to the 3′ untranslated region of p53 mRNA and suppresses p53 protein levels, at least in neuroblastoma and human lung fibroblasts.⁷⁵ Whether there are other more cell context–dependent effects of *MIR125B1* remains to be explored. *MIR125B1* is also highly expressed in germinal center B cells where it down-regulates both IRF4 and PRDM1.⁷⁶

MicroRNAs are often found as multiple copies in the human genome. Given the direct involvement of *MIR125B1* in both myeloid and lymphoid malignancies, it is therefore of interest that a homolog of this gene, *MIR125B2*, is located on chromosome 21q21 (<http://www.mirbase.org/>); whether the extra copy of this microRNA in persons with DS contributes to their increased susceptibility to both BCP-ALL and acute megakaryoblastic leukemias is currently unknown.⁷⁷ High levels of *MIRNA125B2* have been reported in DS AML.⁷⁸ In BCP-ALL, however, expression appears to be restricted to *ETV6-RUNX1* leukemias.⁷⁹

Conclusions

In conclusion, BCP-ALL exhibit a limited number of common recurrent chromosomal translocations, including *EVT6-RUNX1*, *BCR-ABL*, and *MLL-AF4*. Each of these cytogenetically defined subgroups is associated with a specific gene expression signature and a specific response to chemotherapy.^{80,81} BCP-ALL, however, also exhibit a plethora of much rarer *IGH* translocations. Is it important clinically to identify and dissect out these rare genetic events or can they be regarded as curios? Detection of *IGH* translocations in clinical material is relatively simple using the *IGH* “breakapart” probe set in interphase FISH experiments, but determining precisely which partner gene is involved may be difficult because of the large number of involved genes.

The expression of CRLF2 arising as a consequence of either *IGH* translocation or interstitial deletion probably becomes mandatory in clinical samples because of its association with a poor prognosis in otherwise “good-risk” patients. Expression of CRLF2 can be readily detected in clinical material using flow cytometry with commercially available antibodies. However, all *IGH* translocations, even those that are apparently sporadic, merit further scientific and clinical study. First, unlike somatic mutations, which may be either “drivers” or “passengers,” *IGH* translocations invariably act as direct pointers to genes not previously known to be involved in oncogenesis. Molecular cloning of *IGH* translocations has allowed rapid, unambiguous identification of genes that play pivotal roles in disease pathogenesis,¹⁸ and despite their

relative scarcity and diversity in BCP-ALL, this disease appears to be no exception. Second, deregulated target gene expression may be seen more commonly in BCP-ALL resulting from other mechanisms. Third, and perhaps more importantly in terms of clinical application, target genes defined by *IGH* translocations may also lead to new therapeutic approaches. For example, expression of *CRLF2* in BCP-ALL may allow the development of therapeutic antibodies. *BCL9* was originally identified from its involvement in t(1;14)(q21;q32) in BCP-ALL, and its expression indicates constitutive WNT signaling in a cell type-specific fashion.^{31,82} The interaction of *BCL9* with β -catenin is potentially targetable therapeutically, not only for BCP-ALL with the *BCL9* chromosomal translocation but in all forms of WNT-dependent malignancy.⁸³ Similar remarks also apply to *IGF2BP1* and *MIR125B1* that have pathogenetic roles not only in BCP-ALL but also in several other types of malignancy.^{84,85}

Collectively, these data indicate that *IGH* translocations in BCP-ALL may lead to important new mechanistic insights into cancer pathogenesis and may have possible diagnostic and therapeutic significance. The diversity of the targeted genes, including cytokine receptors, transcription factors, and signaling adapter molecules and miRNAs, reflects the heterogeneity of pathogenic mechanisms that underlie these diseases and malignancy as a whole, with no single obvious overarching mechanism to encompass all cases. Thus, both molecular cytogenetic and whole genome approaches in BCP-ALL indicate some common molecular abnormalities, but also a bewildering complexity of potentially “driving” somatic mutations and translocations. This perhaps should not be surprising given that B-cell precursors at different stages of

differentiation have very different gene expression profiles.⁴⁸ Practically, this diversity will have profound implications for the development and rational use of targeted therapies. Development of novel targeted therapeutics may not be possible if the population of affected persons is too small for this to be commercially viable. Even if effective targeted therapeutics are developed, the genetic composition of tumors will require comprehensive assessment from routine clinical material to maximize efficacy and minimize toxicities with such therapeutics.

Acknowledgments

This work was supported by the United Kingdom Medical Research Council, MRC Technology, the Hope Foundation for Cancer Research, Leicester, United Kingdom (Jemima Sellicks Fellowship; P.D.), and the Deutsche Krebshilfe and the KinderKrebsInitiative Buchholz/Holm Seppensen.

Authorship

Contribution: All authors contributed material (some previously unpublished) and reviewed the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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