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Therapeutic targeting of IL-7Rα signaling pathways in ALL treatment

Sarah D. Cramer,[1-3] Peter D. Aplan,[4] and Scott K. Durum[1]

[1]Cytokines and Immunity Section, Cancer and Inflammation Program, National Cancer Institute, National Institutes of Health, Frederick, MD; [2]Comparative Biomedical Scientist Training Program, National Institutes of Health, Bethesda, MD; [3]Department of Veterinary Medicine, University of Maryland, College Park, MD; and [4]Leukemia Biology Section, Genetics Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

Increased understanding of pediatric acute lymphoblastic leukemia (ALL) pathobiology has led to dramatic improvements in patient survival. However, there is still a need to develop targeted therapies to enable reduced chemotherapy intensity and to treat relapsed patients. The interleukin-7 receptor α (IL-7Rα) signaling pathways are prime therapeutic targets because these pathways harbor genetic aberrations in both T-cell ALL and B-cell precursor ALL. Therapeutic targeting of the IL-7Rα signaling pathways may lead to improved outcomes in a subset of patients. (*Blood*. 2016; 128(4):473-478)

Introduction

Treatment of pediatric acute lymphoblastic leukemia (ALL) is a major success of modern medicine. Fifty years ago, children diagnosed with ALL survived for a median of 2 months.[1] Today, the 5-year overall survival rate is >90% in some series.[2] However, survival rates are much lower in children who experience relapse, varying between 21% and 39%.[3,4] In addition, although this overall improvement in outcome is staggering, it comes at a cost. To achieve remission, patients typically undergo 2 to 3 years of chemotherapy.[1] Acute side effects include infections, allergic reactions, thrombosis, pancreatitis, and neurologic impairment. Chronic side effects include osteonecrosis, obesity, secondary malignancies, and neurocognitive deficits.[1]

As we characterize the pathophysiology of ALL, we must use our improved understanding to develop targeted therapies.[5] Targeting molecular lesions in specific patients may enable reduced-intensity chemotherapy and may help to rescue children whom current protocols fail to cure. Although there are many potential targets for therapy, this spotlight will focus on genetic aberrations involving interleukin-7 receptor α (IL-7Rα) signaling pathways.

In health, the IL-7Rα chain forms 2 heterodimeric cytokine receptors (reviewed by Tal et al[6]). The receptor for IL-7 is formed by IL-7Rα pairing with γc, and the receptor for thymic stromal lymphopoietin (TSLP) is formed by IL-7Rα pairing with TSLP receptor (TSLPR) (encoded by the *CRLF2* gene).[6] IL-7 signaling is vital for normal T-cell development and survival of most mature T cells (reviewed by Mazzuchelli and Durum[7]). Conversely, TSLP signaling is involved in stimulation of growth and differentiation of B1 B-cell progenitors.[8] In addition, TSLP is involved with $CD4^+$ T-cell homeostasis, regulatory T-cell development, and dendritic cell activation (reviewed by Liu et al[9]). Finally, TSLP plays a prominent role in the pathogenesis of allergy and asthma (reviewed by Ziegler and Artis[10]). Binding of IL-7 or TSLP to their respective receptors causes activation of the JAK/STAT and PI3K/AKT/mTOR intracellular signaling cascades.[6] The MAPK cascade could also be activated with normal TSLP signaling and with IL-7 signaling in T-cell ALL (T-ALL) and immature B and T cells.[11-13] However, MAPK signaling is not required for the prosurvival and proliferation effects of IL-7 in T-ALL cells, and it does not seem to play a role in IL-7 signaling in normal T cells.[12,14] Because the JAK/STAT and PI3K/AKT/mTOR intracellular signaling cascades seem to be the major players in IL-7Rα signaling, we will focus on them for the remainder of the review.

IL-7Rα signaling pathway perturbations in T-ALL

As might be expected for a receptor that is vital to T-cell development, mutations in *IL-7Rα* have recently been identified in a subset of pediatric T-ALL cases.[15-17] These mutations generally occur in exon 6 of the gene and lead to insertion of multiple amino acids, usually including cysteine. The insertions induce IL-7Rα homodimerization and constitutively activate JAK1 in the absence of IL-7, γc, or JAK3.[16] *IL-7Rα* mutations seem to be more prevalent in HOXA, TLX3, and early T-cell precursor (ETP-ALL) patient subgroups.[16,17] The mutation does not seem to have prognostic implications.[16]

Although mutations in *IL-7Rα* occur in ~10% of pediatric patients, mutations in an upstream regulator of *IL-7Rα*, *NOTCH1*, occur in >50% of T-ALL cases.[18] *NOTCH1* is the most commonly mutated gene in T-ALL and is known to regulate *IL-7Rα* transcription and expression. Increases in IL-7R signaling promoted by NOTCH1 mutations may play a role in NOTCH1 oncogenicity.[19]

Mutations downstream of IL-7R also occur in T-ALL. The JAK/STAT pathway can be activated by several genetic aberrations. Activating mutations in *JAK1* and *JAK3* have been reported, and a *TEL-JAK2* fusion has also been described.[17,20-26] Notably, JAK2 mutations generally seem to be more associated with B-cell precursor ALL (BCP-ALL) than T-ALL. Further downstream, T-ALL patients have also had mutations in *STAT5B*.[27,28] In addition, a negative regulator of JAK-STAT signaling, *PTPN2*, is deleted in some cases of T-ALL.[29]

Activation of the PI3K/AKT/mTOR pathway also occurs in T-ALL secondary to genetic aberrations, and an estimated 47.7% of pediatric cases have deletion or mutation of *PTEN*, *PI3K*, or *AKT*.[30,31] The PI3K/AKT/mTOR-negative regulator *PTEN* is mutated in an estimated 8.7% to 22% of T-ALL cases or deleted in 27.3%.[30,31] PTEN activity can also be decreased by posttranslational effects such as casein kinase 2 (CK2) overexpression, high levels of reactive oxygen species (ROS), or miRNAs.[32,33]

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Furthermore, T-ALL cells from patients proliferate in response to IL-7 signaling, suggesting that targeting this pathway may be useful for many T-ALL patients, not only those with *IL-7Rα* genetic aberrations.[34]

IL-7Rα signaling pathway perturbations in BCP-ALL

In BCP-ALL patients, IL-7Rα pathway perturbations are most commonly caused by genetic aberrations affecting the *CRLF2* gene, though mutations in *IL-7Rα* also occur. In most cases, chromosomal translocations, rearrangements, or gene duplications caused overexpression of *CRLF2*. Translocation leads to *IGH-CRLF2* fusion, whereas interstitial deletion causes *P2RY8-CRLF2* fusion.[35-37] *P2RY8-CRLF2* fusions have been found at a much higher rate (53%) in patients with Down syndrome–associated ALL (DS-ALL).[35] Other chromosomal abnormalities can also lead to *CRLF2* deregulation.[36,37] It appears that rearranged TSLPRs signal conventionally through JAK/STAT and PI3K/AKT/mTOR pathways.[38] Less commonly, the *CRLF2* gene can have a F232C mutation that enables constitutive activation of TSLPR in the absence of IL-7Rα.[39,40] Mutant TSLPR may not signal through the same kinases as wild-type TSLPR, particularly with regards to MAPK, which is downregulated in response to mutant TSLPR/mutant JAK2 signaling.[11]

Notably, *CRLF2* overexpression is not always attributable to structural rearrangement involving the *CRLF2* gene.[41,42] In all, ~15% of patients with BCP-ALL have *CRLF2* overexpression (excluding those with *MLL, TCF3, TEL*, and *BCR/ABL* rearrangements).[37,40,43] Genetic aberrations of *CRLF2* seem to confer a poorer prognosis in most studies.[40,41,44,45] Fusion type may be associated with prognosis.[45] However, not all studies support a poorer prognosis associated with *CRLF2* aberration.[37,46]

Patients with *CRLF2* aberrations often have additional genetic abnormalities including deletion/mutation of *IKZF1* (encoding IKAROS) and mutation or translocation of *JAK2*. Of note, *CRLF2* aberrations can also be paired with concurrent mutations in *IL-7Rα* as well as *JAK1, JAK3*, or *SH2B3* (which encodes the JAK2-negative regulator LNK).[40,41,43] Patients with combined *CRLF2* and *IKZF1* aberrations often have gene expression profiles similar to patients with the *BCR-ABL* fusion protein, leading them to be considered "Philadelphia chromosome–like" (along with other genetic lesions inducing similar gene expression profiles). Patients with a Ph-like gene expression profile have a poor prognosis.[40,47] Mutation or translocation of *JAK2* also occurs independently of *CRLF2* mutations in BCP-ALL.[47-49]

Therapeutic targeting of IL7Rα signaling pathways

Receptor targeting

The mutant IL-7Rα homodimers found in T-ALL depend on formation of disulfide bonds for stability. These bonds could potentially be targeted therapeutically using the reducing agent *N*-acetylcysteine (NAC). In T-ALL cell lines, NAC disrupts homodimerization, and it slows progression of disease in a xenograft model. Although NAC is inexpensive, it requires frequent dosing.[50] In addition to using NAC, development of antibodies or T cells engineered to express chimeric antigen receptors (CAR-T-cells) against IL-7Rα and/ or against mutant homodimers would be potentially therapeutic.[6]

Although antibodies and CAR-T-cells against normal IL-7Rα might be expected to target normal T cells as well as leukemic cells, promising results have been shown with anti-CD19 CAR-T-cells in the treatment of B-ALL. An anti–IL-7Rα CAR-T-cell may have similar effects. Anti-CD19 CAR-T-cell therapy induces severe, temporary B-cell lymphopenia, but B-cell populations rebound after therapy. Similar rebound of T-cell populations might be expected after anti–IL-7Rα CAR-T-cell therapy, given the active thymic function in these patients. In anti-CD19 CAR-T-cell therapy, treatment induces minimal residual disease-negative complete response in 60% of relapsed and refractory B-ALL patients. Therapy serves as an effective bridge to hematopoietic stem cell transplantation, resulting in disease-free survival.[51] Perhaps anti–IL-7Rα CAR-T-cells or antibodies could also be used as a "bridge to transplant." In addition, development of a mutant-specific antibody or CAR-T-cell may reduce potential off-target effects on normal T cells.

For treating CRLF2 genetic aberrations in BCP-ALL, CAR-T-cells against TSLPR have been shown to be effective in treating multiple xenograft models of BCP-ALL.[52] In addition, antibodies against TSLPR have been developed, though these have mostly focused on applications in allergy/asthma to date.[53]

NOTCH1 targeting

In T-ALL, mutated NOTCH1 could potentially be targeted using γ-secretase inhibitors, but these have shown significant side effects in a T-ALL clinical trial (reviewed by Tosello and Ferrando[54]). Alternative approaches to targeting NOTCH1 include using antibodies and proteasome inhibitors.[54-56]

JAK/STAT targeting

Efforts investigating JAK inhibitors to treat neoplasia have focused mainly on the effects of blocking JAK2 in myeloproliferative neoplasms (MPN), polycythemia vera, and myelofibrosis (reviewed by Santos and Verstovsek[57]). In ALL, most studies of JAK inhibition have explored its effects on BCP-ALL. These studies have used primary patient samples or cell lines, including CRLF2-overexpressing cells and Ph-like cells with *IL7Rα*-activating mutation and *SH2B3* mutation. Results from these in vitro and in vivo experiments suggest JAK inhibition may have some efficacy against BCP-ALL.[38,40,43,48,58,59] A recent phase 1 clinical trial of the JAK1/2 inhibitor ruxolitinib suggested that the drug is well tolerated in children.[60] Fewer experimental studies have focused on JAK inhibition in experimental models of T-ALL and ETP-ALL, though results from these studies are promising.[61]

Experience with MPN suggests that resistance to JAK inhibition may develop, fueled by additional mutations in JAK proteins, increased JAK2 expression, or shifting transphosphorylation partners; resistance has been identified in both MPN and T-ALL.[62,63] There are several potential approaches to overcoming resistance to JAK inhibition. New generations of JAK inhibitors are being developed to bind inactive forms of JAK to overcome resistance.[64] Alternatively, JAK inhibitors may be combined with histone deacetylase (HDAC) inhibitors or heat shock protein 90 (HSP90) inhibitors.[65,66] HDAC inhibitors induce hyperacetylation of HSP90, block its chaperone function, and promote JAK2 degradation.[66] Inhibition of HSP90 increases degradation of both wild-type and mutant JAK2 and shows some efficacy against xenograft models of CRLF2-overexpressing BCP-ALL.[65] However, both HSP90 inhibitors and HDAC inhibitors have multiple side effects (reviewed by Hong et al[67]). Phase 1 clinical

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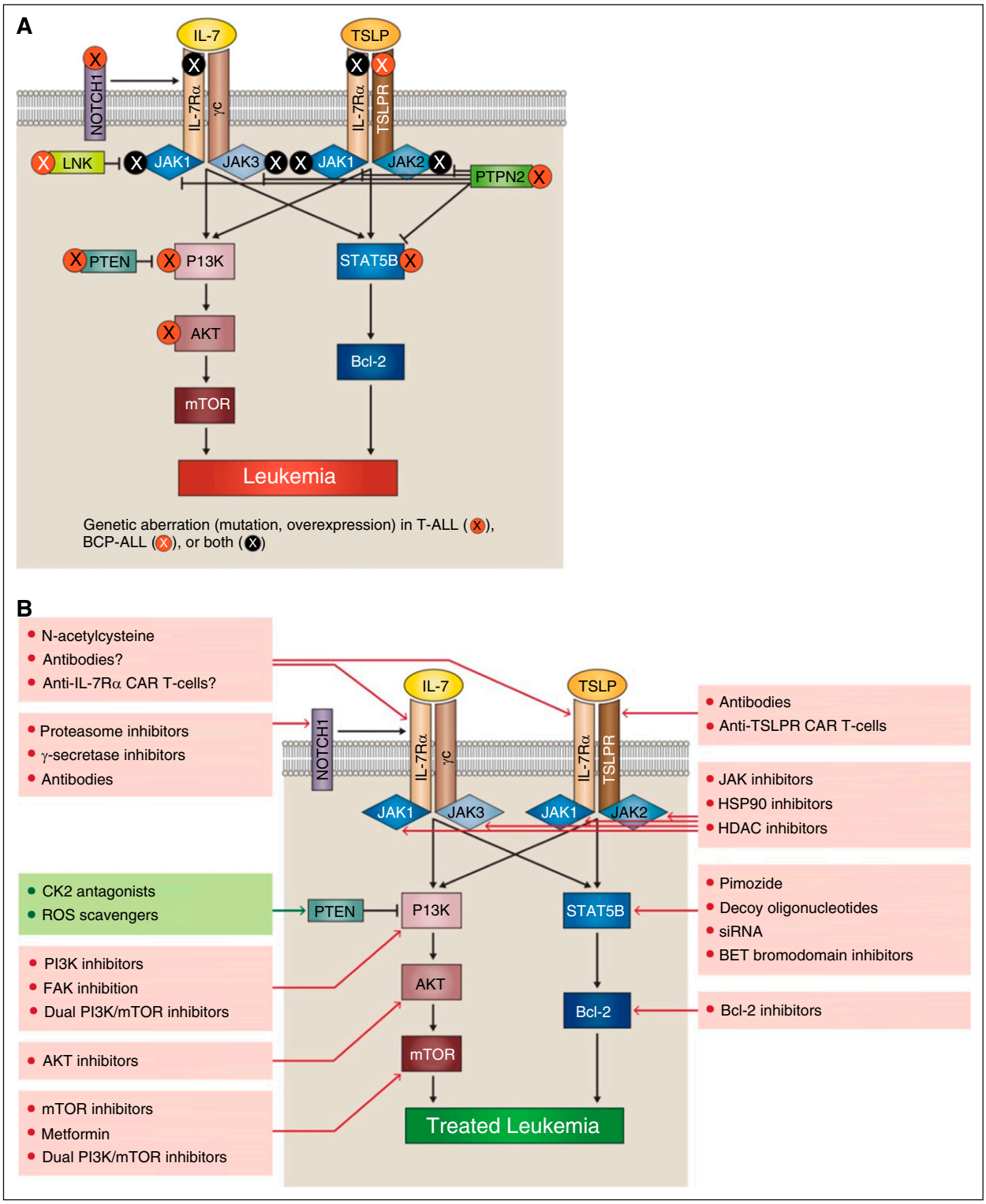

Figure 1. IL-7Rα pathway mutations provide potential targets for acute lymphoblastic leukemia therapy. In both T-ALL and BCP-ALL, mutations can occur at many points within the IL-7Rα signaling pathways (A). Aberrant signaling through these pathways offers multiple potential therapeutic targets (B).

trials of the HDAC inhibitor panobinostat included patients with myeloid and lymphoid malignancies, though none with ALL.[68]

STAT5B targeting is less advanced than JAK targeting (reviewed by Dorritie et al[69]). The drug pimozide targets STAT5B and induces apoptosis in cultures of chronic myelogenous leukemia cells. Alternative efforts to target STAT5B include the use of decoy oligonucleotides in chronic myeloid leukemia and potentially the use of siRNA.[70,71] In an experimental model of BCP-ALL, *STAT5B* gene expression was targeted by epigenetics-based therapy using BET bromodomain inhibitors. The study indicated that bromodomain inhibition induces apoptosis in vitro, improves survival in a xenograft model, and promotes downregulation of *IL-7Rα*.[72] In

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addition, use of a pan–BCL-2 inhibitor, navitoclax, has demonstrated efficacy against STAT5B-mutated cells in vitro.[27]

PI3K/AKT/mTOR targeting

The PI3K/AKT/mTOR pathway can be targeted by inhibitors of PI3K, AKT, and mTOR, dual inhibitors targeting both PI3K and mTOR, or inhibition of other components of the pathway including eukaryotic translation initiation factor 4E and phosphoinositide-dependent protein kinase I (reviewed by Rodon et al[73]).

Studies of PI3K and AKT inhibition have focused on effects in experimental models of T-ALL. PI3K inhibitor NVP-BKM120 induces apoptosis in cell lines and primary T-ALL cells and synergizes with vincristine and doxorubicin in vitro and in vivo.[74] Conversely, although the PI3K inhibitor AS605240 synergizes with glucocorticoids, it antagonizes methotrexate and daunorubicin activity in cell lines, primary T-ALL cells, and murine xenograft models.[75] Dual PI3K and mTOR inhibitors are also effective against T-ALL cell lines and patient samples in vitro and are more effective than individual inhibition of PI3K, AKT, or mTOR.[76] Dual inhibitor BEZ235 synergizes with dexamethasone in vitro and in vivo, suggesting that targeting the PI3K/AKT/mTOR pathway could modulate glucocorticoid resistance in T-ALL.[77] However, dual inhibitor PI-103 has been shown to cause upregulation of NOTCH1 and c-myc; combining PI-103 with c-myc or short-term NOTCH1 inhibition is synergistic and leads to increased death of T-ALL cell lines.[78] In addition, inhibition of the PI3K pathway can lead to activation of the FAK/NF-κB/Bcl-2 pathway, suggesting that inhibition of PI3K may be paired with FAK inhibition for greater efficacy.[79]

AKT inhibition by GSK690693 is effective against both T-ALL and BCP-ALL cell lines in vitro.[80] AKT inhibitor MK-2206 decreases T-ALL cell viability and induces apoptosis in leukemia-initiating cell populations.[81] Combining 3 AKT inhibitors with different mechanisms of action leads to synergistic effects against T-ALL cell lines.[82] AKT inhibition can also lead to reversal of T-ALL glucocorticoid resistance, as demonstrated in cell lines and primografts in vitro as well as in several mouse models.[83]

Inhibition of mTOR has included studies in both T-ALL and BCP-ALL. Rapamycin, the first mTOR inhibitor, is an allosteric inhibitor that does not fully inhibit the mTORC1 and mTORC2 enzymes.[84] Rapamycin shows efficacy against xenografted BCP-ALL with mutated *IL-7Rα* and altered *CRLF2* expression, as well as Ph-like BCP-ALL and a transgenic model of BCP-ALL.[58,85] However, treatment with rapamycin alone is not considered highly effective against T-ALL.[76]

To improve on the efficacy of rapamycin, several other drug types have been developed to target mTOR. Drugs targeting the active site of mTOR are effective against both T-ALL and BCP-ALL cell lines.[86,87] More specific targeting of mTORC1 also induces apoptosis and autophagy in BCP-ALL cell lines and synergizes with AKT inhibitor MK-2206.[87,88] Alternatively, mTORC1 activity can be targeted with metformin, an activator of LKB1/AMPK, which down-modulates mTORC1 activity. Metformin is effective in vitro against T-ALL cell lines, primary cells, and leukemia-initiating cells. There is currently a clinical trial recruiting pediatric patients with relapsed ALL for treatment with a combination of metformin and chemotherapy (NCT01324180).[89] In both T-ALL and BCP-ALL cell lines, inhibition of mTOR synergizes with methotrexate and vincristine, potentially targeting leukemia-initiating cells.[86,90] In addition, inhibition of mTOR may synergize with NOTCH1 or Bcl-2 inhibition, as demonstrated in murine xenografts and T-ALL cell lines and primary samples, respectively.[91,92] Recently, inhibition of the PI3K/AKT/mTOR pathway has been shown to synergize with MEK inhibition in human T-ALL primary cells and BaF3 cells transduced with mutated IL-7Rα signaling pathway members.[93]

In those T-ALL cases where PTEN inhibition by CK2 or ROS leads to activation of the PI3K/AKT/mTOR pathway, antagonists of CK2 or ROS scavengers may help to increase PTEN activity, as suggested by results in T-ALL cell lines and primary cells.[32,94]

Conclusion

In the search for targeted ALL therapies, genetic aberrations in the IL-7Rα signaling pathways offer many potential opportunities for therapeutic intervention (Figure 1). Because drugs targeting IL-7Rα signaling pathways have the potential to reduce cellular proliferation and survival, we believe these drugs could currently be used as adjunctive therapies for many leukemia patients, even those without genetic aberrations involving the IL-7Rα signaling pathways. However, the real promise of these therapies lies in the (it is hoped) near future, when each new case of pediatric leukemia will be sequenced. This will enable clinicians to target an individual leukemia's specific genetic lesions with specific IL-7Rα pathway–directed therapies. The appropriate therapy or therapies will vary depending on the location of the genetic lesion within the signaling pathway. For example, a BCP-ALL patient with overexpressed *CRLF2* might be treated by targeting multiple levels of the signaling pathway, including use of anti-TSLPR CAR-T-cells, monoclonal antibodies, JAK inhibitors, and/or dual PI3K/mTOR inhibitors. A patient with AKT mutation might benefit from AKT or mTOR inhibition. As we move forward, we will need to determine the best use of these therapies to minimize side effects and maximize patient benefit. Perhaps someday, targeted therapy will enable patients with IL-7Rα signaling pathway mutations to be treated with less intensive chemotherapy, and relapsed patients or those patients whom current therapies fail will be successfully treated.

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Authorship

Contribution: S.D.C. researched and wrote the initial manuscript; and P.D.A. and S.K.D. contributed expertise and guidance and edited the manuscript.

Conflict-of-interest disclosure: S.K.D. has applied for a patent titled "IL-7Rα specific antibodies for treating acute lymphoblastic leukemia" (US patent application #62/238 612). The remaining authors declare no competing financial interests.

Correspondence: Scott K. Durum, Center for Cancer Research, National Cancer Institute, Building 560, Room 31-71, Frederick, MD 21702-1201; e-mail: durums@mail.nih.gov.

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References

1. Gutierrez A, Silverman L. Acute lymphoblastic leukemia. In: Orkin S, Fisher D, Ginsburg D, Look A, Lux S, Nathan D, eds. Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Vol. 2. Philadelphia: Elsevier Saunders; 2015:1527-1554.
2. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med.* 2009;360(26): 2730-2741.
3. Nguyen K, Devidas M, Cheng SC, et al; Children's Oncology Group. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. *Leukemia.* 2008;22(12):2142-2150.
4. Freyer DR, Devidas M, La M, et al. Postrelapse survival in childhood acute lymphoblastic leukemia is independent of initial treatment intensity: a report from the Children's Oncology Group. *Blood.* 2011;117(11):3010-3015.
5. Mullighan CG, Hunger SP. Therapy of pediatric ALL: from Bowie to Obama. *Blood.* 2013;122(15): 2531-2532.
6. Tal N, Shochat C, Geron I, Bercovich D, Izraeli S. Interleukin 7 and thymic stromal lymphopoietin: from immunity to leukemia. *Cell Mol Life Sci.* 2014;71(3):365-378.
7. Mazzucchelli RI, Riva A, Durum SK. The human IL-7 receptor gene: deletions, polymorphisms and mutations. *Semin Immunol.* 2012;24(3):225-230.
8. Montecino-Rodriguez E, Leathers H, Dorshkind K. Identification of a B-1 B cell-specified progenitor. *Nat Immunol.* 2006;7(3):293-301.
9. Liu YJ, Soumelis V, Watanabe N, et al. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu Rev Immunol.* 2007;25:193-219.
10. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol.* 2010;11(4):289-293.
11. van Bodegom D, Zhong J, Kopp N, et al. Differences in signaling through the B-cell leukemia oncoprotein CRLF2 in response to TSLP and through mutant JAK2. *Blood.* 2012; 120(14):2853-2863.
12. Barata JT, Silva A, Brandao JG, Nadler LM, Cardoso AA, Boussiotis VA. Activation of PI3K is indispensable for interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. *J Exp Med.* 2004;200(5):659-669.
13. Johnson SE, Shah N, Bajer AA, LeBien TW. IL-7 activates the phosphatidylinositol 3-kinase/AKT pathway in normal human thymocytes but not normal human B cell precursors. *J Immunol.* 2008;180(12):8109-8117.
14. Crawley JB, Willcocks J, Foxwell BMJ. Interleukin-7 induces T cell proliferation in the absence of Erk/MAP kinase activity. *Eur J Immunol.* 1996;26(11):2717-2723.
15. Shochat C, Tal N, Bandapalli OR, et al. Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. *J Exp Med.* 2011;208(5):901-908.
16. Zenatti PP, Ribeiro D, Li W, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. *Nat Genet.* 2011; 43(10):932-939.
17. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature.* 2012;481(7380):157-163.
18. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004; 306(5694):269-271.
19. González-García S, García-Peydró M, Martín-Gayo E, et al. CSL-MAML-dependent Notch1 signaling controls T lineage-specific IL-7Ralpha gene expression in early human thymopoiesis and leukemia. *J Exp Med.* 2009;206(4):779-791.
20. Bains T, Heinrich MC, Loriaux MM, et al. Newly described activating JAK3 mutations in T-cell acute lymphoblastic leukemia. *Leukemia.* 2012; 26(9):2144-2146.
21. Lacronique V, Boureux A, Valle VD, et al. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science.* 1997; 278(5341):1309-1312.
22. Flex E, Petrangeli V, Stella L, et al. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. *J Exp Med.* 2008;205(4): 751-758.
23. Kalender Atak Z, De Keersmaecker K, Gianfelici V, et al. High accuracy mutation detection in leukemia on a selected panel of cancer genes. *PLoS One.* 2012;7(6):e38463.
24. Jeong EG, Kim MS, Nam HK, et al. Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. *Clin Cancer Res.* 2008;14(12): 3716-3721.
25. Asnafi V, Le Noir S, Lhermitte L, et al. JAK1 mutations are not frequent events in adult T-ALL: a GRAALL study. *Br J Haematol.* 2010;148(1): 178-179.
26. Canté-Barrett K, Uitdehaag JC, Meijerink JP. Structural modeling of JAK1 mutations in T-cell acute lymphoblastic leukemia reveals a second contact site between pseudokinase and kinase domains. *Haematologica.* 2016;101(5): e189-e191.
27. Kontro M, Kuusanmäki H, Eldfors S, et al. Novel activating STAT5B mutations as putative drivers of T-cell acute lymphoblastic leukemia. *Leukemia.* 2014;28(8):1738-1742.
28. Bandapalli OR, Schuessele S, Kunz JB, et al. The activating STAT5B N642H mutation is a common abnormality in pediatric T-cell acute lymphoblastic leukemia and confers a higher risk of relapse. *Haematologica.* 2014;99(10):e188-e192.
29. Kleppe M, Lahortiga I, El Chaar T, et al. Deletion of the protein tyrosine phosphatase gene PTPN2 in T-cell acute lymphoblastic leukemia. *Nat Genet.* 2010;42(6):530-535.
30. Gutierrez A, Sanda T, Grebliunaite R, et al. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood.* 2009;114(3):647-650.
31. Remke M, Pfister S, Kox C, et al. High-resolution genomic profiling of childhood T-ALL reveals frequent copy-number alterations affecting the TGF-beta and PI3K-AKT pathways and deletions at 6q15-16.1 as a genomic marker for unfavorable early treatment response. *Blood.* 2009;114(5): 1053-1062.
32. Silva A, Yunes JA, Cardoso BA, et al. PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest.* 2008;118(11): 3762-3774.
33. Mavrakis KJ, Van Der Meulen J, Wolfe AL, et al. A cooperative microRNA-tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL). *Nat Genet.* 2011;43(7):673-678.
34. Barata JT, Cardoso AA, Nadler LM, Boussiotis VA. Interleukin-7 promotes survival and cell cycle progression of T-cell acute lymphoblastic leukemia cells by down-regulating the cyclin-dependent kinase inhibitor p27(kip1). *Blood.* 2001;98(5):1524-1531.
35. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet.* 2009; 41(11):1243-1246.
36. Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood.* 2009;114(13):2688-2698.
37. Yano M, Imamura T, Asai D, et al; Japan Association of Childhood Leukemia Study. An overall characterization of pediatric acute lymphoblastic leukemia with CRLF2 overexpression. *Genes Chromosomes Cancer.* 2014;53(10):815-823.
38. Tasian SK, Doral MY, Borowitz MJ, et al. Aberrant STAT5 and PI3K/mTOR pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic leukemia. *Blood.* 2012; 120(4):833-842.
39. Chapiro E, Russell L, Lainey E, et al. Activating mutation in the TSLPR gene in B-cell precursor lymphoblastic leukemia. *Leukemia.* 2010;24(3): 642-645.
40. Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. *Proc Natl Acad Sci USA.* 2010;107(1):252-257.
41. Chen IM, Harvey RC, Mullighan CG, et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children's Oncology Group study. *Blood.* 2012;119(15):3512-3522.
42. Bugarin C, Sarno J, Palmi C, et al; I-BFM study group. Fine tuning of surface CRLF2 expression and its associated signaling profile in childhood B-cell precursor acute lymphoblastic leukemia. *Haematologica.* 2015;100(6):e229-e232.
43. Roberts KG, Morin RD, Zhang J, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell.* 2012;22(2):153-166.
44. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood.* 2010;115(26):5312-5321.
45. Palmi C, Vendramini E, Silvestri D, et al. Poor prognosis for P2RY8-CRLF2 fusion but not for CRLF2 over-expression in children with intermediate risk B-cell precursor acute lymphoblastic leukemia. *Leukemia.* 2012;26(10): 2245-2253.
46. van der Veer A, Waanders E, Pieters R, et al. Independent prognostic value of BCR-ABL1-like signature and IKZF1 deletion, but not high CRLF2 expression, in children with B-cell precursor ALL. *Blood.* 2013;122(15):2622-2629.
47. Mullighan CG, Zhang J, Harvey RC, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci USA.* 2009;106(23):9414-9418.
48. Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. *Lancet.* 2008;372(9648):1484-1492.
49. Yano M, Imamura T, Asai D, et al. Identification of novel kinase fusion transcripts in paediatric B cell precursor acute lymphoblastic leukaemia with IKZF1 deletion. *Br J Haematol.* 2015;171(5): 813-817.
50. Mansour MR, Reed C, Eisenberg AR, et al. Targeting oncogenic interleukin-7 receptor signalling with N-acetylcysteine in T cell acute lymphoblastic leukaemia. *Br J Haematol.* 2015; 168(2):230-238.

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51. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet.* 2015;385(9967):517-528.

52. Qin H, Cho M, Haso W, et al. Eradication of B-ALL using chimeric antigen receptor-expressing T cells targeting the TSLPR oncoprotein. *Blood.* 2015;126(5):629-639.

53. Borowski A, Vetter T, Kuepper M, et al. Expression analysis and specific blockade of the receptor for human thymic stromal lymphopoietin (TSLP) by novel antibodies to the human TSLPRα receptor chain. *Cytokine.* 2013;61(2):546-555.

54. Tosello V, Ferrando AA. The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. *Ther Adv Hematol.* 2013;4(3):199-210.

55. Agnusdei V, Minuzzo S, Frasson C, et al. Therapeutic antibody targeting of Notch1 in T-acute lymphoblastic leukemia xenografts. *Leukemia.* 2014;28(2):278-288.

56. Koyama D, Kikuchi J, Hiraoka N, et al. Proteasome inhibitors exert cytotoxicity and increase chemosensitivity via transcriptional repression of Notch1 in T-cell acute lymphoblastic leukemia. *Leukemia.* 2014;28(6):1216-1226.

57. Santos FP, Verstovsek S. JAK2 inhibitors: what's the true therapeutic potential? *Blood Rev.* 2011; 25(2):53-63.

58. Maude SL, Tasian SK, Vincent T, et al. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood.* 2012;120(17):3510-3518.

59. Treanor LM, Zhou S, Janke L, et al. Interleukin-7 receptor mutants initiate early T cell precursor leukemia in murine thymocyte progenitors with multipotent potential. *J Exp Med.* 2014;211(4): 701-713.

60. Loh ML, Tasian SK, Rabin KR, et al. A phase 1 dosing study of ruxolitinib in children with relapsed or refractory solid tumors, leukemias, or myeloproliferative neoplasms: A Children's Oncology Group phase 1 consortium study (ADVL1011). *Pediatr Blood Cancer.* 2015;62(10): 1717-1724.

61. Maude SL, Dolai S, Delgado-Martin C, et al. Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. *Blood.* 2015; 125(11):1759-1767.

62. Koppikar P, Bhagwat N, Kilpivaara O, et al. Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. *Nature.* 2012;489(7414):155-159.

63. Springuel L, Hornakova T, Losdyck E, et al. Cooperating JAK1 and JAK3 mutants increase resistance to JAK inhibitors. *Blood.* 2014;124(26): 3924-3931.

64. Meyer SC, Levine RL. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. *Clin Cancer Res.* 2014; 20(8):2051-2059.

65. Weigert O, Lane AA, Bird L, et al. Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. *J Exp Med.* 2012;209(2):259-273.

66. Wang Y, Fiskus W, Chong DG, et al. Cotreatment with panobinostat and JAK2 inhibitor TG101209 attenuates JAK2V617F levels and signaling and exerts synergistic cytotoxic effects against human myeloproliferative neoplastic cells. *Blood.* 2009; 114(24):5024-5033.

67. Hong DS, Banerji U, Tavana B, George GC, Aaron J, Kurzrock R. Targeting the molecular chaperone heat shock protein 90 (HSP90): lessons learned and future directions. *Cancer Treat Rev.* 2013;39(4):375-387.

68. DeAngelo DJ, Spencer A, Bhalla KN, et al. Phase Ia/II, two-arm, open-label, dose-escalation study of oral panobinostat administered via two dosing schedules in patients with advanced hematologic malignancies. *Leukemia.* 2013;27(8):1628-1636.

69. Dorritie KA, McCubrey JA, Johnson DE. STAT transcription factors in hematopoiesis and leukemogenesis: opportunities for therapeutic intervention. *Leukemia.* 2014;28(2):248-257.

70. Wang X, Zeng J, Shi M, et al. Targeted blockage of signal transducer and activator of transcription 5 signaling pathway with decoy oligodeoxynucleotides suppresses leukemic K562 cell growth. *DNA Cell Biol.* 2011;30(2):71-78.

71. Zhang Q, Hossain DM, Nechaev S, et al. TLR9-mediated siRNA delivery for targeting of normal and malignant human hematopoietic cells in vivo. *Blood.* 2013;121(8):1304-1315.

72. Ott CJ, Kopp N, Bird L, et al. BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. *Blood.* 2012; 120(14):2843-2852.

73. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol.* 2013; 10(3):143-153.

74. Lonetti A, Antunes IL, Chiarini F, et al. Activity of the pan-class I phosphoinositide 3-kinase inhibitor NVP-BKM120 in T-cell acute lymphoblastic leukemia. *Leukemia.* 2014;28(6):1196-1206.

75. Silveira AB, Laranjeira AB, Rodrigues GO, et al. PI3K inhibition synergizes with glucocorticoids but antagonizes with methotrexate in T-cell acute lymphoblastic leukemia. *Oncotarget.* 2015;6(15): 13105-13118.

76. Chiarini F, Grimaldi C, Ricci F, et al. Activity of the novel dual phosphatidylinositol 3-kinase/ mammalian target of rapamycin inhibitor NVP-BEZ235 against T-cell acute lymphoblastic leukemia. *Cancer Res.* 2010;70(20):8097-8107.

77. Hall CP, Reynolds CP, Kang MH. Modulation of glucocorticoid resistance in pediatric T-cell acute lymphoblastic leukemia by increasing BIM expression with the PI3K/mTOR inhibitor BEZ235. *Clin Cancer Res.* 2016;22(3):621-632.

78. Shepherd C, Banerjee L, Cheung CW, et al. PI3K/mTOR inhibition upregulates NOTCH-MYC signalling leading to an impaired cytotoxic response. *Leukemia.* 2013;27(3):650-660.

79. You D, Xin J, Volk A, et al. FAK mediates a compensatory survival signal parallel to PI3K-AKT in PTEN-null T-ALL cells. *Cell Reports.* 2015; 10(12):2055-2068.

80. Levy DS, Kahana JA, Kumar R. AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines. *Blood.* 2009;113(8):1723-1729.

81. Simioni C, Neri LM, Tabellini G, et al. Cytotoxic activity of the novel Akt inhibitor, MK-2206, in T-cell acute lymphoblastic leukemia. *Leukemia.* 2012;26(11):2336-2342.

82. Cani A, Simioni C, Martelli AM, et al. Triple Akt inhibition as a new therapeutic strategy in T-cell acute lymphoblastic leukemia. *Oncotarget.* 2015; 6(9):6597-6610.

83. Piovan E, Yu J, Tosello V, et al. Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. *Cancer Cell.* 2013; 24(6):766-776.

84. Janes MR, Vu C, Mallya S, et al. Efficacy of the investigational mTOR kinase inhibitor MLN0128/ INK128 in models of B-cell acute lymphoblastic leukemia. *Leukemia.* 2013;27(3):586-594.

85. Brown VI, Fang J, Alcorn K, et al. Rapamycin is active against B-precursor leukemia in vitro and in vivo, an effect that is modulated by IL-7-mediated signaling. *Proc Natl Acad Sci USA.* 2003;100(25): 15113-15118.

86. Evangelisti C, Ricci F, Tazzari P, et al. Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. *Leukemia.* 2011; 25(5):781-791.

87. Simioni C, Cani A, Martelli AM, et al. Activity of the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic leukemia and its therapeutic potential to prevent Akt reactivation. *Oncotarget.* 2014;5(20):10034-10047.

88. Neri LM, Cani A, Martelli AM, et al. Targeting the PI3K/Akt/mTOR signaling pathway in B-precursor acute lymphoblastic leukemia and its therapeutic potential. *Leukemia.* 2014;28(4):739-748.

89. Grimaldi C, Chiarini F, Tabellini G, et al. AMP-dependent kinase/mammalian target of rapamycin complex 1 signaling in T-cell acute lymphoblastic leukemia: therapeutic implications. *Leukemia.* 2012;26(1):91-100.

90. Teachey DT, Sheen C, Hall J, et al. mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. *Blood.* 2008;112(5):2020-2023.

91. Cullion K, Draheim KM, Hermance N, et al. Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. *Blood.* 2009;113(24): 6172-6181.

92. Iacovelli S, Ricciardi MR, Allegretti M, et al. Co-targeting of Bcl-2 and mTOR pathway triggers synergistic apoptosis in BH3 mimetics resistant acute lymphoblastic leukemia. *Oncotarget.* 2015; 6(31):32089-32103.

93. Canté-Barrett K, Spijkers-Hagelstein JA, Buijs-Gladdines JG, et al. MEK and PI3K-AKT inhibitors synergistically block activated IL7 receptor signaling in T-cell acute lymphoblastic leukemia. *Leukemia.* 2016;May 13.

94. Buontempo F, Orsini E, Martins LR, et al. Cytotoxic activity of the casein kinase 2 inhibitor CX-4945 against T-cell acute lymphoblastic leukemia: targeting the unfolded protein response signaling. *Leukemia.* 2014;28(3):543-553.

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