Novel biological insights in T-cell acute lymphoblastic leukemia

Kaat Durincka, Steven Goossensb,c,d, Sofie Peirsä, Annelynn Wallaerta, Wouter Van Loockea,
Filip Matthijssensa, Tim Pietersa,b,c, Gloria Milanä, Tim Lammensé, Pieter Rondoua, Nadine Van Roya,
Barbara De Moerloosee, Yves Benoité, Jody Haighd, Frank Spelemanf, Bruce Poppei, and
Pieter Van Vlierberghea

a Center for Medical Genetics, Department for Pediatrics, Ghent, Belgium; b Department for Biomedical Molecular Biology, Ghent University, Ghent, Belgium; c Unit for Molecular Oncology, VIB Inflammation Research Center, Ghent, Belgium; d Mammalian Functional Genetics Laboratory, Division of Blood Cancers, Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia; e Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium

(Received 16 May 2015; accepted 24 May 2015)

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive type of blood cancer that accounts for about 15% of pediatric and 25% of adult acute lymphoblastic leukemia (ALL) cases. It is considered as a paradigm for the multistep nature of cancer initiation and progression. Genetic and epigenetic reprogramming events, which transform T-cell precursors into malignant T-ALL lymphoblasts, have been extensively characterized over the past decade. Despite our comprehensive understanding of the genomic landscape of human T-ALL, leukemia patients are still treated by high-dose multiagent chemotherapy, potentially followed by hematopoietic stem cell transplantation. Even with such aggressive treatment regimens, which are often associated with considerable acute and long-term side effects, about 15% of pediatric and 40% of adult T-ALL patients still relapse, owing to acquired therapy resistance, and present with very dismal survival perspectives. Unfortunately, the molecular mechanisms by which residual T-ALL tumor cells survive chemotherapy and act as a reservoir for leukemic progression and hematologic relapse remain poorly understood. Nevertheless, it is expected that enhanced molecular understanding of T-ALL disease biology will ultimately facilitate a targeted therapy driven approach that can reduce chemotherapy-associated toxicities and improve survival of refractory T-ALL patients through personalized salvage therapy. In this review, we summarize recent biological insights into the molecular pathogenesis of T-ALL and speculate how the genetic landscape of T-ALL could trigger the development of novel therapeutic strategies for the treatment of human T-ALL.

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Normal T-cell development is a strictly regulated, multistep process in which hematopoietic progenitor cells differentiate into functionally diverse T-lymphocyte subsets after their migration into the thymus microenvironment. The different checkpoints, covering thymic colonization, lineage commitment, and definitive differentiation [1], are orchestrated by diverse transcriptional regulatory networks and transitions between epigenetic states [2,3] in response to cytokine receptor activation. During this fine-tuned developmental process, inappropiate activation of T-cell acute lymphoblastic leukemia (T-ALL) oncogenes and loss of tumor suppressor gene activity will coordinately push thymic precursors into uncontrolled clonal expansion and cause T-ALL.

T-ALL is an aggressive hematologic cancer that arises from the malignant transformation of T-cell progenitors and occurs in about 15% of pediatric and 25% of adult ALL cases. High-dose multiagent chemotherapy serves as the current standard of care for this tumor entity and is highly effective in the majority of childhood leukemia patients, with overall survival rates reaching 85% in most pediatric protocols. Nevertheless, these aggressive treatment regimens are often associated with severe acute toxicities and long-term side effects, including the development of secondary tumors later in life. Notably, the situation for older leukemia patients is less favorable as compared with
children, with at least 40% of adult T-ALL patients failing current therapy. Despite the introduction of hematopoietic stem cell transplantations for refractory leukemias, the clinical outcome of these high-risk, primary resistant tumors remains extremely poor [4–6].

Different studies have collectively shown that T-ALL can be divided into molecular genetic subgroups that are characterized by unique gene expression signatures and aberrant activation of specific T-ALL transcription factor oncogenes, including MEF2C, HOXA, TLX1, NKX2.1, TLX3, TAL1, LMO1, and LMO2 [6–10]. Moreover, whole-genome T-ALL profiling has provided a fairly complete and comprehensive list of additional genetic defects that are shared among the different genetic subclasses and activate a plethora of oncogenic signaling cascades, including interleukin 7 receptor (IL7R)/Janus kinase (JAK)/signal transducer and activator of transcription (STAT) [11], phosphatidylinositol 3-kinase (PI3K)/Akt [12–14] and Ras/mitogen–extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) [15] signaling. In addition, some of these cooperative genetic lesions also coordinately target more general cellular pathways, as exemplified by aberrant activation of antiapoptotic effector pathways and enhanced cap-dependent translation activity in human T-ALL. Notably, this conversion toward aberrant activation of a discrete set of common cellular or signaling pathways provides unique opportunities for the development of targeted therapies in the context of human T-ALL [16,17].

Now that we have acquired a detailed molecular understanding of the genetic defects that drive human T-ALL at the coding level of the genome, we face a number of interesting challenges that will hopefully be addressed in the near future. First, we need to better understand how specific T-ALL oncogenes and tumor suppressors actually cooperate to drive overt disease and full leukemic transformation. Indeed, we lack a thorough understanding of the genetic interactions between genetic defects that preferentially co-occur in primary human T-ALL samples. Secondly, the potential contribution of the exact order in which genetic lesions are acquired during disease initiation, progression, and/or maintenance should be further investigated. In addition, it is still unclear whether the exact cell of origin for T-cell transformation is truly different for the distinct molecular genetic subtypes of human T-ALL. Moreover, the existence of long-lived preleukemic stem cells (pre-LSCs) in the context of T-ALL is still under debate, and the molecular mechanism that could regulate pre-LSC activity of thymic precursors remains poorly characterized. Finally, epigenetic mechanisms and the noncoding part of the human genome provide an additional layer of complexity, and we are currently only starting to understand the putative oncogenic contributions of deregulated microRNAs (miRNAs), long non-coding RNAs (lncRNAs), enhancer activities, chromatin remodeling, and/or epigenetic changes in the context of malignant T-cell transformation.

In this review, we summarize recent biological insights in the molecular pathogenesis of T-ALL and discuss the impact of these findings on some of the intriguing challenges mentioned above. Moreover, we speculate on how the genetic landscape of T-ALL could trigger the development of novel therapeutic strategies for the treatment of human T-ALL.

**Genetic heterogeneity of immature T-ALL subtypes**

More than a decade ago, attempts were made to classify human T-ALL into different tumor entities based on their transcriptional signature. Indeed, depending on the expression of specific T-ALL transcription factor oncogenes, T-cell leukemia was classified into molecular genetic subgroups reflecting distinct stages of arrest during T-cell development [7].

In these initial studies, early immature T-ALLs were recognized as a transcriptionally distinct leukemia entity with high expression of the basic helix-loop-helix (bHLH) transcription factor lymphoblastic leukemia associated hematopoiesis regulator 1 (LYL1) and the LIM domain only protein 2 (LMO2) [7]. In addition, leukemic blasts from these so-called LYL1⁺T-ALLs collectively expressed early lymphoid/myeloid markers including CD13, CD33, and/or CD34, and most lacked CD4 or CD8 proteins at their surface. Although no unifying molecular alterations could be identified in LYL1⁺ T-ALLs, these leukemias were characterized by a high frequency of deletions on the long arm of chromosome 5 and often lacked deletions on the short arm of chromosome 9 targeting the CDKN2A/CDKN2B tumor suppressor locus [7]. Absence of these 9p deletions, which occur in more than 70% of other T-ALL subtypes [18], is an intriguing feature of immature T-ALL that might be linked to the epigenetic state of the cdkn2a locus during tumor progression. In primitive hematopoietic precursor cells, the polycomb repressive complex 2 (PRC2) mediates silencing of the Ink4a-Arf locus [19,20], suggesting that leukemias that originate from hematopoietic progenitors lack the selective pressure for genomic cdkn2a deletion during T-cell transformation [21,22]. In contrast, this permanent silencing is not present in early thymocytes in which the Ink4a-Arf locus can be reactivated in response to oncogenic signaling [21]. In such model, engagement of cdkn2a expression during tumor initiation creates a strong selective pressure for rare cells to bypass tumor suppression by deleting cdkn2a and, eventually, to emerge as clonal malignancies [22]. Altogether, these studies might provide an explanation for the unequal distribution of CDKN2A/CDKN2B deletions between immature and mature T-ALLs, supporting the view that the cell of origin for some early immature leukemias might have features of a very early hematopoietic progenitor.

More recent efforts have further enhanced our molecular understanding of immature T-ALLs and provided a comprehensive overview of genetic alterations that occur in this particular subtype of human T-ALL. These studies showed that the genetic landscape of immature T-ALL is highly
heterogeneous, with aberrant expression of the MEF2C gene [23,24]; genetic alterations in hematopoietic transcription factors such as RUNX1, GATA3, BCL11B, PU.1, and ETV6 [24]; activating mutations in critical mediators of cytokine receptor and Ras signaling, including NRAS, KRAS, FLT3, IL7R, JAK1, JAK3, SH2B3, and BRAF; mutations in the epigenetic regulators EZH2, EED, SUZ12, MLL2, BMI1, SETD2, and EP300; and mutations in the dynamin coding gene DNMT2 [8,25]. In addition, immature T-ALLs present with lower frequencies of the prototypical NOTCH1 mutations, which occur in the majority of mature T-ALL patient samples and are considered as one of the hallmarks of human T-cell transformation.

For reasons not well understood, the relative percentage of immature T-ALLs in adults seems to be significantly higher as compared with children [26], and some molecular alterations are exclusively present in adult immature T-ALLs [26]. For example, genetic lesions that were initially identified in acute myeloid leukemia and target genes implicated in DNA methylation, such as DNMT3A, IDH1, and IDH2, are uniquely present in adult immature T-ALL and have not been reported in pediatric patients. In that context, it was recently shown that DNMT3A mutations in primary human acute myeloid leukemias (AMLs) occur in both preleukemic hematopoietic stem cells (HSCs) and committed T-cells, suggesting that DNMT3A mutant preleukemic HSCs retain the ability to differentiate into multiple cell types [27]. Therefore, DNMT3A mutations could act as an early leukemogenic event that enables the establishment of a preleukemic reservoir. Such DNMT3A-mutant pre-HSCs could remain quiescent over a certain period in life and eventually progress toward malignant transformation in the myeloid and/or early T-cell lineage, depending on the spectrum of acquired secondary hits. Notably, this hierarchical model, in which preleukemic stem cells precede full malignant transformation, has been previously proposed in the context of T-ALL [28]. The concept of DNMT3A-mutant preleukemic HSCs, which emerge in ageing adults, could partially explain why DNMT3A mutations are not identified in pediatric early T-cell precursor ALL (ETP-ALL) [25], but are mainly present in early immature adult T-ALLs [26,29]. Nevertheless, it is currently unknown whether a similar model could be applied for other lesions that target the DNA methylation machinery, including IDH1 and IDH2 mutations.

Thymocyte self-renewal and preleukemic stem cells in T-ALL development

Preleukemic stem cells (pre-LSCs) are a population of progenitor cells that harbor a few mutations but not the entire complement of genetic alterations necessary to become a leukemia. In contrast to fully transformed leukemic cells, pre-LSCs retain the ability to differentiate into the full spectrum of mature daughter cells. In addition, their stem-cell properties allow clonal expansion and subsequent acquisition of extra oncogenic driver mutations, eventually leading to the onset of a fully transformed tumor.

McCormack and colleagues initially described a population of long-term self-renewing thymocytes many months before the generation of leukemia in the CD2-LMO2gs transgenic mouse model [30], which spontaneously develops T-ALL with an expression profile similar to that of immature T-ALL patients. Unlike the thymus of normal mice, which is continually replenished by progenitors from the bone marrow, the preleukemic thymus of CD2-LMO2gs transgenic mice was self-sustaining from a young age. The self-renewal capacity of CD2-LMO2gs thymocytes was restricted to the CD4+CD8− double-negative precursor T-cells, specifically the CD4+CD8−CD44+CD25+ (DN3) subpopulation. In addition, it was demonstrated that the bHLH transcription factor Lyl1 is an essential, but not a sufficient, factor to program Lmo2-overexpressing DN3 cells into pre-LSCs [31]. More work will need to be done to decipher the exact molecular mechanism explaining how LMO2 controls thymocyte self-renewal. LMO2 itself has no DNA-binding capacity and depends on LYL1 for its transcriptional activity. LYL1 as well requires binding with other helix-loop-helix proteins, including E2A, E47, or transcription factor 12 (TCF12, HEB), to form heterodimers, which can recognize E-box sequences in specific target gene promoters. Recently, it was suggested that LMO2 and LIM domain binding 1 (LDB1) are essential co-factors for bridging two of these heterodimer complexes as a prerequisite for binding at tandem E-boxes throughout the genome [32].

More recently, it was shown that pre-LSCs are Notch1-dependent, and supraphysiologic levels of Notch1 expanded the pool of pre-LSCs and rendered them independent of the thymic microenvironment [33,34]. Nevertheless, it remains to be determined whether gain of preleukemic self-renewal capacity is a true obligatory trait for human T-ALL development. Moreover, it would be interesting to know whether other T-ALL-specific transcription factor oncogenes, including NKX2.1, MEF2C, TLX1, TLX3, or HOXA, also possess the intrinsic capacity to induce self-renewal in T-cell progenitors [28].

Finally, the pre-LSCs discussed above are more chemoradioresistant and could reflect the population that would eventually give rise to tumor relapse after chemotherapy. Recent studies indicate that pre-LSC radioresistance and self-renewal capacity are regulated via distinct molecular pathways [35]. Although Hhex and Kit are not essential for LMO2-mediated leukemia development, Lmo2-transgenic thymocytes utilize Kit-dependent signaling pathways to enable radioresistance.

Prognostic relevance of immature subtypes of human T-ALL

From a clinical perspective, early work suggested that LYL1+ T-ALLs could be associated with reduced survival rates, and
this notion was further supported by the identification of early T-cell-specific V-ets avian erythroblastosis virus E26 oncogene homolog (ERG) and brain and acute leukemia cytoplasmic (BAALC) expression as poor prognostic markers in adult T-ALL [36]. In addition, absence of biallelic deletion of T-cell receptor γ, a marker for early arrest during T-cell differentiation, was associated with induction failure and poor clinical outcome in pediatric T-ALL [37]. Some studies have suggested that RUNX1 [38], DNMT3A [26], IDH1 [26], or IDH2 [26] mutations might confer poor prognosis within the immature subtype of adult T-ALL. Further, specific immature subtypes characterized by aberrant HOXA gene activation, including SET-NUP214- and CALM-AF10-positive leukemias, were associated with higher levels of corticosteroid resistance [39] or dismal survival rates [40]. Finally, in an attempt to start integrating these molecular genetic findings into the clinic, the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) implemented a NOTCH1/FBXW7/RAS/PTEN-based oncogenic risk classification, in which patients with mutated NOTCH1 or FBXW7 who present with a wild-type Ras and PTEN are considered low risk, whereas all other adult T-ALLs are categorized in a high-risk category [41]. Notably, this new risk classification strategy was strongly associated with higher cumulative incidence of relapse and shorter relapse-free and overall survival in the multicenter GRAALL-2003 and GRAALL-2005 clinical trials [42]. Additional inclusion of poor prognostic genetic markers, such as mutational status of DNMT3A, IDH1, and IDH2, should be considered to further improve this novel risk stratification approach for adult T-ALL. Moreover, it should be noted that the prognostic value of this novel risk classification strategy might not be applicable in pediatric treatment protocols given the high overall survival rates for T-ALL in children.

More recently, ETP-ALL was identified as a specific subentity within immature T-ALL with unique immunophenotypic properties, including lack of CD1a and CD8 expression, weak CD5 positivity, and expression of one of the stem-cell/myeloid markers CD13, CD117, CD33, major histocompatibility complex, Class II, DR Beta (HLA-DR), and/or CD34. Most importantly, the initial study that defined ETP-ALL as a novel immature subtype of pediatric human T-ALL reported an extremely poor clinical course for these patients, with 10-year overall survival rates of 19% for ETP-ALL patients, as compared with 84% for other T-ALL subtypes [43]. Although the poor clinical characteristics of this leukemic subtype were subsequently validated in a variety of independent patient series from different international pediatric T-ALL treatment protocols [24,43–45], more recent work has started to question the prognostic relevance of this aggressive T-ALL subtype [23,46]. In that context, a recent large study of 1,144 pediatric T-ALL patients enrolled in the Children’s Oncology Group (COG) study AALL0434 suggested that, despite significantly higher rates of induction failure, pediatric ETP-ALL patients as defined by flow cytometry in a single-reference laboratory, showed 5-year event-free and overall survival rates identical to those of non-ETP-ALLs [47]. These discrepant results suggest that subtle differences in treatment protocols or particular challenges associated with accurate assessment of the ETP-ALL immunophenotype might ultimately affect the therapeutic response for ETP-ALL observed in different studies, institutions, and/or treatment protocols. Nevertheless, given the size of the patient population included in the COG study, it seems unlikely that the ETP-ALL immunophenotype, as originally defined by Coustan-Smith et al. [43], will eventually be implemented as a useful prognostic marker to guide treatment decisions for pediatric T-ALL.

**Novel drivers of T-ALL oncogenesis**

**ZEB2 as a novel transcription factor oncogene in early immature T-ALL**

Although immature T-ALL has been identified as a separate T-ALL disease entity, this genetic subtype is relatively heterogeneous; only few oncogenic transcription factors implicated in immature T-ALL disease biology have been identified to date. The zinc finger E-box binding homeobox 2 protein (ZEB2/SIP1) has an established role in the process of epithelial-mesenchymal transition (EMT) and is implicated in both normal embryonic development and tumorigenesis [48] such as melanoma [49], breast [50], and gastric cancer [51]. Zinc finger E-box binding homeobox 2 (ZEB2) is also abundantly expressed in the hematopoietic system and is essential for differentiation and mobilization of hematopoietic stem cells during embryonic development [52]. However, the role of ZEB2 in normal T-cell development and hematologic malignancies has not been thoroughly investigated.

Recently, Goossens et al. [53] identified the translocation t(2;14)(q22;q32) as a rare but recurrent genetic event in immature T-ALL. Detailed cytogenetic analysis allowed mapping of the translocation breakpoint at 14q32 within the BCL11B locus and within close vicinity of the ZEB2 gene at chromosomal band 2q22. Notably, juxtaposition of BCL11B near putative oncogenes has been previously reported for a number of T-ALL oncogenes including TLX3, NXX2-5, and PU.1 [54,55]. Therefore, this work suggested that sustained expression of the EMT regulator ZEB2 could act as a molecular driver of immature T-ALL development. It is also interesting to note that BCL11B haploinsufficiency has also been implicated in T-cell leukogenesis, as exemplified by the loss-of-function BCL11B deletions and mutations identified in primary T-ALLs [56,57]. Therefore, this particular chromosomal translocation might execute a dual function toward malignant T-cell transformation with concomitant loss of BCL11B and gain of ZEB2, a concept that also applies for the other BCL11B-driven translocations mentioned above.
Using a conditional ROSA26-based Zeb2 gain-of-function mouse model, Goossens et al. [53] showed that hematopoietic overexpression of Zeb2 resulted in spontaneous thymic lymphoma development starting at 5 months of age, indicating that Zeb2 can act as a bona fide oncogene in hematologic T-cell malignancies. Furthermore, breeding of this tumor model in a tumor-prone p53 null background revealed that Zeb2 overexpression shortens tumor latency and drives a gene expression profile that resembles immature T-ALL, including the early T-cell/stem-cell markers c-Kit, Baelc, and Lyt1. Similar activation of a stem-cell-like transcriptional program has previously been described in a CD2-Lmo2 transgenic mouse model [58], in which preleukemic thymocytes acquired Lmo2-driven self-renewal capacity as a prerequisite of full clonal T-cell transformation [30]. Similarly, transplantation experiments using Zeb2-overexpressing lymphoblasts in immunodeficient mice showed that Zeb2-driven tumors contained a higher fraction of leukemia-initiating cells as compared with controls, suggesting some putative commonalities between the Zeb2- and Lmo2-driven tumor models as described above. Interestingly, ZEB proteins also bind tandem E-boxes [59], suggesting a potential overlap or competition for the same regulatory sequences with the above-described LYL1/LMO2 transcriptional regulatory complex [32].

Zeb2 tumors were also characterized by enhanced JAK/STAT signaling through activation of IL7R. This association between ZEB2 and IL7R/JAK/STAT activation is in line with other recent studies that reported alternative in vivo models for immature T-ALL. Indeed, bone marrow transplantation of precursor cells expressing gain-of-function IL7R [60] or JAK3 [61] mutations resulted in the development of a transplantable murine immature T-ALL, reminiscent of the human disease. Altogether, this study convincingly identified ZEB2 as a novel oncogene implicated in immature T-ALL, but the molecular mechanisms by which ZEB2 regulates leukemic stem cell activity, in addition to the spectrum of direct ZEB2 target genes that modulate disease initiation or progression, remains to be established.

The role of noncoding RNAs in T-ALL

Over the last years, genome-wide profiling studies have extensively characterized oncogenic gene expression signatures of molecular genetic subtypes in human T-ALL [7–10]. It has become clear that protein-coding genes only constitute about 2% of the entire genome, suggesting that the human transcriptome is predominantly composed of noncoding RNAs [62]. Besides those RNA species carrying out some of the main housekeeping functions (such as tRNAs, snoRNAs, and tRNAs), diverse classes such as miRNAs, lncRNAs, and circular RNAs constitute novel functional effectors and are crucial regulators of diverse gene expression programs.

MicroRNAs in the pathogenesis of T-ALL

MicroRNAs are a well-known class of small noncoding RNAs, important in both normal development and cancer [63]. The regulatory functions of miRNAs are essential to all levels of hematopoietic development [64]. With respect to T-ALL, a landmark study by Mavrakis et al. [65] identified a set of five microRNAs (miR-19b, miR-20a, miR-26a, miR-92, and miR-223) that cooperatively suppress a network of tumor suppressor genes, including PHF6, PTEN, BIM, and FBXW7. Notably, each of these onco-miRNAs were able to accelerate leukemia onset in a Notch1-induced murine bone marrow transplant model of T-ALL, confirming their in vivo potential. This study was in line with other work that identified the miR-17-92 cluster as one of the most prominent oncogenic miRNA clusters able to induce overt T-cell leukemia in concert with activated NOTCH1 [66,67]. The role of miR-223 in oncogenic T-ALL signaling was also emphasized by others [68] and could be further validated through the identification of miR-223 as a direct target gene of the T-ALL oncogenes NOTCH1 [69] and TAL1, which contribute to T-ALL development by repression of the tumor suppressor gene FBXW7 [70]. Finally, another study identified miR-128-3p as a novel oncogenic miRNA in T-ALL that negatively regulates the expression of the tumor suppressor PHF6 [71] and cooperates with activated NOTCH1 signaling to accelerate T-ALL formation in vivo [71].

In addition to oncogenic miRNAs that contribute to T-cell transformation by inactivation of specific tumor suppressor genes, Sanghvi et al. [72] were able to identify a set of so-called tumor suppressor miRNAs (miR-29, miR-31, miR-150, miR-155, and miR-200) in T-ALL. These miRNAs converged toward posttranscriptional activation of the MYB and HBP1 oncogenes. Another recent T-ALL study identified miR-193b as an additional tumor suppressor miRNA that also regulated MYB oncogene expression, as well as expression of the antiapoptotic factor MCL1 [73].

Altogether, it is clear that a small subset of miRNAs has oncogenic or tumor suppressive properties in the context of malignant T-cell transformation (Fig. 1). Nevertheless, a comprehensive overview of differential miRNA expression between the different molecular genetic subgroups in human T-ALL remains to be established.

Long noncoding RNAs in the pathogenesis of T-ALL

Long noncoding RNAs are defined as transcripts with a length of at least 200 nucleotides that lack protein-coding potential and evolutionary conservation [74]. They are positionally located as antisense, intronic, intergenic, or overlapping transcripts with protein-coding genes [75]. The functional repertoire of lncRNAs can be very diverse, but they act mainly in concert with chromatin modifier enzymes. They can serve as scaffolds bridging between multiple proteins, guides to target chromatin remodelers to their target sites, or control devices that can induce protein conformational changes and thereby activate/inactivate the interacting protein complex [76]. Unlike miRNAs, the role of lncRNAs in
normal and malignant T-cell development is only starting to be fully explored.

Last year, Trimarchi and colleagues published the first landmark study on the identification of a set of lncRNAs under control of aberrant NOTCH1 signaling in T-ALL [77]. They identified LUNAR1 as an oncogenic lncRNA, localized in the nucleus, that is overexpressed in primary T-ALLs, with higher expression in T-ALL cases that harbor activating NOTCH1 mutations. LUNAR1 is located in cis to the IGF1R locus and promotes its expression through a direct interaction between LUNAR1 and an intronic IGF1R enhancer element, as shown by chromosome conformation capture analysis (Hi-C). Moreover, in vitro knockdown of LUNAR1 significantly affected leukemic cell growth owing to decreased insulin-like growth factor 1 receptor (IGF1R) signaling. In addition, the in vivo oncogenic capacity of the LUNAR1 transcript was further supported by xenograft assays, in which tumor cells that lost LUNAR1 expression were outcompeted by the leukemic control population. Importantly, these initial findings were subsequently confirmed by a parallel study [78] in which LUNAR1 was identified as the top candidate of NOTCH1-regulated lncRNAs in T-ALL and normal T-cell development. These studies collectively show that lncRNAs act as an additional layer of complexity in T-ALL disease biology and warrant a further in-depth analysis of lncRNA profiles in an extensive series of disease specimens.

Aberrant enhancer activity in malignant T-cell development

Enhancers are gene regulatory elements that can act in cis or trans and ensure correct spatiotemporal gene expression. It is estimated that there are over one million active enhancers present in all human cells [79]. Hallmark epigenetic features of enhancers include the presence of H3K4me1 and binding by p300, with the absence or presence of H3K27ac further distinguishing between inactive and active enhancers [80]. Context and time-dependent binding of a diversity of transcription factors to these enhancer elements is crucial to ensure proper development and homeostasis [81]. Enhancer activity may act on genes in cis, but are often also implicated in long-range interactions through chromatin looping [82].

Recent studies have comprehensively mapped large enhancer regions that are marked by a high occupancy of bromodomain containing 4 (BRD4), p300, H3K27ac, and the Mediator complex; these are termed the super-enhancers [83]. These large enhancers resemble previously identified locus control regions and associate with lineage-specific transcription factors to establish a correct transcriptional cell identity program [84]. Moreover, given that these large enhancers are often aberrantly acquired at oncogenic driver genes, accurate localization and identification of these super-enhancers can help in identifying relevant cancer genes for a variety of tumor entities [85].
Two parallel T-ALL studies conducted by Mansour et al. [86] and Navarro et al. [87] nicely illustrated the putative role of aberrant enhancer activity in the context of T-cell transformation. Notably, these landmark publications identified a new mechanism for oncogene activation in human T-ALL [86,87], namely, somatic insertions in a regulatory element upstream of the transcriptional start site of the TAL1 oncogene [86,87]. Indeed, small acquired heterozygous alterations targeting a noncoding region of the human genome introduced a de novo recognition site for V-Myb avian myeloblastosis viral oncogene homolog (MYB) near the TAL1 locus [86]. Interestingly, MYB binding at this particular locus resulted in the creation of a somatically acquired super-enhancer and a concomitant epigenetic switch from H3K27me3 to H3K27ac occupancy, which eventually caused increased and monoallelic TALI expression [86]. Therefore, these studies identified a novel genetic mechanism for the generation of oncogenic super-enhancers in malignant T cells [86,87], and their results suggest a general role for MYB in the regulation of T-cell-specific super-enhancer activity [86]. Additional research will be required to verify whether similar mechanisms are also involved in other tumor entities and/or activation of other T-ALL oncogenes.

In T-ALL, it was previously shown that a proportion of relevant NOTCH1 target genes are also under control of long-range enhancers [88]. One interesting example is the massive binding of NOTCH1 at a distal enhancer near the MYC locus, which was recently described by Herranz et al. [89] and subsequently independently validated by others [90]. Most notably, conditional deletion of this single, NOTCH1-controlled MYC enhancer site (N-Me) abolished the initiation and maintenance of NOTCH1 induced leukemia in vivo [89]. Moreover, recurrent and focal duplications of the N-Me locus were found in 5% of T-ALLs, providing an alternative example of genetic alterations that target oncogenic enhancer activity in T-ALL [89].

Epigenetic modulation in T-ALL

Genome-wide or candidate-approach-based studies recently identified recurrent genomic lesions in a variety of genes involved in DNA methylation or posttranslational histone modifications in T-ALL. Therefore, disruption of the epigenetic state and changes in the chromatin structure of normal thymocytes probably represent crucial mediators of malignant T-cell transformation. The reversible nature of epigenetic modifications makes these DNA methyltransferases and histone modifier enzymes attractive targets for therapeutic intervention.

The PRC2 complex functions as a methyltransferase that regulates H3K27me3 levels at specific target genes throughout the genome. The core components of this complex, including EZH2, EED, and SUZ12, were identified as tumor suppressor genes in T-ALL, with mutations in about 25% of adult T-ALL cases. Loss of PRC2 activity resulted in reduced levels of H3K27me3 and further enhanced the NOTCH1-driven oncogenic transcriptional program during malignant T-cell transformation [91].

Lysine (K)-specific demethylase 6A (KDM6A, MYB) is a H3K27 demethylase that exerts an opposite function on gene transcription as compared with the PRC2 complex. By contrast, some studies have reported loss-of-function mutations targeting UTX in human T-ALL [92–94], suggesting that both chromatin remodelers can exert tumor suppressor functions during T-cell transformation. Interestingly, Van der Meulen et al. [94] exclusively identified UTX mutations in male T-ALL patients and showed that UTX escapes chromosome X inactivation in female T-ALL blasts, providing a possible explanation for the skewed gender distribution observed in T-ALL. These findings are also clinically relevant, given that T-ALL driven by UTX inactivation exhibits collateral sensitivity to pharmacologic H3K27me3 inhibition [94].

Finally, the critical role of unbalanced H3K27me3 levels in the initiation and maintenance of T-ALL was further exemplified by a recent study that identified an essential oncogenic role for the H3K27 demethylase Lysine (K)-specific demethylase 6B (JMJD3) in the pathogenesis of T-ALL [93]. Indeed, JMJD3 was critically required for the maintenance of oncogenic NOTCH1 signaling during T-cell transformation and was overexpressed in primary T-ALL. JMJD3 inhibition by the small molecule GSKJ4 could restrain T-ALL tumor growth, suggesting that this epigenetic inhibitor could serve as another promising therapeutic strategy for the treatment of T-ALL [93].

Opportunities for targeted therapy in T-ALL

The NOTCH1 signaling pathway

T-ALL is a genetically heterogeneous disorder in which multiple oncogenic and loss-of-function mutations cooperate to establish leukemia. Nevertheless, the concept of oncogene addiction supports the idea that targeting single oncogenes should be sufficient to develop effective oncogene-based therapeutic opportunities [95].

The prototype example in the context of T-ALL is the inhibition of the proteolytic cleavage of the transmembrane NOTCH1 receptor by the presenilin/γ-secretase complex using γ-secretase inhibitors (GSIs). Problematically, the accumulation of goblet cells in the digestive system, induced by GSI treatment through upregulation of Kruppel-like factor 4 (KLF4) levels, leads to gastrointestinal toxicity. Combination therapy with glucocorticoid administration effectively reduces the toxic effects in the gut [96]. It was recently shown that GSI-resistant T-ALLs could benefit from a combination of vincristine and GSI treatment, since GSIs were shown to enhance the apoptotic effect induced by the chemotherapeutic agent [97]. Similarly, a preclinical study using a clinically relevant GSI in combination with dexamethasone showed synergistic results in glucocorticoid-resistant leukemias [98].
Alternative strategies to target the NOTCH1 pathway are still being developed and include specific NOTCH1-inhibitory antibodies and stapled peptides that target the NOTCH1 transcriptional complex [99,100]. For example, the synthetic peptide SAHM1 that directly interferes at the level of protein-protein interactions required for the NOTCH1 transcriptional complex shows a higher potency to inhibit NOTCH1 signaling in comparison to GSI [99].

Other strategies involve therapeutic targeting of downstream signaling components of the NOTCH pathway. For example, pharmacologic inhibition or genetic ablation of IGFIr, a direct NOTCH1 target gene, inhibits growth and viability of T-ALL cells and might influence the leukemia-initiating cell activity of NOTCH1-induced tumors [101]. Additionally, an elegant high-throughput compound screening approach identified the sarco/endoplasmic reticulum calcium ATPase (SERCA) channels as novel therapeutic targets in NOTCH1-induced T-ALL [102]. SERCA inhibition by the small molecule thapsigargin selectively impaired NOTCH signaling and demonstrated antileukemic activity in both vitro and in vivo model systems [102]. Finally, Schnell and colleagues confirmed the critical role of Hes family BHLH transcription factor 1 (HES1) as downstream component of NOTCH1 signaling in T-ALL and revealed that perhexiline could evoke a strong in vitro and in vivo antileukemic response by reverting the HES1-driven gene expression signature, providing a new lead for targeted T-ALL treatment linked to hyperactive NOTCH1.

Pharmacologic BCL-2 inhibition in T-ALL

As mentioned earlier, immature T-ALLs are often characterized by aberrant IL7R/JAK/STAT activation, and this signaling cascade will eventually converge toward STAT5-mediated activation of the antiapoptotic factor BCL-2 [116]. In addition, antiapoptotic genes show a spatio-temporal expression pattern during T-cell differentiation, with the highest levels of BCL-2 in early T-cell precursors [117].

Given this, pharmacologic inhibition of BCL-2 has been suggested as a promising new therapeutic strategy in immature subtypes of human T-ALL. Indeed, three independent studies [117–119] recently showed that immature T-ALLs display an increased sensitivity toward the highly specific BCL-2 inhibitor ABT-199 [120]. In addition, synergistic effects were reported between ABT-199 and conventional chemotherapeutics that are currently used in T-ALL treatment schedules. Finally, preclinical models of patient derived xenografts confirmed ABT-199 sensitivity in specific T-ALL subtypes providing, additional rationale for including T-ALL patients in clinical trials using this particular drug [117–119].

The PI3K-Akt-mTOR pathway

The PI3K/Akt/mammalian target of rapamycin (mTOR) pathway controls multiple cellular responses, including
metabolic regulation, cell growth, and survival. Activation of PI3K by growth factor stimuli results in the generation of phosphatidylinositol triphosphate (PIP3) in the plasma membrane and subsequent activation of the Akt kinase and downstream target proteins, including mTOR. Importantly, the phosphatase and tensin homologue (PTEN) tumor suppressor negatively regulates PI3K/Akt/mTOR signaling by dephosphorylation of PIP3 [121].

In human T-ALL, constitutive activation of the PI3K/Akt/mTOR signal transduction pathway is achieved by deletions or mutations targeting PTEN in about 15% of cases [122,123] and sporadic gain-of-function mutations in Akt, PIK3R1, or PIK3CA [123–125]. Moreover, normal and malignant thymocytes rapidly activate the PI3K/Akt/mTOR signaling by dephosphorylation of PIP3 [121].

Figure 2. Molecular mechanisms for IL7R/JAK/STAT activation in T-cell acute lymphoblastic leukemia (T-ALL). Graphical overview of oncogenic mechanisms that can drive aberrant IL7R/JAK/STAT signaling in human T-ALL. The left panel illustrates indirect mechanisms, which will eventually result in enhanced IL7R expression and ligand-dependent pathway activation, including aberrant NOTCH1 signaling or sustained expression of ZEB2. The right panel documents the direct mechanism of pathway activation, which result in ligand-independent stimulation, including activating IL7R, JAK1, or JAK3 mutations. Therapeutic targeting of this oncogenic signaling pathway could be achieved by the JAK1/2 inhibitor ruxolitinib or the JAK3 inhibitor tofacitinib. Moreover, aberrant IL7R/JAK/STAT activation will eventually converge toward STAT5-mediated activation of the antiapoptotic factor BCL-2. Activation of this antiapoptotic factor could also be exploited by the BH3-mimetic BCL-2 inhibitor ABT-199.

to show that the Akt pathway is critically involved in expansion of the pool of leukemia-propagating cells that may ultimately give rise to hematologic relapse [131].

Given the aberrant activation of the PI3K/Akt/mTOR pathway, this signaling cascade has been evaluated as novel therapeutic target in T-ALL. The mTOR inhibitor rapamycin showed promising results in preclinical models [132] and might modulate glucocorticoid resistance in T-ALL [133]. However, inhibition of mTOR can hyperactivate Akt by a feedback loop between mTOR, PI3K, and Akt [134]. Therefore, dual PI3K/mTOR small-molecule inhibitors have been developed [135]. They show strong cytotoxic activity against T-ALL cell lines and lymphoblasts obtained from primary human leukemia patients [136]. In addition, direct Akt inhibition leads to rapid cell death in some T-ALL cell lines and primary patient samples [122,137]. At the level of PI3Ks, elegant work recently showed that the p110δ and p110γ isoforms of PI3K are both critically required to sustain T-ALL development in a mouse model induced by conditional loss of Pten in T cells [138]. Moreover, a specific p110δ/p110γ dual inhibitor prolonged the survival of Pten null mice and showed promising effects in Pten-deficient primary human T-ALL tumor cells [138]. Therapeutic targeting of cancer cells by exploiting their addiction to specific PI3K isoforms might be particularly relevant in the context of limiting toxicities that would be associated with pan-PI3K inhibitors.
Targeting general transcription machinery in T-ALL

Impacting on the activity of large enhancers may provide unique opportunities for intervention with oncogenic transcription networks, a concept that has now been firmly established by the use of the BRD4 inhibitor JQ1 in a variety of preclinical tumor models, including T-ALL. Interestingly, chemical inhibition of CDK7, an important component of the transcription factor IIH complex (TFIIH), which regulates transcription elongation activity [139], has recently been achieved by the development of a novel CDK7 inhibitor named THZ1. This molecule directly impacts CDK7 kinase activity, which is required for regulation of the TFIIH complex and therefore globally dampens mRNA transcription of a small set of critical genes involved in T-ALL tumorigenesis [140]. Besides the remarkable in vitro and in vivo antitumor activity observed in T-ALL [140], the anticancer effectiveness of the THZ1 compound was also shown for other tumor entities, such as MYCN-driven neuroblastoma [141] and small-cell lung cancer [142]. Nevertheless, the actual transcriptional response downstream of CDK7 inhibition still needs to be further characterized for most tumors, and the degree of overlap with JQ1-driven BRD4 inhibition remains largely unexplored.

Targeting cap-dependent translation in the biology of T-ALL

Translation of most cellular mRNAs is mediated by a cap structure at the 5′ end of mRNAs. The initiation of this process, which is termed cap-dependent translation initiation, involves a tightly controlled multiprotein initiation complex that consists of omnipresent eukaryotic initiating factors, including the cap-binding protein eIF4E and the RNA helicase eIF4A.

Notably, two recent landmark publications showed that T-ALL cells strictly depend on cap-dependent translation for their survival [143,144]. Indeed, sustained expression of eIF4A or eIF4E was shown to accelerate tumor development in vivo, and strong antileukemic/apoptotic effects were observed using eIF4A (silvestrol)- or eIF4E (4EGI-1)-specific inhibitors [143,144]. This critical dependency for leukemic survival could, at least in part, be explained by a variety of signaling cascades that eventually converge toward enhanced cap-dependent translation activity in human T-ALL through aberrant activation of mTORC1 and mTORC2 [145]. Examples of such signaling pathways include aberrant NOTCH1 activation, enhanced PI3K/Akt signaling through PTEN inactivation, and aberrant receptor tyrosine kinase activity through IL7R or JAK mutations [145]. Moreover, enhanced cap-dependent translation in T-ALL will eventually result in more efficient translation of specific mRNAs with previously established roles in the pathogenesis of T-ALL and unique structural 5′ untranslated region features [143,144].

Mechanisms of disease relapse in T-ALL

The biological basis for disease relapse in T-ALL is poorly understood. The identification and characterization of leu-kemia stem cells might provide novel insights into the mechanisms that mediate disease recurrence in T-ALL and will have important implications for drug development and preclinical disease modeling.

Initial studies have shown that multiple different leukemic T-cell subpopulations at diagnosis have intrinsic repopulation capacity in immunodeficient recipient mice [146–149]. More importantly, pairwise genetic comparison of human T-ALL samples at diagnosis, with corresponding leukemic cells obtained after in vivo engraftment, showed that the xenograft leukemias often contained additional genetic defects targeting known T-ALL oncogenes and tumor suppressors including PTEN, MYC, MYB, and CDKN2A [150]. Interestingly, these genetic abnormalities were present in minor leukemic subclones at diagnosis, suggesting a clonal relationship between the relapse clone and a chemoresistant subpopulation at diagnosis. Competitive engraftment experiments using genetically modified primary leukemia cells showed that, for example, loss of PTEN is able to drive enhanced leukemia-initiating capacity in primary human T-ALL patient samples [150].

These genomic analyses [151] suggest that relapse in T-ALL is mainly mediated by oncogenic hits that are already present in minor leukemic subclones at diagnosis and does not simply result from mutations of specific drug-resistance genes. However, this idea has recently been challenged by the identification of mutations targeting the NT5C2 gene in about 20% of relapsed T-cell ALL patients [152,153]. NT5C2 encodes a 5′-nucleotidase enzyme that can dephosphorylate thiopurine nucleotides, thereby inactivating the purine analogues 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), which are routinely used in T-ALL maintenance therapy. NT5C2 mutations probably represent gain-of-function alleles, since they are clustered in specific regions of the cytosolic 5′-nucleotidase II (NT5C2) protein. Indeed, recombinant NT5C2 mutant protein showed higher enzymatic activity, and expression of NT5C2 mutations in human T-ALL cell lines conferred resistance to the nucleoside analogues 6-MP and 6-TG [152]. Together, these studies document an important role for nucleoside analogue metabolism in the progression and chemoresistance of T-ALL.

With the discovery of the chemo/radioresistant preleu-kemic stem cells, it is becoming increasingly clear that treatment of bulk leukemic cells may not be sufficient to discard the cell of origin. Although the remaining pre-LSCs are not able to cause immediate overt leukemia, their clonal expansion would allow an accumulation of extra oncogenic hits, causing relapse over time. Therefore, it will be important to further study how pre-LSCs are controlled and how they can be therapeutically targeted. Since pre-LSCs appear to be dependent on Notch1 [33], inhibition of NOTCH
Table 1. Opportunities for targeted therapy in T-ALL

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<th>Pathway</th>
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| NOTCH1                   | Activating mutations in the NOTCH1 gene are present in over 50% of T-ALL cases [6]. | • NOTCH1 inhibitory antibodies and stapled peptides (e.g., SAHMI)  
• γ-secretase inhibitors (e.g., compound E)  
• IGF1R inhibitors (e.g., BMS-536924)  
• SERTCA inhibitors (e.g., thapsigargin)  
• HES1-signature antagonists (e.g., perhexiline)  
• JAK1/JAK2 inhibitors (e.g., ruxolitinib)  
• JAK3 inhibitors (e.g., tofacitinib)  
• STAT5 inhibitors |
| IL7R/JAK/STAT            | Activation of pathway in T-ALL by:                                        | • BCL-2 inhibitors (e.g., ABT-199)                                                                                   |
|                          | • Gain-of-function mutations in IL7R, JAK1, JAK3, and STAT5B or loss of PTPN2 | • mTOR inhibitors (e.g., rapamycin)                                                                                   |
|                          | • Overexpression of ZEB2                                                  | • PI3K/MTOR inhibitors (e.g., CAL-130)                                                                             |
|                          | • Activation of NOTCH1 pathway                                            | • dual PI3K/mTOR inhibitors (e.g., PI-103)                                                                          |
| Mitochondrial apoptosis  | Overexpression of BCL-2 is typical for immature T-ALL.                   | • Akt inhibitors (e.g., A443654)                                                                                     |
| PI3K/Akt/mTOR            | IL7R/JAK/STAT signaling also leads to BCL-2 upregulation                | • JMJD3 inhibitors (e.g., GSKJ4)                                                                                     |
| H3K27 demethylation      | JMJD3 is overexpressed and oncogenic in T-ALL                            | • BRD4 inhibitors (e.g., JQ1)                                                                                       |
| General transcription     | Oncogenic driver genes are often associated with super-enhancers and are very strongly transcribed | • CDK7 inhibitors (e.g., THZ1)                                                                                       |
| Cap-dependent translation| T-ALL cells depend on cap-dependent translation for their survival         | • elf4A inhibitors (e.g., silvestrol)                                                                              |
|                          |                                                                          | • elf4E inhibitors (e.g., 4EGI-1)                                                                                  |

Notably, the high survival rates reported in pediatric T-ALL protocols could result from overtreatment of a significant fraction of children. Therefore, and given the long-term side effects associated with intensive chemotherapy, risk stratification in future pediatric T-ALL treatment protocols should be further optimized based on our enhanced understanding of T-ALL disease biology. On the other hand, further reduction of chemotherapy can also be achieved by translation of our molecular genetic findings into novel targeted therapies for the treatment of human T-ALL. In that context, a variety of preclinical studies have reported promising therapeutic effects for particular small-molecule inhibitors targeting specific oncogenic pathways (Table 1). Hopefully, some of these novel therapeutic strategies can be implemented in daily clinical practice in complement with low-dose chemotherapy. This will require an accurate definition of the specific T-ALL patient population that might benefit from these novel targeted therapies.

Conclusion

T-ALL originates from T-cell precursors at different stages of their development and is characterized by distinct and well characterized molecular genetic subtypes. Children affected by this disease respond fairly well to high-dose chemotherapy regimens. Unfortunately, the clinical response in adults remains problematic, and therapeutic options for relapsed T-ALL patients remain scarce.

Notably, the high survival rates reported in pediatric T-ALL protocols could result from overtreatment of a significant fraction of children. Therefore, and given the long-term side effects associated with intensive chemotherapy, risk stratification in future pediatric T-ALL treatment protocols should be further optimized based on our enhanced understanding of T-ALL disease biology. On the other hand, further reduction of chemotherapy can also be achieved by translation of our molecular genetic findings into novel targeted therapies for the treatment of human T-ALL. In that context, a variety of preclinical studies have reported promising therapeutic effects for particular small-molecule inhibitors targeting specific oncogenic pathways (Table 1). Hopefully, some of these novel therapeutic strategies can be implemented in daily clinical practice in complement with low-dose chemotherapy. This will require an accurate definition of the specific T-ALL patient population that might benefit from these novel targeted therapies.

Acknowledgments

This work was supported by the Fund for Scientific Research Flanders (research projects G.0202.09; G.0869.10N to F Speleman; and G065614, 3GA00113N, and G.0C47.13N to P Van Vlierbergh). S. Geossens received a postdoctoral grant. S. Peirs, A. Wallaert, and K. Durinck received PhD grants. B. Poppe is a senior clinical investigator. P. Van Vlierbergh is the recipient of an Odysseus Grant. This work was also supported by the Belgian Foundation against Cancer, the Flemish Liga against Cancer (via postdoctoral grant to F. Matthiasse), Ghent University (GOA grant 12051203 to F. Speleman), the Cancer Plan from the Federal Public Service of Health (grants to F. Speleman and Y. Benoît), the Children Cancer Fund Ghent (grants to F. Speleman and Y. Benoît), and the Belgian Program of Interuniversity Poles of Attraction (grant no.36509110 to F. Speleman). Additional funding was provided by an Australian National Health and Medical Research Council grant (no. APP1047995) to J. Haigh.

Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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