The clonal evolution of leukemic stem cells in T-cell acute lymphoblastic leukemia

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Purpose of review
Recent genome sequencing studies have identified a broad spectrum of gene mutations in T-cell acute lymphoblastic leukemia (T-ALL). The purpose of this review is to outline the latest advances in our understanding of how these mutations contribute to the formation of T-ALL.

Recent findings
Aberrant expression of transcription factors that control hematopoiesis can induce an aberrant stem cell-like program in T-cell progenitors, allowing the emergence of an ancestral or preleukemic stem cell (pre-LSC). In contrast, gain-of-function mutations of genes involved in signaling pathways regulating T-cell development, such as NOTCH1, interleukin-7, KIT and FLT3, are insufficient *per se* to initiate T-ALL but promote pre-LSC growth independent of the thymic niche. Loss-of-function mutations of epigenetic regulators, such as DNMT3A, have been identified in T-ALL, but their role in leukemogenesis remains to be defined.

Summary
Relapse is associated with clonal evolution from a population of pre-LSCs that acquire the whole set of malignant mutations leading to a full-blown T-ALL. Understanding the genetic events that underpin the pre-LSC will be crucial for reducing the risk of relapse.

Keywords
cell of origin, clonal evolution, leukemic stem cell, T-cell acute lymphoblastic leukemia

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) is a genetically heterogeneous cancer, with 20% of childhood patients and the majority of adult patients dying from resistant or relapsed disease [1]. The prognosis is particularly bleak for patients with early thymocyte progenitor (ETP)-ALL, which is characterized by a stem cell-like phenotype with distinct genetic mutations involving transcription factors, epigenetic regulators and signaling pathways, important for T-cell development [2,3]. Recent sequencing studies of T-ALL have confirmed the presence of these mutations as well as novel recurrent mutations in the tumor suppressor CNOT3, ribosomal proteins (RPL5 and RPL10) and in the setting of relapse, the NT5C2 gene, which inactivates nucleoside-analogue chemotherapy drugs [4,5]. One interesting observation is that the number and type of mutations is age dependent. Mutations of genes involved in hematopoietic stem cell (HSC) development, such as *SCL* and *MYB* [6], and ribosomal function, such as *RPL5* and *RPL10* [4], are almost exclusively present in childhood T-ALL. In contrast, adult T-ALL have on average twice as many mutations, with those involving *TLX1, FBXW7, CNOT3* and *PHF6* occurring almost exclusively in older patients [4]. The difference in mutational profile may reflect differences in cell of origin: prenatal versus natal and T-cell progenitor versus HSC.

An important study by the Ferrando group, which sequenced a small number of matched diagnostic and relapsed T-ALL samples, elegantly supports the data for B-cell ALL [7] wherein the clone responsible for relapse frequently arises from an ancestral or preleukemic stem cell (pre-LSC) that harbors some but not all of the mutations in the diagnostic clone [5]. Further studies in T-ALL are

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KEY POINTS

- Pre-LSCs are ancestral cells within the leukemia responsible for relapse.
- Mutations of transcription factors can generate pre-LSCs.
- Activating mutations of signaling pathways important for T-cell development allow pre-LSCs to expand and escape the thymic niche.
- Mutations of epigenetic regulators are commonly found in T-ALL but how they contribute to leukemogenesis remains to be defined.

required to characterize clonal evolution, but the scenario is likely to be similar to human acute myeloid leukemia, wherein the existence of pre-LSCs with mutations of epigenetic regulators, such as DNMT3A, have been demonstrated by deep-targeted sequencing of diagnostic, remission and relapsed samples [8,9▪▪]. Clonal diversity appears to be recapitulated in xenografts of T-ALL in immunodeficient mice, suggesting that mouse models may be an alternate method to understand the drivers of pre-LSCs and determine whether targeting these cells can prevent relapse [10]. For the purpose of this review, we functionally define the pre-LSC as a cell containing the initiating genetic event that confers self-renewal capacity, such that it can propagate leukemia in immunodeficient mice after many months. In contrast, leukemic stem cells (LSCs) are derived from pre-LSCs after acquiring additional genetic events that allow them to rapidly (weeks) generate leukemia in immunodeficient mice (Fig. 1). Now that the recurrent genetic mutations of T-ALL have been largely identified, a major question is how these mutations contribute to T-ALL: specifically which ones can initiate disease and in which cell type, HSC or T-cell progenitor. A better understanding of the role of these mutations is crucial for designing new therapeutic approaches to successfully target the pre-LSC for curing T-ALL.

CREATING PRELEUKEMIC STEM CELLS BY THE BASIC HELIX–LOOP–HELIX TRANSCRIPTION FACTOR COMPLEX

Recent transcript and genome sequencing studies have identified known and a number of new chromosomal rearrangements in T-ALL [11]. These rearrangements lead to aberrant T-cell expression of a broad range of transcription factors, including the basic helix–loop–helix (bHLH) factors *SCL/TAL1, LYL1* and *MYC*; LIM-only domain cofactors, such as *LMO1* and *LMO2*; the homeobox genes *TLX1/HOX11, TLX3/HOX11L2, NKX2.1, NKX2.2, NKX2.5* and *HOXA*; and *MYB*, *TAN1* and MEF2C [1,3,12]. Gene expression profiling shows that these oncogenes are associated with distinct T-ALL subgroups with the poor prognosis, immature T-ALL subgroup defined by high expression of LMO2, LYL1 and rearrangements of MEF2C [11]. It remains unclear why different transcription factors are implicated in distinct subgroups of T-ALL although it may relate to the origin of the malignancy as elegantly demonstrated by the use of a mouse model that utilized stage-specific transposon mutagenesis [13].

Many of these oncogenes are aberrantly activated in T-cell development by juxtaposition near cis-regulatory elements of T-cell receptor genes or by removal of negative regulatory elements from the oncogene promoter [14]. A recent comprehensive analysis of breakpoint sites suggests that most occur by erroneous repair of double-strand breaks rather than mistargeting of the V(D)J recombination machinery [15]. The LMO2 locus is also a hot-spot for γ-retroviral vector insertion, most recently reported in gene therapy trials for Wiskott–Aldrich syndrome [16].

Direct experimental evidence that these transcription factors can initiate T-ALL is relatively limited. To our knowledge, the only transcription factors shown to be capable of establishing pre-LSCs from T-cell progenitors are those forming the bHLH complex (SCL/TAL1, LYL1, LMO1 and LMO2). Using transgenic mouse models with T-cell specific promoters, enforced expression of these factors confers aberrant self-renewal of T-cells, which establishes a pool of cells that can acquire the additional gene events required for progression to overt T-ALL [17–19].

Several potential targets of the bHLH complex that might contribute to its ability to induce a self-renewal program have been proposed in recent studies. Using a new *LMO2* transgenic mouse model, Smith *et al.* [20] demonstrated that one important transcriptional target for LMO2 is the homeobox gene *HHEX*. In this study, conditional inactivation of Hhex markedly attenuated the development of T-ALL. Chromatin immunoprecipitation of the SCL/TAL1 complex in T-ALL cells has identified a core regulatory transcriptional complex that includes not only LMO1/2 but also GATA3 and RUNX1 [21▪]. This feed-forward regulatory loop recapitulates a similar scenario observed in normal HSCs [22]. Interestingly, mutations of both GATA3 and RUNX1 have been identified in T-ALL, but how these affect the function of the SCL/TAL1 regulatory complex is unknown. Finally, microRNAs may be

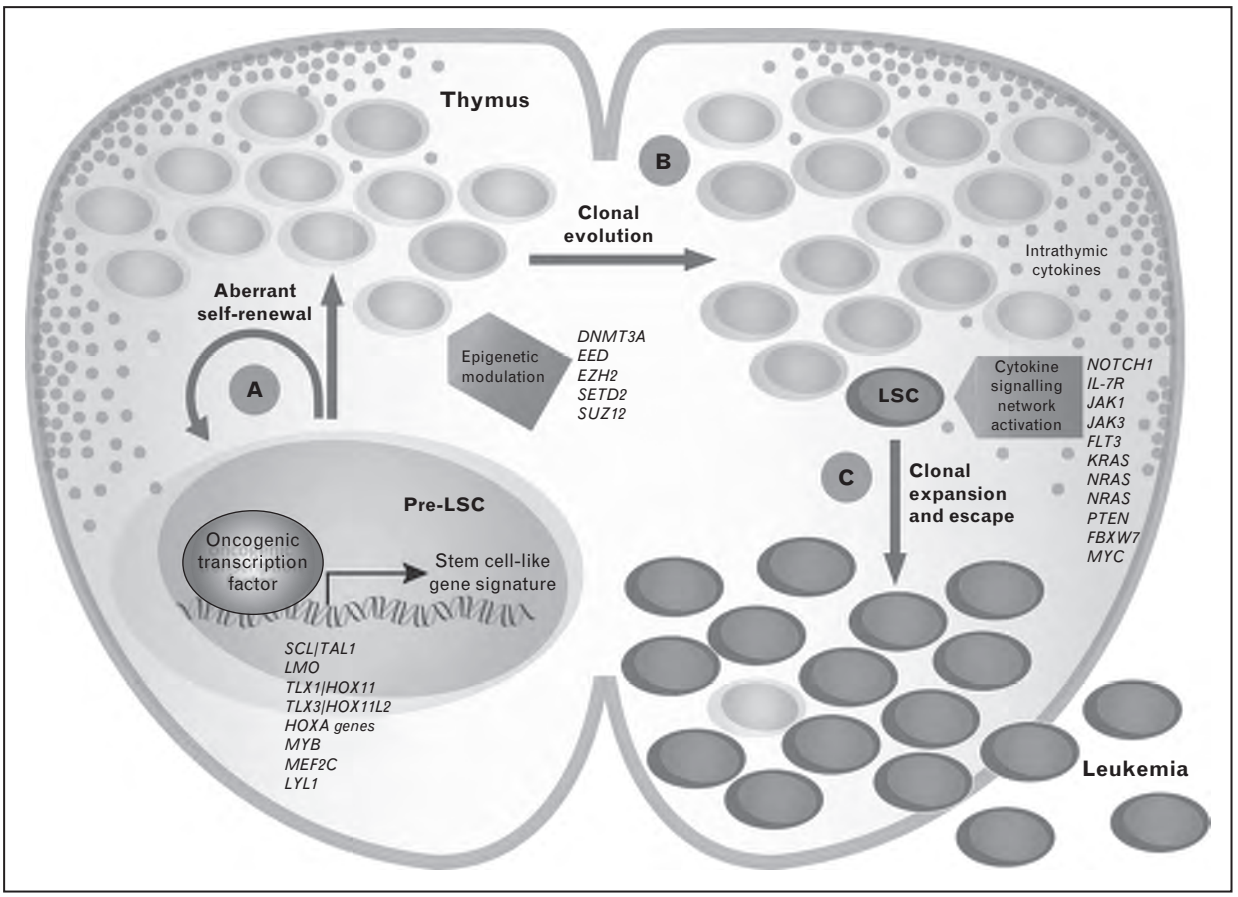

FIGURE 1. A hierarchical model of T-ALL proposing the contribution of different mutations to the leukemogenic process. (A) Genetic lesions inducing the ectopic expression of transcription factors induce the expression of a stem cell-like gene signature in the immature T-cell progenitors to generate the preleukemic stem cells (pre-LSCs). (B) Self-renewal of pre-LSCs allows acquisition of mutations in epigenetic regulators to form leukemic stem cells (LSCs). (C) The acquisition of activating mutations in cytokine signaling pathways promotes clonal expansion of LSCs, which can now escape from the thymic niche to generate overt T-ALL. T-ALL, T-cell acute lymphoblastic leukemia.

important targets of the bHLH complex that could initiate a self-renewal program [23▪,24]. For example, SCL/TAL1 could promote expression of miR-223, which can target the E3 ubiquitin ligase FBXW7, thereby reducing proteosomal degradation of a host of known T-ALL oncogenes, including NOTCH1, MYB, MYC and CYCLIN E [23▪]. Consistent with the role of these master regulators to induce a stem cell identity, the LMO2 complex has been shown to bind the *miR-223* promoter in normal human $CD34^{+}$ stem cells [25].

ROLE OF OTHER TRANSCRIPTION FACTORS

Much less is known about how aberrant expression of transcription factors other than the bHLH complex initiate T-ALL. In part, this lack of understanding reflects the limited number of T-cell-specific models of these oncogenic factors. Transgenic models of transcription factors that induce a more mature T-ALL, such as TLX1 driven off the *LCK* promoter, have been recently reported [26]. Here, mice develop an aneuploid and mature T-ALL akin to the human TLX subtype, but definitive thymocyte transplant experiments from young mice have not been performed to determine whether TLX1 can directly induce self-renewal of T-cell progenitors. In the case of c-Myc, Loosveld *et al.* [27] recently showed that increased expression of c-Myc in T-cell progenitors following a sporadic V(D)J rearrangement was insufficient to initiate leukemia in a transgenic mouse .

ROLE OF EPIGENETIC REGULATORS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Whole-exome sequencing studies in T-ALL have recently identified recurrent loss-of-function mutations in DNA (cytosine-5)-methyltransferase

3A (*DNMT3A*) and genes coding for members of the Polycomb Repressive Complex 2 (PRC2) [1,28]. Methylation of cytosine at the 5-position (5mC) in CpG islands by DNMT3A is associated with transcriptional silencing [29]. Conditional knockout studies in normal HSCs suggest that DNMT3A is required for differentiation by silencing stem cell genes [30]. Consistent with a role for DNMT3A in silencing the stem cell program, *DNMT3A* mutation is associated with improved HSC function, a differentiation block and hypomethylation of genomic regions containing genes frequently overexpressed in leukemias [30,31]. Recent clonal evolution studies found that *DNMT3A* loss of function arises early in leukemogenesis [9▪▪,32–35]. Although speculative, it is possible that loss-of-function mutations of *DNMT3A* in immature thymocytes may be sufficient to generate pre-LSCs. Alternatively, long-lived HSCs with *DNMT3A* mutations might give rise to T-ALL if they acquire a second mutation that favors T-cell leukemia.

Homozygous or heterozygous loss-of-function mutations of EZH2 and SUZ12, components of PRC2, are observed in 25% of all T-ALL [36▪▪]. The PRC2 complex is the writer of the predominant repressive histone modification H3K27me3. Consistent with a tumor suppressor role, conditional deletion of *EZH2* in HSCs using the inducible *MxCre* transgene was sufficient to induce T-ALL development [37]. However, unlike DNMT3A, loss of PRC2 function leads to loss of HSC activity [38,39]. Therefore, it seems unlikely that mutations of PRC2 complex are capable of inducing self-renewal of T-cells. Mutations of the PRC2 complex frequently cooccur with activating mutations of Notch1, suggesting they cooperate. Indeed, Notch binding in T-ALL overlaps most closely with PRC2 binding sites, suggesting that loss of PRC2 repressor function in T-ALL reinforces the gene activation by Notch1 [36▪▪,40]. Accordingly, T-ALL with mutations of Notch and PRC2 may be responsive to inhibitors of H3K27 demethylases.

SIGNALING PATHWAYS NETWORK INVOLVED IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PROGRESSION

T-cell development is tightly regulated by cytokines, such as IL-7, FLT3-ligand, SCF (KIT-ligand) and DLL (NOTCH ligands), produced by the thymic microenvironment [41]. Somatic gain-of-function mutations of *IL-7R-α*, which are more common in ETP-ALL, are associated with constitutive IL-7 signaling and increased proliferation in T-ALL samples [3,42–44]. However, recent in-vivo studies suggest that activating IL-7 signaling in T-cell progenitors is insufficient to initiate leukemia [45▪▪]. To address the oncogenic potential of IL-7R-α mutations, Yokoyama *et al.* [45▪▪] retrovirally transduced HSCs and different hematopoietic progenitors using an IL-7R-α chain mutant previously identified in a T-ALL cell line. Overexpression of mutant IL-7R-α in T-cell progenitors did not induce T-ALL, whereas constitutive activation of IL-7 signaling in transduced HSCs caused oligoclonal myeloproliferative disease, indicating that IL-7R-α mutations alone are not sufficient to initiate leukemia. In contrast, activation of IL-7 signaling can synergize with other mutations to expand the pool of leukemic cells, thereby accelerating the development of T-ALL [46].

Activating mutations of the Notch signaling pathway are found in over 60% of T-ALLs [47]. Mutations are likely to be secondary events because constitutive activation of Notch signaling is unable to induce self-renewal of T-cell progenitors [18,48–51]. Rather, activation of Notch expands the LSC pool by multiple mechanisms, including activation and stabilization of c-Myc [52,53▪▪], the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway [48,54] and cyclin-dependent kinases, CDK4 and CDK6 [55]. Targeting each of these pathways downstream of Notch has been shown to have therapeutic potential either alone or in combination with Notch inhibitors [40,48,53▪▪,56,57]. A recent study by Kelliher's group also showed that MYC silencing using shRNAs and inhibition using BET bromodomain 4 (Brd4) inhibitor reduced the frequency of LSCs, measured by the engraftment of leukemic cells in transplanted recipients [58]. These results strongly suggest that mutations leading to constitutive NOTCH1 signaling and increased MYC expression are important for the maintenance of LSCs, but are not sufficient to induce leukemogenesis. Given these cytokine signals are provided by the thymic microenvironment, it seems likely that gain-of-function mutations in these signaling pathways allow pre-LSCs to escape the confines of the niche necessary for overt T-ALL [59].

Although activating mutations of most signaling pathways do not appear to be capable of initiating T-ALL, one exception may be the RAS signaling pathway that is most commonly mutated in ETP-ALL [1,3,60]. *NRas* overexpression confers an aberrant self-renewal potential to multipotent progenitors [61] and induces T-cell expansion [62], suggesting that oncogenic RAS could potentially generate pre-LSCs of T-cell origin.

CONCLUSION

The recent identification of pre-LSCs in human acute myeloid leukemia and their importance in relapse argues that understanding the genetic events

involved in the initiation of T-ALL is not merely an academic exercise but may be essential for improving outcomes in patients with T-ALL. The strongest evidence currently lies with the bHLH transcription factors, which can generate pre-LSCs from T-cell progenitors. The self-renewal of these pre-LSCs allows them to acquire other mutations, such as signaling pathways involved in T-cell differentiation, which on their own are insufficient to initiate the leukemic program but allow growth of pre-LSCs independent of the thymic microenvironment. Although therapies targeting those mutations that expand LSCs may have therapeutic efficacy, targeting the pre-LSC may for long-term cures.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Van Vlierberghe P, Ambesi-Impiombato A, De Keersmaecker K, *et al.* Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. Blood 2013; 122:74–82.
2. Van Vlierberghe P, Ambesi-Impiombato A, Perez-Garcia A, *et al.* ETV6 mutations in early immature human T cell leukemias. J Exp Med 2011; 208:2571–2579.
3. Zhang J, Ding L, Holmfeldt L, *et al.* The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012; 481:157–163.
4. De Keersmaecker K, Atak ZK, Li N, *et al.* Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 2013; 45:186–190.
5. Tzoneva G, Perez-Garcia A, Carpenter Z, *et al.* Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat Med 2013; 19:368–371.
6. Tremblay CS, Hoang T, Hoang T. Early T cell differentiation lessons from T-cell acute lymphoblastic leukemia. Prog Mol Biol Transl Sci 2010; 92:121–156.
7. Mullighan CG, Phillips LA, Su X, *et al.* Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 2008; 322:1377–1380.
8. Corces-Zimmerman MR, Hong WJ, Weissman IL, *et al.* Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A 2014; 111:2548–2553.
9. ■■ Shlush LI, Zandi S, Mitchell A, *et al.* Identification of preleukaemic haematopoietic stem cells in acute leukaemia. Nature 2014; 506:328–333.

This study provides experimental evidence of long-lived, treatment-resistant pre-LSCs in human leukemia, supporting the rationale for targeting the founder mutation.

10. Clappier E, Gerby B, Sigaux F, *et al.* Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse. J Exp Med 2011; 208:653–661.
11. Homminga I, Pieters R, Langerak AW, *et al.* Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. Cancer Cell 2011; 19:484–497.
12. Zuurbier L, Gutierrez A, Mullighan CG, *et al.* Immature MEF2C-dysregulated T-cell leukemia patients have an early T-cell precursor acute lymphoblastic leukemia gene signature and typically have nonrearranged T-cell receptors. Haematologica 2014; 99:94–102.
13. Berquam-Vrieze KE, Nannapaneni K, Brett BT, *et al.* Cell of origin strongly influences genetic selection in a mouse model of T-ALL. Blood 2011; 118:4646–4656.
14. Dik WA, Nadel B, Przybylski GK, *et al.* Different chromosomal breakpoints impact the level of LMO2 expression in T-ALL. Blood 2007; 110:388–392.
15. Larmonie NS, Dik WA, Meijerink JP, *et al.* Breakpoint sites disclose the role of the V(D)J recombination machinery in the formation of T-cell receptor (TCR) and non-TCR associated aberrations in T-cell acute lymphoblastic leukemia. Haematologica 2013; 98:1173–1184.
16. Braun CJ, Boztug K, Paruzynski A, *et al.* Gene therapy for Wiskott-Aldrich syndrome: long-term efficacy and genotoxicity. Sci Transl Med 2014; 6:227ra33.
17. McCormack MP, Young LF, Vasudevan S, *et al.* The Lmo2 oncogene initiates leukemia in mice by inducing thymocyte self-renewal. Science 2010; 327:879–883.
18. Tremblay M, Tremblay CS, Herblot S, *et al.* Modeling T-cell acute lymphoblastic leukemia induced by the SCL and LMO1 oncogenes. Genes Dev 2010; 24:1093–1105.
19. Cleveland SM, Smith S, Tripathi R, *et al.* Lmo2 induces hematopoietic stem cell-like features in T-cell progenitor cells prior to leukemia. Stem Cells 2013; 31:882–894.
20. Smith S, Tripathi R, Goodings C, *et al.* LIM domain only-2 (LMO2) induces T-cell leukemia by two distinct pathways. PLoS One 2014; 9:e85883.
21. ■ Sanda T, Lawton LN, Barrasa MI, *et al.* Core transcriptional regulatory circuit controlled by the TAL1 complex in human T cell acute lymphoblastic leukemia. Cancer Cell 2012; 22:209–221.

Using ChIP-seq, this study provides evidence for an intergated transcriptional network in T-ALL analogous to normal HSCs.

22. Wilson NK, Foster SD, Wang X, *et al.* Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. Cell Stem Cell 2010; 7:532–544.
23. ■ Mansour MR, Sanda T, Lawton LN, *et al.* The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. J Exp Med 2013; 210:1545–1557.

This study demonstrates how the hHLH complex can activate the Notch signaling pathway by activating a microRNA.

24. Correia NC, Durinck K, Leite AP, *et al.* Novel TAL1 targets beyond protein-coding genes: identification of TAL1-regulated microRNAs in T-cell acute lymphoblastic leukemia. Leukemia 2013; 27:1603–1606.
25. Beck D, Thoms JA, Perera D, *et al.* Genome-wide analysis of transcriptional regulators in human HSPCs reveals a densely interconnected network of coding and noncoding genes. Blood 2013; 122:e12–e22.
26. De Keersmaecker K, Real PJ, Della Gatta G, *et al.* The TLX1 oncogene drives aneuploidy in T cell transformation. Nat Med 2010; 16:1321–1328.
27. Loosveld M, Bonnet M, Gon S, *et al.* MYC fails to efficiently shape malignant transformation in T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer 2014; 53:52–66.
28. Grossmann V, Haferlach C, Weissmann S, *et al.* The molecular profile of adult T-cell acute lymphoblastic leukemia: mutations in RUNX1 and DNMT3A are associated with poor prognosis in T-ALL. Genes Chromosomes Cancer 2013; 52:410–422.
29. Butler JS, Dent SY. The role of chromatin modifiers in normal and malignant hematopoiesis. Blood 2013; 121:3076–3084.
30. Challen GA, Sun D, Jeong M, *et al.* Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2012; 44:23–31.
31. Jeong M, Sun D, Luo M, *et al.* Large conserved domains of low DNA methylation maintained by Dnmt3a. Nat Genet 2014; 46:17–23.
32. Shih AH, Abdel-Wahab O, Patel JP, *et al.* The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer 2012; 12:599–612.
33. Fried I, Bodner C, Pichler MM, *et al.* Frequency, onset and clinical impact of somatic DNMT3A mutations in therapy-related and secondary acute myeloid leukemia. Haematologica 2012; 97:246–250.
34. Ding L, Ley TJ, Larson DE, *et al.* Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature 2012; 481:506–510.
35. Welch JS, Ley TJ, Link DC, *et al.* The origin and evolution of mutations in acute myeloid leukemia. Cell 2012; 150:264–278.
36. ■■ Ntziachristos P, Tsirigos A, Van Vlierberghe P, *et al.* Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat Med 2012; 18:298–301.

This study provides insights into how mutations of the polycomb repressor complex contribute to T-ALL.

37. Simon C, Chagraoui J, Krosl J, *et al.* A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. Genes Dev 2012; 26:651–656.
38. Bracken AP, Dietrich N, Pasini D, *et al.* Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev 2006; 20:1123–1136.
39. Xie H, Xu J, Hsu JH, *et al.* Polycomb repressive complex 2 regulates normal hematopoietic stem cell function in a developmental-stage-specific manner. Cell Stem Cell 2014; 14:68–80.
40. Piovan E, Yu J, Tosello V, *et al.* Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. Cancer Cell 2013; 24:766–776.

41. Rothenberg EV, Moore JE, Yui MA. Launching the T-cell-lineage developmental programme. Nat Rev Immunol 2008; 8:9–21.

42. Barata JT, Cardoso AA, Nadler LM, *et al.* 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; 98:1524–1531.

43. Zenatti PP, Ribeiro D, Li W, *et al.* Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet 2011; 43:932–939.

44. Ribeiro D, Melao A, Barata JT. IL-7R-mediated signaling in T-cell acute lymphoblastic leukemia. Adv Biol Regul 2013; 53:211–222.

45. ■■ Yokoyama K, Yokoyama N, Izawa K, *et al.* In vivo leukemogenic potential of an interleukin 7 receptor alpha chain mutant in hematopoietic stem and progenitor cells. Blood 2013; 122:4259–4263.

This is the first study to demonstrate that IL-7R gain of function is insufficient to initiate T-ALL.

46. Silva A, Laranjeira AB, Martins LR, *et al.* IL-7 contributes to the progression of human T-cell acute lymphoblastic leukemias. Cancer Res 2011; 71:4780–4789.

47. Weng AP, Ferrando AA, Lee W, *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004; 306:269–271.

48. Blackburn JS, Liu S, Raiser DM, *et al.* Notch signaling expands a pre-malignant pool of T-cell acute lymphoblastic leukemia clones without affecting leukemia-propagating cell frequency. Leukemia 2012; 26:2069–2078.

49. Chiang MY, Xu L, Shestova O, *et al.* Leukemia-associated NOTCH1 alleles are weak tumor initiators but accelerate K-ras-initiated leukemia. J Clin Invest 2008; 118:3181–3194.

50. Li X, Gounari F, Protopopov A, *et al.* Oncogenesis of T-ALL and nonmalignant consequences of overexpressing intracellular NOTCH1. J Exp Med 2008; 205:2851–2861.

51. Chiang MY, Shestova O, Xu L, *et al.* Divergent effects of supraphysiologic Notch signals on leukemia stem cells and hematopoietic stem cells. Blood 2013; 121:905–917.

52. Weng AP, Millholland JM, Yashiro-Ohtani Y, *et al.* c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. Genes Dev 2006; 20:2096–2109.

53. ■■ King B, Trimarchi T, Reavie L, *et al.* The ubiquitin ligase FBXW7 modulates leukemia-initiating cell activity by regulating MYC stability. Cell 2013; 153:1552–1566.

This study highlights the role of FBXW7 mutations in leukemogenesis and demonstrates that MYC is not sufficient to induce T-ALL. These results may have important consequences for the development of novel therapies to prevent relapse in T-ALL.

54. Chan SM, Weng AP, Tibshirani R, *et al.* Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood 2007; 110:278–286.

55. Joshi I, Minter LM, Telfer J, *et al.* Notch signaling mediates G1/S cell-cycle progression in T cells via cyclin D3 and its dependent kinases. Blood 2009; 113:1689–1698.

56. Sawai CM, Freund J, Oh P, *et al.* Therapeutic targeting of the cyclin D3:CDK4/6 complex in T cell leukemia. Cancer Cell 2012; 22:452–465.

57. 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 2013. doi: 10.1038/leu. 2013.369.

58. Roderick JE, Tesell J, Shultz LD, *et al.* c-Myc inhibition prevents leukemia initiation in mice and impairs the growth of relapsed and induction failure pediatric T-ALL cells. Blood 2014; 123:1040–1050.

59. Hagenbeek TJ, Wu X, Choy L, *et al.* Murine Pten T-ALL requires nonredundant PI3K/mTOR and DLL4/Notch1 signals for maintenance and gammac/TCR signals for thymic exit. Cancer Lett 2014; 346:237–248.

60. Atak ZK, De Keersmaecker K, Gianfelici V, *et al.* High accuracy mutation detection in leukemia on a selected panel of cancer genes. Plos One 2012; 7:7; e38463.

61. Li Q, Bohin N, Wen T, *et al.* Oncogenic Nras has bimodal effects on stem cells that sustainably increase competitiveness. Nature 2013; 504:143–147.

62. Lassen LB, Ballarin-Gonzalez B, Schmitz A, *et al.* Nras overexpression results in granulocytosis, T-cell expansion and early lethality in mice. PLoS One 2012; 7:e42216.