

The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia

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ABSTRACT

Transcription factor IKZF1 (IKAROS) acts as a critical regulator of lymphoid differentiation and is frequently deleted or mutated in B-cell precursor acute lymphoblastic leukemia. *IKZF1* gene defects are associated with inferior treatment outcome in both childhood and adult B-cell precursor acute lymphoblastic leukemia and occur in more than 70% of *BCR-ABL1*-positive and *BCR-ABL1*-like cases of acute lymphoblastic leukemia. Over the past few years, much has been learned about the tumor suppressive function of IKZF1 during leukemia development and the molecular pathways that relate to its impact on treatment outcome. In this review, we provide a concise overview on the role of IKZF1 during normal lymphopoiesis and the pathways that contribute to leukemia pathogenesis as a consequence 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.

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%;¹ however, relapses still pose a significant clinical challenge due to resistance to chemotherapy of the recurrent disease.¹ Both in pediatric and adult BCP-ALL, specific genetic subtypes with distinct prognostic outcomes can be identified.² 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.² In addition to these gross chromosomal rearrangements, deletions or mutations affecting the B-cell transcription factor *IKZF1*, are a strong and independent predictor of poor outcome in BCP-ALL.^{3,4} 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 consequences of *IKZF1* loss for the pathogenesis of BCP-ALL. Finally, we discuss some of the molecular mechanisms by which *IKZF1* gene alterations may contribute to therapy resistance.

Transcription regulation by IKAROS zinc-finger protein 1

The IKAROS family of transcription factors consists of five different IKAROS zinc-finger proteins (IKZF1-IKZF5) that are able to bind DNA directly at the core

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motif A/GGGAA through their N-terminal zinc-finger domain.^{5,6} 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,⁷⁻¹⁰ 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.^{11,12} 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.^{13,14} Other transcriptional co-factors that can associate with IKZF1 and mediate gene regulation include CtBP, CtIP and SWI/SNF-related complex.¹⁵⁻¹⁷ On the other hand, IKZF1 may itself participate in transcription initiation through direct interactions with the general transcription factors TFIIB and TBP.¹⁶ IKZF1 also controls transcription elongation via association with protein phosphatase 1 α and cyclin-dependent kinase 9 (CDK9), the enzymatic component of the positive transcription elongation factor b.¹⁸⁻²⁰ IKZF1-mediated transfer of protein phosphatase 1 α 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.¹⁸

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.²⁰⁻²² Conversely, casein kinase II inhibition enhances the transcriptional repressor function of IKZF1.²³ On the other hand, 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

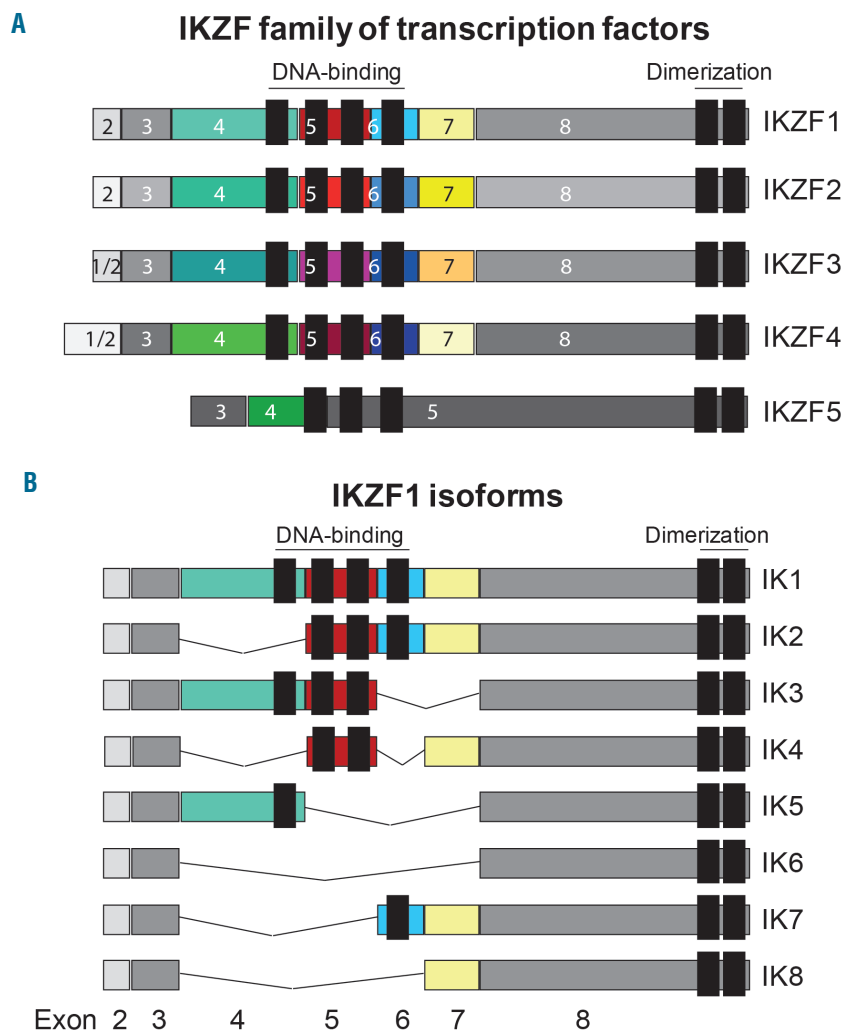


Figure 1. Overview of the human family of IKAROS zinc-finger (IKZF) transcription factors and IKZF1 isoforms. (A) Schematic representation of the five IKZF proteins (IKZF1-IKZF5), including the N-terminal zinc-fingers that define the DNA-binding domain and the two C-terminal zinc-fingers representing the dimerization domain. The colored boxes indicate the individual regions within the protein that are encoded by distinct exons. (B) The common IKZF1 splice variants (IK1-IK8) are shown, including the shorter isoforms that are generated by alternative splicing. The splice variants lacking exons 4 and 5 (IK6-IK8) represent dominant-negative isoforms of IKZF1.

localization and DNA binding activity.^{24,25} Sumoylation of IKZF1 on lysine residues occurs within the nucleus and seems to interfere with transcriptional repression.^{26,27} It was previously shown that IKZF1 is also subject to ubiquitination,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-122.²⁸ These immunomodulatory drugs promote proteosomal degradation of IKZF1 and IKZF3 by redirecting the substrate specificity of the CRL4^{CRBN} ubiquitin ligase complex.^{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 *Ikzf1* knockout mouse models have demonstrated that IKZF1 function is not only required at different stages of lymphopoiesis,^{12,31,32} but also for normal myeloid, megakaryocyte and erythroid differentiation.³³⁻³⁶ *Ikzf1*-deficient mice (*Ikzf1*^{null/null}) lack all B cells, natural killer cells, plasmacytoid dendritic cells and fetal T cells^{31,37} (Figure 2). Nonetheless, post-natal *Ikzf1*-null mice harbor early T lineage progenitors within the thymus and export mature T

cells to the periphery.³⁸ Mice homozygous mutant for a hypomorphic allele of *Ikzf1* (*Ikzf1*^{L/L}) show reduced B-cell progenitors in the bone marrow compartment, but still generate normal counts of mature B2 cells.³⁹ These splenic B cells display alterations in isotype selection during immunoglobulin class switch recombination and a hyperproliferation phenotype upon antigenic stimulation.^{40,41} Although spontaneous progression to B-cell ALL is not observed in *Ikzf1*^{L/L} mice, haplodeficient *Ikzf1*^{L/+} animals demonstrate an accelerated onset of B-cell leukemia in combination with a *BCR-ABL1* transgene.⁴² Moreover, all *Ikzf1*^{L/L} mice develop thymic lymphoma within a period of 10 months through activation of the Notch pathway.⁴³ *Ikzf1* mutant mice expressing dominant-negative isoforms of IKZF1 (*Ikzf1*^{DN/DN} and *Ikzf1*^{Plstc/Plstc}) demonstrate a widespread failure of hematopoiesis,^{44,45} highlighting the importance of IKAROS transcription factors in hemato-lymphoid differentiation. Notably, heterozygous *Ikzf1* mutant mice develop T-cell malignancies with very high penetrance and short latency in the case of the dominant-negative isoforms,^{46,47} while this phenotype is less obvious in *Ikzf1*^{+/-} mice.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

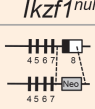
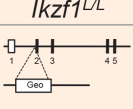
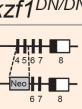

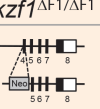

Mouse strain	<i>Ikzf1</i> ^{null/null}	<i>Ikzf1</i> ^{L/L}	<i>Ikzf1</i> ^{DN/DN}	<i>Ikzf1</i> ^{Plstc/Plstc}	<i>Ikzf1</i> ^{ΔF1/ΔF1}	<i>Ikzf1</i> ^{ΔF4/ΔF4}
Knockout allele	 Deletion Exon 8	 LacZ knock-in Exon 2 (Hypomorphic allele)	 Deletion Exon 4-5 (ZF1-3)	 Mutation Exon 4 (ZF3) p.H191R	 Deletion Exon 4 (ZF1)	 Deletion Exon 6 (ZF4)
B-lymphoid phenotype	• B cells absent	• Mild block B cell development; • Activated B cells; • Disturbed Ig class switch recombination	• B cells absent	• B cells absent	• Block at pre-B cell stage; • Reduction of large pre-BII cells	• Mild reduction progenitor B cells; • Increase of large pre-BII cells
T-lymphoid phenotype	• Fetal T cells absent; • Post-natal T cells activated; • Skewing towards CD4 ⁺ lineage	• Normal thymic cellularity; • Activated thymocytes and T cells	• T cells absent	• T cells absent	• Fetal T cells present; • Mild reduction thymic cellularity	• Fetal T cells absent; • Reduced thymic cellularity
Hematopoietic phenotype	• Mild reduction in HSC activity; • NK cells and pDCs absent	• NK cells and pDCs absent; • Increase of neutrophil precursors in fetal liver	• Strong reduction in HSC activity; • Reduction of erythroid progenitors • NK cells and pDCs absent	• Loss of LT-HSC pool at E15.5; • Increase of GMPs at E15.5; • Fatal fetal anemia	• NK cells and pDCs present	• NK cells and pDCs absent
T-lineage malignancies	• Fraction <i>Ikzf1</i> ^{null/+} mice develops T-cell malignancy	• All <i>Ikzf1</i> ^{L/L} mice develop thymic lymphoma in 10 mo	• All <i>Ikzf1</i> ^{DN/+} mice develop thymic lymphoma in 4 mo	• Most <i>Ikzf1</i> ^{Plstc/+} mice develop T cell malignancy in 4 mo	• None	• All <i>Ikzf1</i> ^{F4/F4} mice develop thymic lymphoma in 10 mo
References	Wang <i>et al.</i> , 1996 ³¹ Yoshida <i>et al.</i> , 2006 ³³ Avitahl <i>et al.</i> , 1999 ⁵⁰ Winandy <i>et al.</i> , 1999 ⁵¹	Kirstetter <i>et al.</i> , 2002 ³⁹ Sellars <i>et al.</i> , 2009 ⁴⁰ Heizmann <i>et al.</i> , 2016 ⁴¹ Dumortier <i>et al.</i> , 2006 ⁴³	Georgopoulos <i>et al.</i> , 1994 ⁴⁴ Winandy <i>et al.</i> , 1995 ⁴⁶	Papathanasiou <i>et al.</i> , 2003 ⁴⁵ Manta <i>et al.</i> , 2007 ⁴⁷	Schjerven <i>et al.</i> , 2013 ³⁷	Schjerven <i>et al.</i> , 2013 ³⁷

Figure 2. Summary of the observed phenotypes in the different constitutive *Ikzf1* knockout mouse models. The knockout allele shows a schematic representation at which position the deletion or mutation is present in the mouse *Ikzf1* 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; pDC: plasmacytoid dendritic cells; LT-HSC: long-term hematopoietic stem cell; GMPs: granulocyte-macrophage precursors; mo: months.

multipotent progenitors.⁴⁹ 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.^{50,51} 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.^{12,32,52} 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*, *Foxo1* and *Ebf1*.^{12,32,53} Many of those regulatory activities during B-lineage differentiation are navigated by super-enhancer networks controlled by IKZF1 and other B-cell master transcription factors.⁵⁴ 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.⁵⁴

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 (*Ikzf1*^{ΔF1/ΔF1}), or exon 6 encoding zinc-finger 4 (*Ikzf1*^{ΔF4/ΔF4}).⁵⁷ Germline deletion of either exon 4 or 6 results in decreased B-cell precursors with a stronger developmental block in *Ikzf1*^{ΔF1/ΔF1} mice, especially at the pre-B-cell stage.⁵⁷ In contrast, the fraction of large pre-B cells is strongly increased in *Ikzf1*^{ΔF4/ΔF4} 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.^{57,55} Conditional deletion of exon 5 (*Ikzf1*^{E5^{ΔA}}), 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.⁵⁶ 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.⁵⁷⁻⁶⁰ 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 4-8.⁶¹ 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.^{62,63} In addition, IKZF1 function is compromised by insertions, frameshift and missense mutations, which represent ~7% of *IKZF1* alterations in BCP-ALL.⁶³

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*.⁶⁴ 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%),⁶⁵ and *BCR-ABL1*-like ALL (~70%), which is characterized by a range of genetic alterations driving cytokine receptor and kinase signaling.^{3,66-68} 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.^{65,69,70} *IKZF1* deletions are also rarely detected in *ETV6-RUNX1*-positive BCP-ALL (3%), *TCF3*-rearranged (~3%) and *MLL*-rearranged (~5%) B-cell ALL.^{58,71,72} The distribution of *IKZF1* deletions among the remaining subtypes, including hyperdiploid and B-other leukemia, ranges from 15%-20%.⁷²

IKZF1 acts as a critical tumor suppressor in mouse T-lymphoid malignancies,^{43,46,47} 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.^{58,65,71,73} 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*).⁷⁴ *IKZF1* alterations have also been reported in myeloproliferative neoplasms,⁷⁵ and both pediatric and adult acute myeloid leukemia harbor *IKZF1* deletions that affect its function.^{76,77} 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,^{3,4,78} even within the high-risk group of *BCR-ABL1*-positive ALL.^{79,80} 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,⁷² including high hyperploidy.⁸¹ Indeed, *IKZF1* deletion represents one of the strongest independent predictors of poor treatment outcome in childhood BCP-ALL.^{71,72,82} 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.⁸³⁻⁸⁶ 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.⁴⁸ 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.^{64,87-90} 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.^{65,69,91,92} The latter group is associated with concomitant *JAK1* and *JAK2* activating mutations.⁹¹ Similarly, *IKZF1* alterations are highly prevalent in tyrosine kinase-activating lesions that define *BCR-ABL1*-like ALL.^{67,68} 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.⁹³ 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 skews *BCR-ABL1*-mediated leukemia from an exclusive myeloproliferative disease towards a combined myeloid and B-lymphoid disease.⁶⁵ Introducing *p19^{Arf}*-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.⁶⁵

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^{48,58} (Figure 3). BTG1 belongs to the BTG/TOB antiproliferative (APRO) family of proteins,⁹⁴ which control gene transcription by their ability to interact with specific transcription factors, such as nuclear receptors and homeobox proteins,^{95,96} the CCR4-NOT transcriptional regulatory complex,⁹⁷ or through recruitment of protein arginine methyl transferase PRMT1.⁹⁸ In addition, BTG1 through interaction with the CCR4-NOT, may also regulate mRNA deadenylation and consequently mRNA decay.^{99,100} Mice deficient for *Big1* show a partial block in B-cell development,

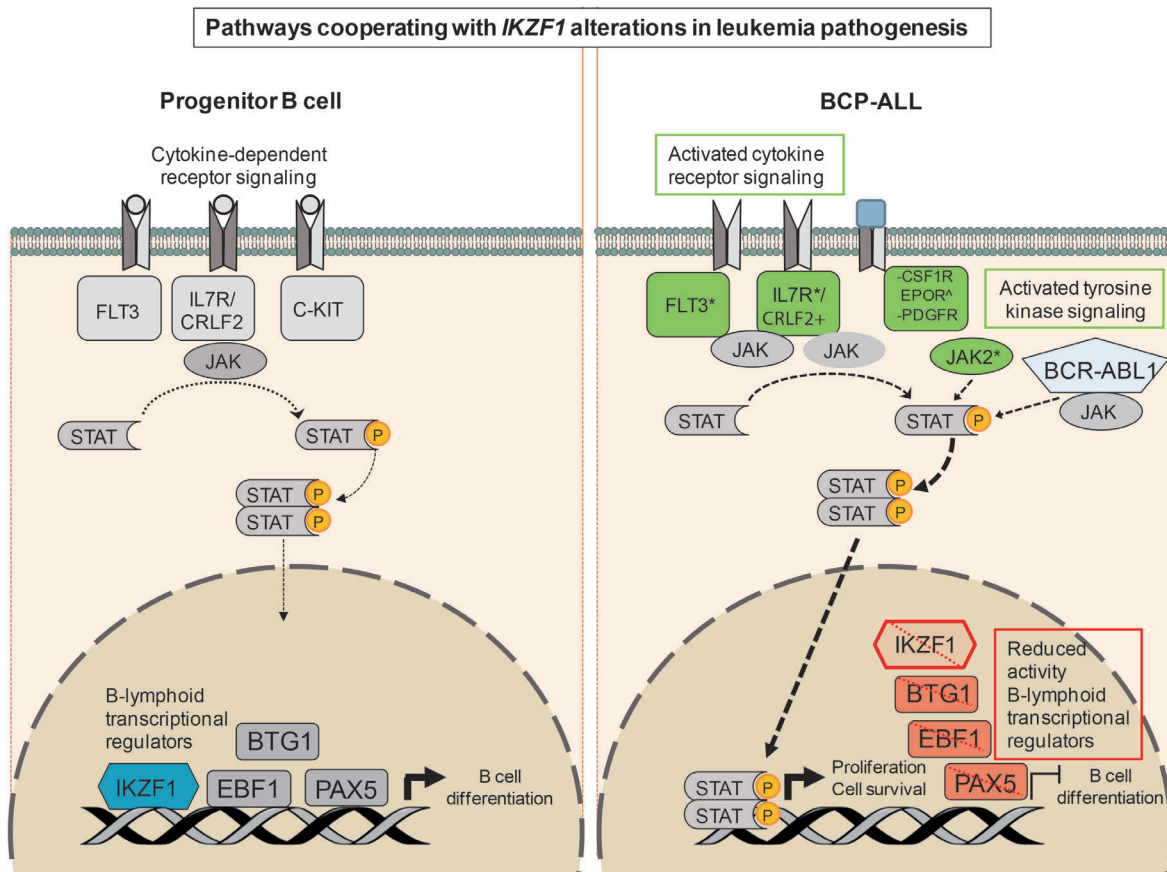


Figure 3. Pathways cooperating with *IKZF1* alterations in leukemia pathogenesis. Pathways involving cytokine receptor signaling and B-cell differentiation by lymphoid transcriptional regulators in normal progenitor B cells are schematically indicated on the left. Alterations of these pathways co-occur 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 results 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.¹⁰¹ These studies have demonstrated that BTG1, together with BTG2, is required to suppress a T-lineage inappropriate expression program in progenitor B cells. Thus, mono-allelic 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.¹⁰² 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*.^{12,32,37,53} 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.¹⁰³ 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*.^{104,105} 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.¹⁰⁴ 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.⁵⁵

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.^{55,106} A critical target

gene within this subgroup includes *Ctndd1* 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.¹⁰⁷ Activation of *CTNND1* expression is observed in samples from patients with *IKZF1* deletions,¹⁰⁸ and inactivation of p120-catenin reduces the proliferative capacity of *BCR-ABL1*-positive leukemic cells.^{55,106} 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).^{12,56} 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.^{63,109} FAK pathway upregulation is also observed in *BCR-ABL1*-positive BCP-ALL, especially in the context of IK6 expression.¹⁰⁹ Moreover, FAK inhibition potentiates the responsiveness to the ABL inhibitor dasatinib in a xenograft model system and improves survival.¹⁰⁹

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.¹¹⁰ Inducible reconstitution of functional IKZF1 in patient-derived *IKZF1*-deleted B-ALL cells results in activation of the LKB1-AMPK energy-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 TXNIP and CNR2, 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.^{63,105} (Figure 4). Activation of *THY1* expression has been linked to enhanced self-renewal,⁶³ and *Ikzf1* has been shown to regulate expression of multiple genes involved in cell cycle regulation, including *Cdkn1a*, *Cdkn2a*, and *Cdk6*.^{53,55} In mouse progenitor B cells and human B-ALL, *BCL6* and *MYC* have been identified as IKZF1 targets,^{32,111-113} 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.^{63,80} 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 relocalization of leukemic

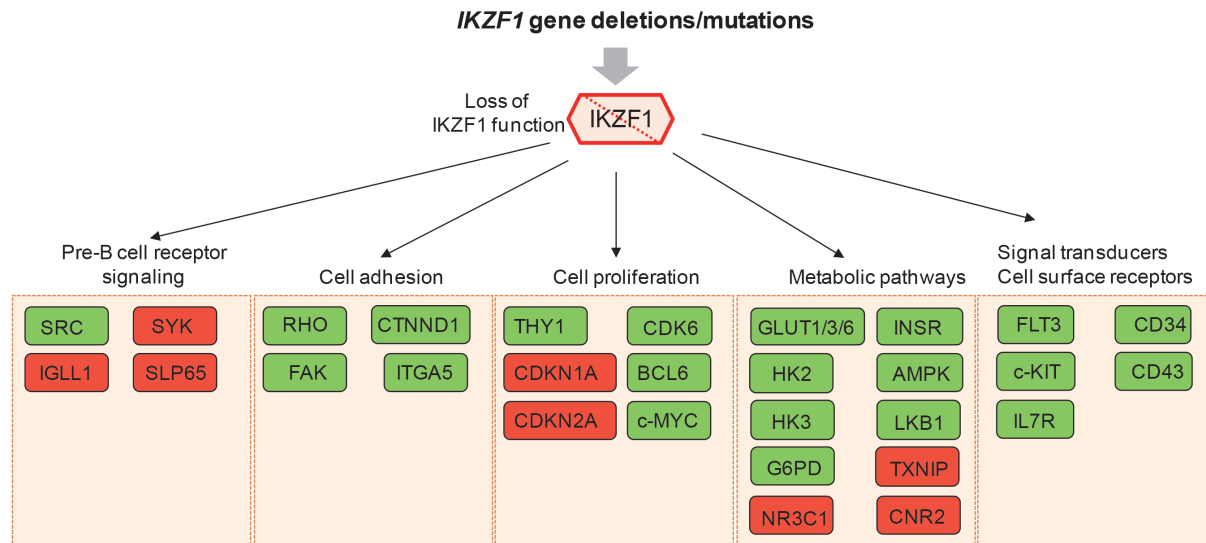


Figure 4. Effector pathways downstream of IKZF1 involved in leukemia pathogenesis. Loss of IKZF1 function due to *IKZF1* gene deletions and mutations affects multiple pathways, including pre-B-cell receptor signaling, cell adhesion and proliferation, metabolic pathways and signal transducers and cell surface receptors. IKZF1 affects the expression of defined key molecules within each of these pathways, as indicated in the boxes. Green boxes define targets that are upregulated upon loss of IKZF1 function, while red boxes represent repressed targets. SRC: sarcoma proto-oncogene tyrosine kinase; SYK: spleen tyrosine kinase; IGLL1: immunoglobulin lambda-like polypeptide 1; SLP65: B-cell linker; RHO: RHO family of GTPases; CTNND1: catenin delta 1/p120 catenin; FAK: focal adhesion kinase; ITGA5: integrin subunit alpha 5; THY1: thymus cell antigen 1; CDK6: cyclin dependent kinase 6; CDKN1A: cyclin dependent kinase inhibitor 1A; BCL6: B-cell lymphoma 6; CDKN2A: cyclin dependent kinase inhibitor 2A; c-MYC: cellular myelocytomatosis oncogene; GLUT1/3/6: glucose transporter 1/3/6; INSR: insulin receptor; HK2: hexokinase 2; HK3: hexokinase 3; AMPK: AMP-activated protein kinase; LKB1: liver kinase B1; G6PD: glucose-6-phosphate dehydrogenase; TXNIP: thioredoxin interacting protein; NR3C1: nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor); CNR2: cannabinoid receptor 2; FLT3: FMS related tyrosine kinase 3; CD34: hematopoietic progenitor cell antigen; c-KIT: KIT receptor tyrosine kinase; CD43: sialophorin; IL7R: interleukin 7 receptor.

cells to the bone marrow niche. Indeed, FAK inhibition re-sensitizes *BCR-ABL1* leukemic cells to tyrosine kinase inhibitor therapy.¹⁰⁹ 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.⁶⁵ 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*,^{114,115} but different mechanisms have been proposed. IKZF1 actively represses genes of the phosphatidylinositol-3 kinase pathway, including *PIK3CD* and *PIK3C2B*.²⁵ Disruption of IKZF1 function, and subsequent activation of the PI3K/AKT/mTOR pathway can promote glucocorticoid resistance.^{116,117} IKZF1 controls expression of several genes involved in glucose and energy supply.¹¹⁰ 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 IK6.¹¹⁰ 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 inde-

pendently of altered NR3C1 mRNA and protein expression.¹¹⁴ Indeed, IKZF1 itself appears to regulate NR3C1-dependent gene transcription.¹¹⁴ 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.⁴⁸ Finally, IKZF1 target gene *EMP1*,¹⁰⁶ 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.¹¹⁸ 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-

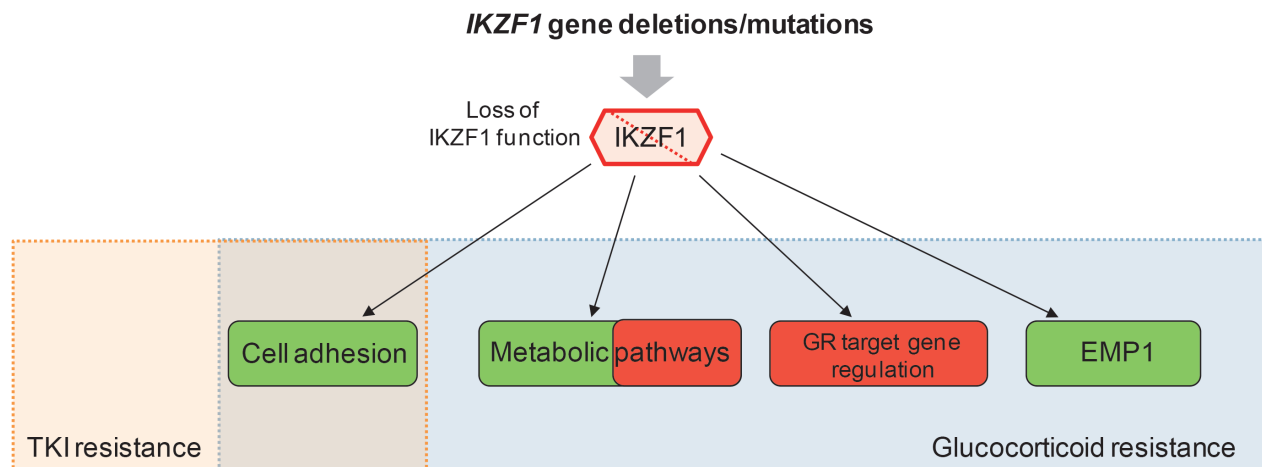


Figure 5. *IKZF1* alterations mediate therapy resistance. Overview of *IKZF1*-affected pathways contributing to tyrosine kinase inhibitor (TKI) resistance and glucocorticoid (GC) resistance. Enhanced cell adhesion due to loss of *IKZF1* function has been shown to contribute to both TKI and GC resistance. Deregulation of metabolic pathways, such as LKB1/AMPK signaling and glucose metabolism, attenuated glucocorticoid receptor (GR) target gene regulation and upregulation of epithelial membrane protein 1 (EMP1) have been implicated in mediating GC resistance of *IKZF1*-deleted BCP-ALL. Green boxes indicate activated targets or pathways, while red boxes define attenuated pathways. Targets within the metabolic pathway can either promote or inhibit GC resistance.

ALL. However, more recently the role of *IKZF1* deletions as an independent prognostic marker has been challenged,¹¹⁹ as has the specific contributions of whole gene *versus* intragenic dominant-negative *IKZF1* deletions.⁸⁶ One potential explanation for such disparities may relate to differences in scheduling and dosing of specific therapeutic 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-

steroid pulses during maintenance therapy,⁸¹ 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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