Introduction

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common malignancy in children and involves uncontrolled expansion of B-lymphoid progenitors in the bone marrow. The disease is frequently initiated by a chromosomal translocation but becomes manifest only when leukemic progenitors in the bone marrow have accumulated a number of additional gene deletions and mutations that drive disease progression. With current treatment protocols long-term survival approaches 90%; however, relapses still pose a significant clinical challenge due to resistance to chemotherapy of the recurrent disease. Both in pediatric and adult BCP-ALL, specific genetic subtypes with distinct prognostic outcomes can be identified. Some of these subtypes, such as hyperdiploid ALL and ETV6-RUNX1-rearranged ALL are associated with a favorable outcome, while other genetic hallmarks, such as MLL gene rearrangements, hypodiploidy, intrachromosomal translocation of chromosome 21 (iAMP21), or the presence of the t(9;22) BCR-ABL1 translocation predict poor outcome. Moreover, the presence of a gene expression profile similar to that of BCR-ABL1-positive ALL, which frequently involves genetic alterations that deregulate cytokine receptor and/or tyrosine kinase signaling, is similarly associated with poor outcome. Together with its role as a critical regulator of B-cell development and a leukemia tumor suppressor, there is mounting evidence that IKZF1 loss also affects signaling pathways that modulate therapy response.

Here, we provide an overview of the complex role of transcription factor IKZF1 during normal lymphopoiesis and the consequences of altered IKZF1 function. Furthermore, we discuss different mechanisms by which IKZF1 alterations impose therapy resistance on leukemic cells, including enhanced cell adhesion and modulation of glucocorticoid response.
motif A/GGGAA through their N-terminal zinc-finger domain. Furthermore, all IKAROS family members harbor two additional C-terminal zinc-fingers required for homo- and heterodimerization between the different IKZF proteins (Figure 1A). The formation of homo- or heterodimers between IKAROS zinc-finger proteins with a functional DNA binding domain strongly enhances their DNA affinity and transcriptional activity. However, a common feature of IKZF1 and related family members is the presence of shorter variants due to alternative splicing. These variants often lack DNA binding activity but retain the ability to interact with full-length IKZF1-IKZF5, thereby creating dominant-negative isoforms. A well-known splice variant of both the mouse and human IKZF1 gene is the IK6 isoform, which lacks exons 4 to 7 that encode the four N-terminal zinc-fingers representing the DNA binding domain (Figure 1B).

IKZF1 mainly regulates gene expression through association with the nucleosome remodeling and deacetylase complex, which includes histone deacetylases HDAC1, HDAC2 and the ATP-dependent chromatin remodeling proteins CHD3 and CHD4. The nucleosome remodeling and deacetylase complex is involved in both transcriptional repression as well as gene activation by IKZF1. Gene silencing by IKZF1 is also facilitated through interaction with Polycomb repressive complex 2, which promotes histone H3 lysine 27 trimethylation to maintain genes in an inactive state. Other transcriptional co-factors that can associate with IKZF1 and mediate gene regulation include CtBP, CtIP and SWI/SNF-related complex. On the other hand, IKZF1 may itself participate in transcription initiation through direct interactions with the general transcription factors TFIIA and TBP. IKZF1 also controls transcription elongation via association with protein phosphatase 1α and cyclin-dependent kinase 9 (CDK9), the enzymatic component of the positive transcription elongation factor b. IKZF1-mediated transfer of protein phosphatase 1α to CDK9 promotes activation of positive transcription elongation factor b and recruitment to gene regulatory regions, thereby facilitating transcription elongation of IKZF1-target genes in hematopoietic cells.

Distinct post-translational modifications are able to modify the function of IKZF1. Phosphorylation of IKZF1 at multiple serine and threonine residues by casein kinase II impairs its function as a transcription factor. Conversely, casein kinase II inhibition enhances the transcriptional repressor function of IKZF1. Dual-specificity kinases BTK and SYK both phosphorylate IKZF1 on specific serine residues in close proximity of the DNA binding domain to augment its nuclear activity.
localization and DNA binding activity. \textsuperscript{24,25} Sumoylation of IKZF1 on lysine residues occurs within the nucleus and seems to interfere with transcriptional repression. \textsuperscript{24,27} It was previously shown that IKZF1 is also subject to ubiquitination,\textsuperscript{20} and there is now renewed interest in this pathway, since both IKZF1 and IKZF3 are targets of the immunomodulatory drugs thalidomide, lenalidomide, pomalidomide and CC-122.\textsuperscript{28} These immunomodulatory drugs promote proteosomal degradation of IKZF1 and IKZF3 by redirecting the substrate specificity of the CRL4<sub>CRBN</sub> ubiquitin ligase complex.\textsuperscript{29,30} Immunomodulatory drugs show therapeutic effects in a broad range of hematologic malignancies through their ability to target the malignant cells and modulate the immune system and its microenvironment.

**IKZF1 is essential for normal lymphopoiesis**

Studies performed in both constitutive and conditional <i>Ikzf1</i> knockout mouse models have demonstrated that IKZF1 function is not only required at different stages of lymphopoiesis,\textsuperscript{12,31,32} but also for normal myeloid, megakaryocyte and erythroblast differentiation.\textsuperscript{33-36} <i>Ikzf1</i>-deficient mice (<i>Ikzf1</i> null/null) lack all B cells, natural killer cells, plasmacytoid dendritic cells and fetal T cells\textsuperscript{31,37} (Figure 2). Nonetheless, post-natal <i>Ikzf1</i>-null mice harbor early T lineage progenitors within the thymus and export mature T cells to the periphery.\textsuperscript{38} Mice homozygous mutant for a hypomorphic allele of <i>Ikzf1</i> (<i>Ikzf1</i><sup>Hyp</sup>) show reduced B-cell progenitors in the bone marrow compartment, but still generate normal counts of mature B2 cells.\textsuperscript{39} These splenic B cells display alterations in isotype selection during immunoglobulin class switch recombination and a hyperproliferation phenotype upon antigenic stimulation.\textsuperscript{40,41} Although spontaneous progression to B-cell ALL is not observed in <i>Ikzf1</i><sup>Hyp</sup> mice, haplodeficient <i>Ikzf1</i><sup>L/−</sup> animals demonstrate an accelerated onset of B-cell leukemia in combination with a <i>BCR-ABL1</i> transgene.\textsuperscript{42} Moreover, all <i>Ikzf1</i><sup>L/−</sup> mice develop thymic lymphoma within a period of 10 months through activation of the Notch pathway.\textsuperscript{43} <i>Ikzf1</i><sup>L/−</sup> mutant mice expressing dominant-negative isoforms of IKZF1 (<i>Ikzf1</i><sup>L/−</sup><sub>DN</sub> and <i>Ikzf1</i><sup>L/−</sup><sub>Plstc</sub>) demonstrate a widespread failure of hematopoiesis,\textsuperscript{44,45} highlighting the importance of IKAROS transcription factors in hematolymphoid differentiation. Notably, heterozygous <i>Ikzf1</i> mutant mice develop T-cell malignancies with very high penetrance and short latency in the case of the dominant-negative isoforms,\textsuperscript{46,47} while this phenotype is less obvious in <i>Ikzf1</i><sup>L/−</sup> mice.\textsuperscript{48}

Detailed gene expression profiling has revealed that IKZF1 is essential for the generation of common lymphoid progenitors by priming lymphoid lineage-specific signatures in hematopoietic stem cells and lymphoid-primed

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<th>Mouse strain</th>
<th>&lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;null/null&lt;/sup&gt;</th>
<th>&lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;</th>
<th>&lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;&lt;sub&gt;DN&lt;/sub&gt;</th>
<th>&lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;&lt;sub&gt;Plstc/Plstc&lt;/sub&gt;</th>
<th>&lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−/L/−&lt;/sup&gt;</th>
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<td>B-lymphoid phenotype</td>
<td>• B cells absent</td>
<td>• Mild block B cell development; • Activated B cells; • Disturbed Ig class switch recombination</td>
<td>• B cells absent</td>
<td>• Block at pre-B cell stage; • Reduction of large pre-B cells</td>
<td>• Mild reduction progenitor B cells; • Increase of large pre-B cells</td>
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<td>T-lymphoid phenotype</td>
<td>• Fetal T cells absent; • Post-natal T cells activated; • Skewing towards CD4&lt;sup&gt;+&lt;/sup&gt; lineage</td>
<td>• Normal thymic cellularity; • Activated thymocytes and T cells</td>
<td>• T cells absent</td>
<td>• Mild reduction thymic cellularity</td>
<td>• Fetal T cells present; • Reduced thymic cellularity</td>
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<td>Hematopoietic phenotype</td>
<td>• Mild reduction in HSC activity; • NK cells and pDCs absent; • Increase of neutrophil precursors in fetal liver</td>
<td>• Strong reduction in HSC activity; • Reduction of erythroblast progenitors; • NK cells and pDCs absent</td>
<td>• Loss of LT-HSC pool at E15.5; • Increase of GMPs at E15.5; • Fatal fetal anemia</td>
<td>• NK cells and pDCs present</td>
<td>• NK cells and pDCs absent</td>
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<td>T-lineage malignancies</td>
<td>• Fraction &lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;null&lt;/sup&gt;/null mice develop T-cell malignancy</td>
<td>• All &lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt; mice develop thymic lymphoma in 10 mo</td>
<td>• All &lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;&lt;sub&gt;DN&lt;/sub&gt; mice develop thymic lymphoma in 4 mo</td>
<td>• Most &lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;&lt;sub&gt;Plstc&lt;/sub&gt; mice develop T cell malignancy in 4 mo</td>
<td>• None</td>
<td>• All &lt;i&gt;Ikzf1&lt;/i&gt;&lt;sup&gt;L/−&lt;/sup&gt;&lt;sub&gt;Plstc&lt;/sub&gt; mice develop thymic lymphoma in 10 mo</td>
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**References**

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Manta et al., 2007\textsuperscript{47}  
Schierven et al., 2013\textsuperscript{37}  
Schierven et al., 2013\textsuperscript{37}

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Figure 2. Summary of the observed phenotypes in the different constitutive <i>Ikzf1</i> knockout mouse models. The knockout allele shows a schematic representation at which position the deletion or mutation is present in the mouse <i>Ikzf1</i> gene. DN: dominant negative; Plstc: ENU-induced dominant-negative point mutation, called Plastic Neo: neomycin gene; βGeo: fusion between LacZ and neomycin gene; ZF: zinc-finger; HSC: hematopoietic stem cell; NK: natural killer; pDCs: plasmacytoid dendritic cells; LT-HSC: long-term hematopoietic stem cell; GMPs: granulocyte-macrophage precursors; mo: months.
multipotent progenitors. At different stages of T-lineage differentiation and development, IKZF1 is engaged by setting thresholds for (pre-)T-cell receptor-controlled checkpoints as well as T-cell activation downstream of interleukin-2 receptor signaling. In B-cell progenitors, Ikzf1 is required to induce Rag1 and Rag2 expression, and mediates chromatin accessibility during immunoglobulin gene rearrangement and allelic exclusion at the Igk locus. During pre-B-cell differentiation, IKZF1 regulates the transcription of genes implicated in pre-B-cell receptor signaling, cell survival, stromal-cell adhesion and B-cell commitment, such as Pax5, Foxn1 and EBF1. Many of these regulatory activities during B-lineage differentiation are navigated by super-enhancer networks controlled by IKZF1 and other B-cell master transcription factors.

Besides regulating expression of B-lymphoid genes, IKZF1 is actively involved in repression of a lineage-inappropriate transcriptional program normally prevalent in epithelial and mesenchymal precursors.

To further delineate the function of the individual zinc-fingers within the DNA-binding domain of IKZF1 in B-lymphopoiesis, Ikzf1 mouse mutants have been generated with targeted deletion of exon 4, which encodes zinc-finger 1 (Ikezf1ΔF4/ΔF4), or exon 6 encoding zinc-finger 4 (Ikezf1ΔF6/ΔF6). Germline deletion of either exon 4 or 6 results in decreased B-cell precursors with a stronger developmental block in Ikezf1ΔF4/ΔF4 mice, especially at the pre-B-cell stage. In contrast, the fraction of large pre-B cells is strongly increased in Ikezf1ΔF6/ΔF6 mice as compared to wild-type control animals. Interestingly, deletion of zinc-finger 4, but not zinc-finger 1, accelerates the onset of BCR-ABL1-mediated B-cell leukemia. Conditional deletion of exon 5 (Ikezf1ΔF5/ΔF5), which encodes zinc-fingers 2 and 3, at the stage of common lymphoid progenitors also results in an expansion of large pre-B cells within the bone marrow compartment, which is followed by a subsequent block in the transition to small pre-B cells. These findings indicate that N-terminal zinc-fingers 2, 3 and 4 of IKZF1 limit cell proliferation and survival at the time of active pre-B-cell receptor signaling, while zinc-fingers 1, 2 and 3 are absolutely required for the transition to the pre-B-cell stage.

**IKZF1 gene lesions drive leukemia development and relapse**

In the past decade, complementary genome-wide approaches have been employed to identify the genetic drivers implicated in the pathogenesis of ALL. Those studies revealed that the IKZF1 gene, which is located on chromosome band 7p12.2, is recurrently affected by different types of genetic alterations in BCP-ALL. Analysis of copy number alterations has demonstrated that IKZF1 gene deletions are present in about 15% of cases of childhood BCP-ALL and 40%-50% of adult patients with BCP-ALL. These deletions frequently involve the whole gene (DEL1-8) that results in loss of expression of wild-type IKZF1, as well as focal deletions that alter the function of IKZF1, such as the dominant-negative isoform IK6 (DEL4-7). Other common variants include deletions affecting exons 2-3, exons 2-7 and exons 4-8. In most cases these are monoallelic IKZF1 deletions where one functional copy of IKZF1 is retained, although biallelic deletions are also observed in a fraction of BCP-ALL cases. In addition, IKZF1 function is compromised by insertions, frameshift and missense mutations, which represent ~7% of IKZF1 alterations in BCP-ALL. Furthermore, rare in-frame gene fusions involving IKZF1 have been identified by RNA sequencing in BCP-ALL, including IKZF1-NUTM1, IKZF1-SETD5 and the reciprocal SETD5-IKZF1. However, it remains to be established whether these IKZF1 gene fusions are pathogenic and contribute to leukemia development.

An interesting feature is the strongly increased prevalence of IKZF1 deletions and mutations in high-risk BCP-ALL cases with an activated tyrosine kinase profile, particularly BCR-ABL1-positive ALL (~85%), and BCR-ABL1-like ALL (~70%), which is characterized by a range of genetic alterations driving cytokine receptor and kinase signaling. Similarly, IKZF1 deletions and mutations are highly abundant in chronic myeloid leukemia that has progressed to lymphoid blast crises, but IKZF1 alterations are virtually absent in chronic-phase and myeloid blast crisis chronic myeloid leukemia. IKZF1 deletions are also rarely detected in ETV6-RUNX1-positive BCP-ALL (3%), TCF3-rearranged (~3%) and MLL-rearranged (~5%) B-ALL. The distribution of IKZF1 deletions among the remaining subtypes, including hyperdiploid and B-other leukemia, ranges from 15%-20%. IKZF1 acts as a critical tumor suppressor in mouse T-lymphoid malignancies, but IKZF1 gene lesions are not very prevalent in T-ALL. Copy number alterations and mutations affecting the IKZF1 gene can be detected in ~4% of T-ALL. Notably, IKZF1 alterations occur in ~13% of early T-cell precursor ALL, a high-risk subtype of T-ALL characterized by recurrent mutations activating tyrosine kinases (FLT3, JAK1, JAK3) and cytokine signaling (IL7R).

IKZF1 alterations have also been reported in myeloproliferative neoplasms, and both pediatric and adult acute myeloid leukemia harbor IKZF1 deletions that affect its function. Thus, the tumor suppressive activity of IKZF1 is not uniquely restricted towards the lymphoid lineage and extends to a broader range of hematologic malignancies.

Besides its critical role in the pathogenesis of leukemia, IKZF1 alterations are also associated with adverse prognosis in BCP-ALL even within the high-risk group of BCR-ABL1-positive ALL. Notably, the occurrence and prognostic impact of IKZF1 alterations is not restricted to high-risk cases, but is also observed in standard-risk B-ALL subtypes, including high hyperdiploidy. Indeed, IKZF1 deletion represents one of the strongest independent predictors of poor treatment outcome in childhood BCP-ALL. Similar data have been reported in adult BCP-ALL, where loss-of-function gene deletions of IKZF1 predict poor treatment outcome in BCR-ABL1-negative cases. Interestingly, the presence of other co-occurring gene lesions may either enhance or negate the prognostic value of IKZF1 deletions. For instance, focal deletions affecting both transcriptional regulator BTG1 and IKZF1 represent a high-risk group with a worse outcome than those with IKZF1 alterations alone. On the other hand, the BCP-ALL subtype characterized by deregulation of transcription factors ERG and DUX4 has a favorable outcome, despite the presence of IKZF1 deletions in approximately 40% of these patients. An explanation for this latter observation remains elusive.

**Genetic alterations that cooperate with IKZF1 deletions in B-cell precursor acute lymphoblastic leukemia**

There is accumulating evidence that recurrent chromosomal aberrations present in BCP-ALL, such as BCR-ABL1...
translocations or CRLF2 rearrangements, act as driver lesions and represent early events in leukemia development. Genome-wide analysis has established that several other genetic alterations cooperate before B-cell leukemia becomes manifest. Gene lesions that inactivate the lymphoid transcription factor IKZF1 are frequently observed in BCR-ABL1-positive and CRLF2-rearranged BCP-ALL. Similarly, IKZF1 alterations are highly prevalent in tyrosine kinase-activating lesions that define BCR-ABL1-like ALL. These include rearrangements involving ABL1/ABL2, CSF1R, EPOR, JAK2 and PDGFRB, or sequence mutations affecting FLT3, IL7R or SH2B3. Indeed, loss of IKZF1 may permit more effective STAT5 target gene regulation downstream of these pathways. Collectively, these findings argue that loss of IKZF1 function strongly cooperates with activated tyrosine kinase signaling pathways linked to enhanced progenitor B-cell proliferation and immortalization (Figure 3).

The predilection for IKZF1 gene alterations in BCR-ABL1-mediated lymphoid versus myeloid malignancies has been further corroborated in mouse studies. In a bone marrow transplantation model using lineage-negative hematopoietic progenitor cells, it was shown that expression of IK6 skewed BCR-ABL1-mediated leukemia from an exclusive myeloproliferative disease towards a combined myeloid and B-lymphoid disease. Introducing p19Arf-deficiency further strengthens this trend towards uniformly induced B-cell ALL. This is in agreement with the finding that BCR-ABL1-positive BCP-ALL is characterized by the co-occurrence of IKZF1 and CDKN2A gene deletions.

Another group of genetic changes that frequently co-occur with IKZF1 alterations in BCP-ALL include gene deletions affecting lymphoid transcription factors, such as EBF1 and PAX5, and the transcriptional co-factor BTG1, which control gene transcription by their ability to interact with specific transcription factors, such as nuclear receptors and homeobox proteins, or through recruitment of protein arginine methyl transferase FRMT1. In addition, BTG1 through interaction with the CCR4-NOT, may also regulate mRNA deadenylation and consequently mRNA decay. Mice deficient for Btg1 show a partial block in B-cell development,

![Figure 3. Pathways cooperating with IKZF1 alterations in leukemia pathogenesis.](image-url)

Pathways involving cytokine receptor signaling and B-cell differentiation by lymphoid transcriptional regulators in normal progenitor B cells are schematically indicated on the left. Alterations of these pathways co-occur frequently with IKZF1 deletions and mutations in B-cell progenitor acute lymphoblastic leukemia (BCP-ALL) as indicated on the right. These include, activating mutations in FLT3, IL7R, JAK2, upregulation of CRLF2, C-terminal truncations or upregulation of EPOR, chromosomal translocations generating fusion proteins with PDGFR or CSF1R, and BCR-ABL1, which collectively result in activated cytokine receptor and tyrosine kinase signaling leading to STAT activation. In addition, IKZF1 alterations co-occur with gene deletions affecting the activity of B-lymphoid transcriptional regulators EBF1, PAX5 and BTG1, which results in a block of B-cell differentiation. FLT3: FMS related tyrosine kinase 3; IL7R: interleukin 7 receptor; CRLF2: cytokine receptor like factor 2; C-KIT: mast/stem cell growth factor receptor Kit; JAK, Janus kinase; STAT: signal transducer and activator of transcription; BTG1: B-cell translocation gene 1; EBF1: early B-cell factor 1; PAX5: paired box 5; IKZF1: IKAROS family zinc finger 1; CSF1R: colony-stimulating factor 1 receptor; EPOR: erythropoietin receptor; PDGFR: platelet-derived growth factor receptor.
which is even more evident in Btg1−/−;Btg2−/− mice. These studies have demonstrated that BTG1, together with BTG2, is required to suppress a T-lineage inappropriate expression program in progenitor B cells. Thus, monoallelic gene deletions of IKZF1 in combination with EBF1, Pax5 or BTG1 may contribute to a more prominent block in B-cell development and increased proliferative expansion of precursor B cells. Indeed, intercrossing haplodeficient Ikzf1 animals with heterozygous Ebf1 or Pax5 knockout mice promotes the onset of ALL, giving rise to both B-ALL and T-ALL. On the other hand, Btg1-deficiency specifically accelerates the development of T-ALL in Ikzf1−/− mice, which suggests that B-lineage-restricted mouse models will be required to establish their synergistic action in the pathogenesis of B-ALL.

**Effector pathways downstream of IKZF1 involved in leukemia pathogenesis**

Since lymphoid transcription factors are commonly deleted in BCP-ALL, the tumor suppressive functions of IKZF1 and other B-cell master regulators, such as EBF1 and Pax5, have been mostly linked to the suppression of their B-cell differentiation programs in these leukemic cells. However, this would not fully explain the predilection of IKZF1 alterations in BCR-ABL1-positive and BCR-ABL1-like leukemia, suggesting that IKZF1 also regulates other molecular pathways. Furthermore, loss of IKZF1 function probably affects different target genes in human leukemic cells as compared to mouse progenitor B cells, which could even be distinct from those deregulated by expression of dominant-negative isoforms, such as Ik6. Nonetheless, mouse studies performed over the past 5 years have been very instrumental in deciphering the transcriptional networks downstream of IKZF1. Thus, gene expression profiling in different Ikzf1 knockout mouse models combined with genome-wide chromatin immunoprecipitation studies has uncovered IKZF1-specific targets that are not only linked to lymphoid lineage commitment and B-cell differentiation, but also to leukemia development.

A large group of those Ikzf1-target genes can be classified as signal transducers, some of which drive early lymphoid differentiation, such as c-Kit, Flt3 and Il7r. Adult ALL samples harboring IKZF1 deletions display increased expression of Il7r together with reduced expression of Sh2B3, which represents a defined subset of high-risk B-ALL. Other genes differentially expressed in Ikzf1-mutant mice are important for pre-B-cell receptor signaling, and several of these IKZF1 targets appear to be deregulated in BCR-ABL1-positive B-ALL, including IglL1, Syk, and Slp65. Indeed, defective pre-B-cell receptor function is a hallmark of BCR-ABL1-positive ALL, and loss of IKZF1 function enhances Src phosphorylation at the expense of the Syk/Slp65 pathway activation, which is required for pre-B-cell differentiation. Besides transcriptional regulation of signal transducers, Ikzf1 controls the expression of cell surface receptors, such as Cd34 and Cd43, and these molecules confer a leukemic growth advantage to IKZF1-mutated BCR-ABL1-positive B-ALL cells.

Another group of IKZF1 target genes identified in mouse progenitor B cells seems to converge on a cellular network coupling cell surface protein expression with intracellular Wnt and Rho signaling as well as catenin-driven gene regulation inside the nucleus. A critical target gene within this subgroup includes Cnmd1 encoding p120-catenin. This is a multifunctional protein that regulates cadherin stability at the cell membrane, activation of the Rho family of GTPases in the cytoplasm and Wnt/β-catenin target genes within the nucleus by interacting with Kaiso. Activation of CTNNB1 expression is observed in samples from patients with IKZF1 deletions, and inactivation of p120-catenin reduces the proliferative capacity of BCR-ABL1-positive leukemic cells. A related downstream effector pathway of IKZF1 that plays an eminent role during mouse B-cell development is integrin-dependent survival signaling, which involves activation of focal adhesion kinase (FAK). In mouse models of BCR-ABL1-positive B-ALL, perturbation of Ikzf1, including loss-of-function deletions and expression of Ik6, leads to activation of an adhesive phenotype, which correlates with overexpression of FAK. FAK pathway upregulation is also observed in BCR-ABL1-positive BCP-ALL, especially in the context of Ik6 expression. Moreover, FAK inhibition potentiates the responsiveness to the ABL inhibitor dasatinib in a xenograft model system and improves survival.

Recently, it has been proposed that the B-lymphoid transcriptional program regulated by IKZF1, as well as Pax5, acts as a metabolic barrier against malignant transformation of B-cell precursor cells. Inducible reconstitution of functional IKZF1 in patient-derived IKZF1-deleted B-ALL cells results in activation of the Lkb1-Ampk energy-stress-sensor pathway, and decreased protein levels of the insulin receptor, the glucose transporters GLUT1, GLUT3 and GLUT6, as well as the effectors of glucose metabolism, such as HK2, HK3, and G6PD. On the other hand, the expression of glucose-transport inhibitors, such as Tnxf1 and Cnrd2, are strongly induced by IKZF1. Consequently, these IKZF1-reconstituted B-ALL cells transit into a state of chronic energy deficit. Thus, this ‘metabolic gatekeeper’ function of IKZF1 may force silent pre-leukemic clones that carry potentially oncogenic lesions to remain in a latent state.

Besides imposing a change on pre-B-cell receptor signaling, cell adhesion and metabolic state, IKZF1 alterations in combination with BCR-ABL1 expression also result in acquisition of stem cell-like features and enhanced self-renewal of progenitor B cells (Figure 4). Activation of Thy1 expression has been linked to enhanced self-renewal, and Ikzf1 has been shown to regulate expression of multiple genes involved in cell cycle regulation, including Cdk14a, Cdkn2a, and Cdk6. In mouse progenitor B cells and human B-ALL, Bcl6 and Myc have been identified as IKZF1 targets, and probably both contribute to enhanced cell proliferation of IKZF1-deleted B-ALL. However, it remains to be established whether targeting these pathways has therapeutic potential in high-risk B-ALL patients.

**IKZF1 alterations mediate therapy resistance**

The presence of IKZF1 gene lesions in BCR-ABL1-positive B-ALL results in inferior treatment outcome and mouse xenograft models suggest that IKZF1 loss contributes to resistance to tyrosine kinase inhibitor-based therapy. Reactivation of cell adhesion pathways by perturbation of IKZF1 function leads to elevation of key adhesion molecules, such as integrins (ItgA5) and CD90, and adhesion regulators, such as FAK, as well as increased phosphorylation of FAK itself, which permits relocation of leukemic
cells to the bone marrow niche. Indeed, FAK inhibition re-sensitizes BCR-ABL1 leukemic cells to tyrosine kinase inhibitor therapy. Similar results are observed after treatment with retinoids, specifically retinoid X receptor agonists, which induce expression of wild-type IKZF1, but not IK6, thereby abrogating expression of stem cell and adhesion molecules. Although these studies have provided important clues about how IKZF1 deletions alter treatment response especially in the context of BCR-ABL1-positive ALL, alternative mechanisms of therapy resistance may exist besides protection through cell interactions within the bone marrow microenvironment.

Synthetic glucocorticoids, such as prednisolone, constitute essential drugs in the treatment of ALL patients and glucocorticoid resistance remains a substantial problem in the treatment of BCP-ALL. There is accumulating evidence that IKZF1 deletions mediate prednisolone resistance in vivo, but different mechanisms have been proposed. IKZF1 actively represses genes of the phosphatidylinositol-3 kinase pathway, including PIK3CD and PIK3C2B. Disruption of IKZF1 function, and subsequent activation of the PI3K/AKT/mTOR pathway can promote glucocorticoid resistance. IKZF1 controls expression of several genes involved in glucose and energy supply. This metabolic program may alter the threshold for responses to glucocorticoids in BCP-ALL. Specifically, the glucocorticoid receptor NR3C1 was reported to be a target of IKZF1 in pre-B ALL cells, and downregulation of NR3C1 protein levels could be observed upon expression of IK6. However, studies performed in murine Ikzf1−/− B cells and human BCP-ALL cell lines with short hairpin-mediated IKZF1 knockdown have demonstrated that loss of IKZF1 function induces glucocorticoid resistance independently of altered NR3C1 mRNA and protein expression. Indeed, IKZF1 itself appears to regulate NR3C1-dependent gene transcription. The transcriptional regulator BTG1 has been identified as a modifier of IKZF1-mediated resistance to glucocorticoid therapy and the combined loss of BTG1 and IKZF1 leads to an even stronger inhibition of glucocorticoid-induced cell death. Finally, IKZF1 target gene EMP1, which itself represents a poor prognostic factor in pediatric ALL, was shown to regulate the response to prednisolone, but also, on the other hand, to affect normal leukemic cell viability and proliferation. Collectively, these findings demonstrate that IKZF1, through modulation of different signaling pathways and acting directly on glucocorticoid target genes, alters treatment response, thereby mediating therapy resistance in BCP-ALL (Figure 5).

Conclusions and perspectives

From this review it becomes clear that loss of IKZF1 function affects a broad variety of biological pathways which may all contribute to leukemia development. Moreover, the recently established roles for IKZF1 in cell adhesion, metabolism and glucocorticoid-dependent target gene regulation seem to be important determinants of therapy resistance. Preclinical studies are helping with the identification of molecular pathways that can be exploited for targeted therapy of IKZF1-deleted BCP-ALL.

Over the past decade, a large series of studies conducted in both childhood and adult ALL have provided clear evidence that IKZF1 alterations predict adverse outcome in BCP-ALL, both in BCR-ABL1-positive and -negative B-
ALL. However, more recently the role of IKZF1 deletions as an independent prognostic marker has been challenged, as has the specific contributions of whole gene versus intragenic dominant-negative IKZF1 deletions. One potential explanation for such disparities may relate to differences in scheduling and dosing of specific therapeutics agents between different treatment protocols. It will, therefore, be important to study these protocol-dependent differences in order to define what is currently the most efficient treatment for IKZF1-deleted ALL. Certain adjustments, such as the addition of vincristine-stro pulses during maintenance therapy, may already prevent relapses. For the near future, more systematic screens aimed at determining specific vulnerabilities of IKZF1-deleted ALL may lead to the identification of targeted therapies that can re-sensitize this high-risk ALL subgroup to curative treatment.

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