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Pediatric T-Cell Acute Lymphoblastic Leukemia

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Running title: T-ALL

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The most common pediatric malignancy is acute lymphoblastic leukemia (ALL), of which T-cell ALL (T-ALL) comprises 10-15% of cases. T-ALL arises in the thymus from an immature thymocyte as a consequence of a stepwise accumulation of genetic and epigenetic aberrations. Crucial biological processes such as differentiation, self-renewal capacity, proliferation, and apoptosis are targeted and deranged by several types of neoplasia-associated genetic alteration, for example translocations, deletions, and mutations of genes that code for proteins involved in signaling transduction, epigenetic regulation, and transcription. Epigenetically, T-ALL is characterized by gene expression changes caused by hypermethylation of tumor suppressor genes, histone modifications, and miRNA and lncRNA abnormalities. Although some genetic and gene expression patterns have been associated with certain clinical features, such as immunophenotypic subtype and outcome, none has of yet generally been implemented in clinical routine for treatment decisions. The recent advent of massive parallel sequencing technologies has dramatically increased our knowledge of the genetic blueprint of T-ALL, revealing numerous fusion genes as well as novel gene mutations. The challenges now are to integrate all genetic and epigenetic data into a coherent understanding of the pathogenesis of T-ALL and to translate the wealth of information gained in the last few years into clinical use in the form of improved risk stratification and targeted therapies. Here, we provide an overview of pediatric T-ALL with an emphasis on the acquired genetic alterations that result in this disease.

A BRIEF HISTORICAL BACKGROUND

> "Several cases exist with a great excess of white blood cells (…) Blood of such patients contains so many white blood cells that at first glance I thought they contained purulent matter. In fact I believe the excess of white blood cells is due to an arrest of maturation of blood. From my theory on the origin of blood cells, the overabundance of white blood cells should be the result of an arrest of the development of intermediate cells."

This was written in 1844 by the French physician Alfred Donné, a pioneer in using microscopic examinations for the study of disease (Degos, 2001). Donné examined the blood from patients with splenomegaly and noted that what had previously been thought of as "pus in the blood" was in fact leukocytosis. He suggested, quite presciently, that the excess of white blood cells (WBC) could be due to a maturation arrest (Donné, 1844; Degos, 2001). However, the first reports recognizing leukemia as a distinct clinical entity are more often attributed to John Hughes Bennett and Rudolf Virchow. In 1845, Bennett, a professor at the Institutes of Medicine in Edinburgh, performed an autopsy on a 28-year-old man with hepatosplenomegaly. He published an extensive post mortem report, in which he proposed that the peripheral blood (PB) abnormalities were independent of inflammation, concluding that the patient had had a systemic blood disorder. Bennett called it leucocythemia (Bennett, 1845; Piller, 2001). A few months later, Virchow, a German pathologist at the Charité Hospital in Berlin, reported similar findings based on an autopsy on a 50-year-old woman with edema, splenomegaly, and nose bleeding. When examining the PB, a skewed distribution of erythrocytes and WBC compared with normal blood was found. His initial publication was entitled "Weisses Blut" ("white blood") but in a paper a few years later, Virchow named the disease "Leukämie" (from Greek *leukos* "clear, white" and *haima* "blood") (Virchow, 1845, 1848).

The first case of pediatric leukemia was reported in 1850 by Henry William Fuller, who described a 9-year-old girl presenting at the St George's Hospital in London with frequent hemorrhages, enlarged spleen, and leukocytosis; she died after a few months. Although subsequently often believed that she had had acute leukemia, a later review of Fuller's

detailed notes suggests that she actually may have had chronic myeloid leukemia (Fuller, 1850; Piller, 2001). In fact, the distinction between acute and chronic leukemia was first made seven years after Fuller's publication by Nikolaus Friedrich and leukemias originating from myeloid and lymphoid lineages were not distinguished until almost 30 years later by Paul Ehrlich (Friedrich, 1857; Ehrlich, 1879; Piller, 2001). Interestingly, the relatively high prevalence of acute leukemia in children was not recognized until 1917 when an epidemiological survey on 1,457 cases of leukemia was performed by Gordon Ward while serving as a medical officer with the Royal Army Medical Corps (Ward, 1917).

More than 50 years later, in 1973, Drs. Luis Borella and Luisa Sen, both at St. Jude Children's Research Hospital in Memphis, described a 7-year-old boy with ALL who had a pronounced leukocytosis, with the lymphoblasts displaying T-cell surface markers, and hepatosplenomegaly (but with no clinical or radiologic evidence of thymic enlargement); two years later, they established that T-ALL is a clinically as well as biologically distinct disease entity (Borella and Sen, 1973; Sen and Borella, 1975). Thus, T-ALL is a surprisingly recent member of the acute leukemia group.

THE PLACE AND CELL OF ORIGIN

The thymus is a mediastinal organ located right behind the sternum. During the early years of life, the thymus is prominent but with increasing age a loss of tissue mass and structure takes place, a process called involution (Shanley et al., 2009). The ancient Greeks knew about the thymus − the name is believed to derive either from the Greek word thumos, meaning spirit, soul, and courage, or from the plant Thymus vulgaris, possibly due to a vague resemblance of the thymus to the leaf of the common thyme (Lavini, 2008). However, the function of the thymus remained elusive for centuries. It was not until the early 1960s that Jacques Miller, at the Chester Beatty Research Institute in London, showed that the thymus

plays a major role in lymphopoiesis and that it performs an important immunological function (Miller, 1961). Today, it is well known that the thymus orchestrates the complex symphony of the T-cell differentiation process that results in functional, self-tolerant T-cells (Miller, 2011).

The thymus is dependent upon the input of progenitor cells, thymocytes, from the bone marrow (BM). Early thymic progenitors – the most immature thymocytes – enter the thymus, make a loop through the outer cortex, and then return to the inner medulla. During this journey throughout the thymus, maturation occurs, as manifested by the assembly of the T-cell receptor (*TCR*) genes (Figs. 1A and 2). This differentiation route is highly intricate – somatic rearrangements of the *TCR* genes must be completed and the functionality and tolerance of the TCRs need to be scrutinized by both positive and negative selection (Fig. 1A). The mature T-cells exit the thymus and begin to circulate in the blood as well as through different lymphatic and non-lymphatic organs (Fig. 1C). However, less than 5% of the thymocytes generated in the thymus leave as mature T-cells – this loss of more than 95% of aspiring thymocytes is the price we have to pay for an effective, self-tolerant immune defense (Egerton et al., 1990).

Acquired genetic and epigenetic changes in an immature thymocyte result in either T-ALL or T-cell lymphoblastic lymphoma (T-LBL) (Fig. 1B), both of which are included as T lymphoblastic leukemia/lymphoma in the World Health Organization classification (Swerdlow et al., 2008). T-ALL and T-LBL are usually considered to be different manifestations of the same disease, with the extent of BM involvement being the main feature to distinguish T-ALL (>25% BM blasts) from T-LBL; the latter corresponds to a nodal distribution of the disease, often with a large mediastinal mass, with no or minimal signs of PB or BM involvement. However, even though these entities display overlapping clinical and immunophenotypic features, some studies have identified distinct gene expression profiles,

indicating a greater biological difference between T-ALL and T-LBL than previously surmised (Basso et al., 2011).

SYMPTOMS AND SIGNS

The malignant clone arising in the thymus proliferates and propagates throughout the body, mainly in the BM, PB, lymph nodes, and central nervous system (CNS) (Fig. 1D). This spread results in the signs and symptoms associated with T-ALL, such as suppressed BM function, leukocytosis, neurological abnormalities, and respiratory difficulties.

The expansion of immature lymphoblasts in the BM represses hematopoiesis, resulting in a deficiency of normal PB cells. Fever, recurrent infections, fatigue, paleness, and skin/mucosal bleeding are frequent, and so are bone pain and arthralgia (Bernbeck et al., 2009). A mediastinal mass (~60%) and CNS involvement (~10%) are common in T-ALL and result in dyspnea, headache, nausea, and visual impairment. T-ALL is characterized by a high WBC count (median 60-70 x 10^9/l) and peripheral lymphoblasts; almost 50% of patients have hyperleukocytosis (>100 x 10^9/l) (Bernbeck et al., 2009; Karrman et al., 2009a; Toft et al., 2013). The latter is associated with neurological (headache, mental changes, seizures, and brain hemorrhage), pulmonary (respiratory symptoms, hypoxia, and pulmonary infiltrates on X-ray), and metabolic (tumor lysis syndrome: hyperuricemia, hyperkalemia, and hyperphosphatemia) complications.

IMMUNOPHENOTYPIC FEATURES

Because T-ALL and B-cell precursor (BCP) ALL lymphoblasts are morphologically indistinguishable, immunophenotypic analyses are essential to identify the lineage involved (Swerdlow et al., 2008). The common immunophenotypic theme of T-ALL is the expression of a set of intracellular or cell surface antigens, collectively termed T-cell markers. CD3,

expressed in the cytoplasm or on the cell surface, is T-lineage specific and hence of great diagnostic importance. CD3 associates with membrane-bound TCRs resulting in TCR complexes, the functionality of which is necessary for the intrathymic survival of T-cells. The DNA polymerase terminal deoxynucleotidyl transferase (TdT) is usually expressed in T-lymphoblasts and so are, variably, CD1a, CD2, CD4, CD5, CD7, and CD8, involved in antigen presentation (CD1), cell-cell interactions and adhesion (CD2 and CD7), assisting the TCR receptor (CD4 and CD8), and modulating immune response (CD5). In addition, aberrant expression of one or both of the myeloid-associated CD13 and CD33 is seen in 20-30% of cases (Swerdlow et al., 2008).

As normal thymocytes mature, they are characterized by a change in the expression of CD markers; to some extent, the immunophenotypic features of T-ALL lymphoblasts mirror the normal, physiological maturation process. The constellation of CD markers in a T-ALL case may hence suggest at which stage the leukemic differentiation block occurred (Berquam-Vrieze et al., 2011). Four subgroups of T-ALL have been defined according to their CD patterns: 1) pro-T (T-I): cCD3+/CD7+/CD2-/CD1a-/CD34+/-; 2) pre-T (T-II): cCD3+/CD7+/CD2+/CD1a-/CD34+/-; 3) cortical T (T-III): cCD3+/CD7+/CD2+/CD1a+/CD34-; and 4) medullary T (T-IV): cCD3+/CD7+/CD2+/CD1a-/CD34-/membrane CD3+. These differ slightly prognostically in the sense that pro- and pre-T-ALL cases tend to have a worse and the cortical and medullary subtypes a better outcome (Schrappe et al., 2011). The T-I and T-II stages are double negative for CD4 and CD8 and the T-III is double positive for CD4 and CD8, whereas the more mature T-IV is single positive for either CD4 or CD8 (Bene et al., 1995; Swerdlow et al., 2008). T-ALL cases corresponding to specific stages often display a gene expression profile resulting from deregulation of a particular oncogene, such as *MEF2C*, *TLX1* (a.k.a. *HOX11*), and *TAL1* in the immature, early, and late cortical stages, respectively (Ferrando et al., 2002; Homminga et al., 2011). Thus,

gene expression patterns can be used, albeit indirectly, to identify underlying, pathogenetically important genetic changes.

A recently delineated subtype of T-ALL – the early T-cell precursor ALL (ETP ALL) − is thought to originate from the earliest thymic immigrants; however, some data suggest a more differentiated cell of origin (Berquam-Vrieze et al., 2011). ETP ALL cases, which comprise 5-15% of T-ALL, display a unique constellation of cell surface markers: CD1a-/CD8-/CD5(+)/cCD3+/CD7+ and expression of one or more of the myeloid- or stem cell-associated markers, CD13, CD33, CD34, CD117, HLA-DR, CD11b, and CD65. Interestingly, the gene expression profiles of ETP ALL are similar to those of hematopoietic stem cells and myeloid progenitors and, furthermore, ETP ALL cases frequently harbor mutations in genes commonly mutated in acute myeloid leukemia, such as *DNMT3A*, *ETV6*, *FLT3*, *GATA3*, *IDH1*, *IDH2*, *JAK3*, *NRAS*, and *RUNX1* (Zhang et al., 2012; Haydu and Ferrando, 2013). Initially, patients with ETP ALL were considered to have a very dismal prognosis but more recently, using current treatment strategies, the outcome has improved (Coustan-Smith et al., 2009; Patrick et al., 2014; Conter et al., 2016).

TCR REARRANGEMENTS

The *TCR* genes in T-cells are, similar to the immunoglobulin genes (*IG*) in B-cells, rearranged in a specific order during the differentiation process, starting with *TRD* (at 14q11.2 coding for δ protein), followed by *TRG* (7p14.1, γ protein), *TRB* (7q34, β protein), and *TRA* (14q11.2, α protein). Recombination of *TRA* leads to deletion of *TRD* because it is located within *TRA* (Fig. 2). Each T-cell harbors a unique *TCR* rearrangement, like a DNA fingerprint, due to combinatorial and junctional diversification during the V(D)J recombination process (Nemazee, 2006). Because T-ALL is a clonal disease, an identical *TCR* rearrangement, initially generated in the ancestral cell, will be present in all lymphoblasts.

Hence, in contrast to an inflammatory process that gives rise to a polyclonal T-cell response, the malignant T-cells display *TCR* monoclonality, which can be used as a leukemic marker in minimal residual disease (MRD) monitoring. Although the monoclonal legitimate *TCR* rearrangement as such does not participate in the pathogenesis of T-ALL, a recent study showed that persistent TCR signaling possesses a potent tumor-suppressive function, a property which may prove suitable for therapeutic interventions (Trinquand et al., 2016). More than 90% of T-ALL cases carry at least one monoclonal rearrangement, most often involving *TRG* and *TRB* (Kraszewska et al., 2012). In a small subset of cases, however, there is no biallelic *TRG* rearrangement; this represents an immature form of T-ALL associated with a poor outcome (Szczepański et al., 2000; Gutierrez et al., 2010a). Cases without *TRG* rearrangements partially overlap with ETP ALL in the sense that such rearrangements also are infrequent in ETP ALL; furthermore, the gene expression profiles are similar in the two groups. However, although they express some CD markers in common, the former cases only rarely meet the immunophenotypic criteria for ETP ALL (Coustan-Smith et al., 2009; Gutierrez et al., 2010a).

It should be stressed that cross-lineage *IG* rearrangements occur in 10-20% of T-ALL and *TCR* rearrangements are found in up to 70% of BCP ALL; thus, their presence cannot be used for lineage assignment (Swerdlow et al., 2008).

EPIDEMIOLOGY AND ETIOLOGY

Epidemiology

Leukemia is the most common pediatric cancer, accounting for one-third of all cases, with an incidence of ~4/100,000 children/year. The vast majority (80-85%) is ALL, of which T-ALL comprises 10-15% (Hjalgrim et al., 2003; Siegel et al., 2012). Patient characteristics differ between BCP ALL and T-ALL. The former has a marked incidence peak between 2 and

5 years of age, with a male/female ratio of 1.2, whereas the latter has a flatter incidence curve, with a median age of 9 years, and a marked male predominance – boys have a threefold increased risk of T-ALL (Schneider et al., 2000; Hjalgrim et al., 2003; Ballerini et al., 2008; Karrman et al., 2009a). The reasons for the age difference between BCP ALL and T-ALL are not fully understood, but the fact that prenatal initiation of pediatric BCP ALL is common whereas genetic changes in T-ALL only rarely arise in utero may partly explain the later occurrence of T-ALL (Ford et al., 1997; Gale et al., 1997; Fischer et al., 2007; Eguchi-Ishimae et al., 2008; Mansur et al., 2015). As to the skewed gender distribution, the reason(s) for this is unknown, although inactivating mutations or deletions of X-linked tumor suppressor genes have been suggested to play a role. Possible candidate genes include *KDM6A* (a.k.a. *UTX*) at Xp11.3, *PHF6* at Xq26.2, and *RPL10* at Xq28, which code for lysine (K)-specific demethylase 6A, PHD finger protein 6, and ribosomal protein L10, respectively. However, both *PHF6* and *RPL10* appear to undergo X inactivation in females (Ariës and Gutierrez, 2015), making them less likely to be involved in the preponderance of males with T-ALL. In contrast, *KDM6A*, which is preferentially mutated in male T-ALL cases, escapes X inactivation and could hence contribute to the gender bias (Van Vlierberghe et al., 2010; De Keersmaecker et al., 2013; Van der Meulen et al., 2015).

Etiology

Etiological studies have mainly focused on BCP and mature B-cell ALL – the relatively low incidence of T-ALL makes it difficult to obtain robust etiological data for this subtype. This notwithstanding, investigations of causative mechanisms for BCP ALL may, at least to some extent, be relevant also to T-ALL, given that both diseases originate from hematopoietic progenitor cells.

Environmental Exposures

The only definite external cause of ALL is ionizing radiation (Wakeford, 2008). Thus, both pre- and postnatal exposures are now kept to a minimum – irradiation should hence not contribute significantly to cases occurring today. Several other risk factors have been proposed, such as parental smoking, paint and household chemicals, pesticides, maternal diet, traffic fumes, and electric fields, but so far none has been shown convincingly to add to the incidence of childhood ALL (Eden, 2010). That infections and/or the body's response to them may promote leukemia development has been postulated in two separate, albeit somewhat overlapping, theories: "population mixing" and "delayed-infection" (Kinlen, 2004; Greaves, 2006). Circumstantial evidence for the "delayed-infection" hypothesis comes from investigations showing that children who are exposed to common infections at an early age, measured by proxy as day-care attendance and antibiotic prescriptions, have a reduced risk of ALL (Urayama et al., 2010; Gradel and Kærlev, 2015). It should be emphasized, however, that these studies were mainly based on BCP ALL cases.

A rare cause of T-ALL has, unfortunately, been shown to be iatrogenic. A number of children with immunodeficiency syndromes treated by retrovirus-mediated gene therapy subsequently developed T-ALL. This could be attributed to vectors integrating near oncogenes, most commonly *LMO2*, resulting in deregulated expression of the target genes, akin to the effect of *TCR* translocations in T-ALL, as discussed below (Hacein-Bey-Abina et al., 2003; Braun et al., 2014). Needless to say, this dampened the enthusiasm for gene therapy quite dramatically, putting the entire field under close scrutiny.

Genetic Predisposition

Is there an inherent difference among children as to how susceptible they are to develop ALL? Although a few syndromes due to germline mutations in high penetrance genes do

confer an increased risk of ALL, such as ataxia-telangiectasia, caused by mutations in the cell cycle checkpoint serine/threonine protein kinase gene *ATM* at 11q22.3 and associated with T-ALL, hereditary syndromes are a rare cause of ALL (Seif, 2011). However, a study of late T-ALL relapses, occurring ≥2.5 years after diagnosis, revealed that the leukemia- and clone-specific markers were completely different between diagnosis and recurrence in eight of the 22 investigated cases, suggestive of new primary T-ALLs rather than relapses (Szczepański et al., 2011). One explanation for this could be that patients with two “separate” T-ALLs are genetically predisposed to develop this disease; indeed, one of the patients in that study harbored a constitutional del(11)(p12p13), previously associated with T-ALL as an acquired abnormality (Van Vlierberghe et al., 2006). However, the existence of a pre-leukemic, ancestral clone could also give rise to two seemingly distinct leukemias (Shlush et al., 2014). This possibility can of course be addressed by next generation sequencing (NGS) analyses of paired samples in order to find identical mutations in the “different” leukemias (Kunz et al., 2015).

Although constitutional high penetrance alleles are a rare cause of ALL, frequent but low-penetrant alleles may play a role. Indeed, genome-wide association studies have identified several polymorphisms in genes, such as *ARID5B* (located in 10q21.2), *CDKN2A* (9p21.3), *CEBPE* (14q11.2), *GATA3* (10p14), *IKZF1* (7p12.2), and *PIP4K2A* (10p12.2), that influence the risk, albeit with low impact, of childhood ALL. However, these polymorphisms have primarily been associated with an increased risk of BCP ALL; in fact, only *CDKN2A* polymorphisms have been clearly associated with T-ALL to date (Papaemmanuil et al., 2009; Treviño et al., 2009; Sherborne et al., 2010; Perez-Andreu et al., 2013; Xu et al., 2013).

If genetic and/or environmental risk factors play a role in the etiology of ALL, one would expect that siblings of children with ALL would have an increased risk of this disease but that is generally not the case (Winther et al., 2001). However, a few studies have indicated

an ALL-subtype concordance among siblings affected by multiple cases of pediatric ALL (Mansur et al., 2011; Schmiegelow et al., 2012). Notably, Schmiegelow et al. (2012) found that in five of the six families reported where the first case was T-ALL, the subsequent case (or cases) was also T-ALL. This indicates that certain, but presently unknown, etiologic/genetic factors may be specific for T-ALL.

SURVIVAL AND MRD

The survival rates of childhood ALL have improved dramatically since the 1960s, when only 5-10% of patients were cured (Pui and Evans, 2013); at present, the probabilities of 5-year event-free survival and overall survival for BCP ALL exceed 80% and 90%, respectively (Pui et al., 2015). However, the event-free and overall survival for children, adolescents, and younger adults with T-ALL is generally inferior, just over 70% and 80%, respectively (Table 1), with relapsed T-ALL having a particularly dismal outcome (~20%). Hopefully, treatment that targets specific leukemia-specific mutations, many of which are now being identified by NGS, will improve the prognosis (Roti and Stegmaier, 2014).

Considering the poor survival after relapse, it is of vital importance to identify patients already early on during treatment who are at increased risk of such an event. Thus, MRD monitoring has proved important to evaluate the effect of the treatment given, thereby assessing the risk of relapse. MRD analysis can, in principle, be performed by three different approaches: 1) real-time quantitative PCR (qPCR) analyses of monoclonal *IG*/*TCR* rearrangements; 2) flow cytometric detection of aberrant leukemic immunophenotypes; and 3) qPCR analyses of leukemia-specific fusion genes and/or gene mutations. The latter approach is seldom used in T-ALL because only a minority of cases carries a suitable target (van Dongen et al., 2015). Furthermore, because detection of MRD by flow cytometry has proved less sensitive than qPCR in T-ALL (Vaitkevičienė et al., 2011), MRD evaluation in clinical

routine is most often done by qPCR analyses of the *TCR* genes (Bandapalli et al., 2013; Jenkinson et al., 2013; Toft et al., 2013). The methodological sensitivity of qPCR is somewhere between 10^{-4} and 10^{-5}, i.e., one leukemic cell among 10,000-100,000 normal cells can be detected (Teachey and Hunger, 2013). The importance of MRD analyses is clearly exemplified by the AIEOP-BFM-ALL 2000 study that showed that MRD $\geq 10^{-3}$ at day 79 constituted the most powerful predictor for relapse of T-ALL (Schrappe et al., 2011).

GENETICS OF T-ALL

In the last decade, it has become evident that both genetic and epigenetic abnormalities play an essential role in the leukemogenic process underlying T-ALL, as will be reviewed in the following paragraphs.

Karyotypic Features

Conventional chromosome banding analyses reveal clonal abnormalities in 55-75% of T-ALL cases (Table 2), a frequency clearly lower than that of 85-90% in childhood BCP ALL (Zachariadis et al., 2011). However, the proportion of aberrant T-ALL increases quite substantially if interphase fluorescence in situ hybridization (FISH) or single nucleotide polymorphism array (SNP-A) analyses are performed (Karrman et al., 2015). Hence, chromosomal changes in T-ALL often escape cytogenetic detection. The reasons for this are manifold, including poor chromosome morphology, analysis of non-neoplastic dividing BM cells, and the presence of cytogenetically cryptic abnormalities, such as deletions and duplications <5-10 Mb and translocations involving chromosome bands of similar size and banding pattern.

Modal Chromosome Number Distribution

Close to 70% of karyotypically abnormal pediatric T-ALL cases have 46 chromosomes, i.e., they are pseudodiploid; the modal chromosome numbers of most other cases are 45 or 47-49 (Fig. 3A). Thus, the ploidy groups that constitute specific entities in BCP ALL – near-haploidy, low hypodiploidy, high hyperdiploidy, near-triploidy, and near-tetraploidy – are, in all practice, not seen in T-cell ALL, with the exception of rare cases of tetraploid T-ALL (Paulsson and Johansson, 2009; Lemež et al., 2010).

Number and Types of Chromosomal Abnormalities

T-ALL is only rarely karyotypically complex; in fact, approximately 50% of cytogenetically abnormal childhood T-ALL cases harbor only one chromosomal aberration; two, three, and four changes are found in ~25%, ~10%, and ~8% of cases, respectively (Fig. 3B). Thus, prior to interphase FISH, SNP-A, and molecular genetic analyses revealing several additional rearrangements and mutations in T-ALL, there was little evidence for a multistep oncogenic process underlying T-ALL, something that is now widely accepted (Zhang et al., 2012). Structural chromosome changes are much more common than trisomies/monosomies: 65% of cases carry only structural abnormalities, 25% both structural and numerical changes, and 10% only gains/losses. This holds true also for T-ALL with single chromosomal changes, 90% of which are structural and 10% numerical (Mitelman et al., 2016).

Thus, in conclusion, T-ALL is typically karyotypically characterized by the presence of only one or a few structural chromosome changes.

Frequent Chromosome Abnormalities and Their Molecular Genetic Correlates

A database search of all published, cytogenetically abnormal pediatric T-ALL cases (Mitelman et al., 2016) reveals that the most common aberrations are translocations or inversions involving the *TCR* loci (~30% of aberrant cases), deletions of 6q (~20%), loss of

9p material through deletions or unbalanced translocations (~15%), and trisomy 8, deletions of 11q, and loss of 12p (5-10%), frequencies on a par with those in larger population-based series of childhood T-ALL (Heerema et al., 1998; Schneider et al., 2000; Karrman et al., 2009a). In cases with single changes, the proportion of *TCR* translocations/inversions remains approximately 30%, whereas the frequencies of 6q and 9p deletions (10%) and +8 and loss of 11q and 12p (<5%) decrease. Thus, based on cytogenetic data alone, *TCR* translocations are primary abnormalities whereas the unbalanced changes are secondary, in line with neoplasia-associated chromosome aberrations in general (Johansson et al., 1996).

There is ample evidence that the functionally important outcome of the 9p deletions in T-ALL is loss of *CDKN2A*, encoding the cell cycle regulator cyclin-dependent kinase inhibitor 2A (Kawamata et al., 2008; Mullighan et al., 2008; Yu et al., 2011; Karrman et al., 2015). As to the genes targeted by del(6q), it is surprising and frustrating that they remain, like the Scarlet Pimpernel, elusive – not least considering that a relatively high frequency of 6q deletions was identified in T-ALL already in the late 1970s (Mitelman et al., 2016). However, the minimally deleted region has been delineated to 6q15-16, in which three genes that code for the caspase 8 associated protein 2 (*CASP8AP2*), the EPH tyrosine kinase receptor A7 (*EPHA7*), and the glutamate receptor, ionotropic, kainate 2 (*GRIK2*) have been suggested as candidate tumor suppressors (Sinclair et al., 2004; Remke et al., 2009; López-Nieva et al., 2012). Little is also known about the molecular genetic consequences of the 11q deletions, although loss of the *ATM* gene has been proposed as one likely target (Krieger et al., 2010). As regards trisomy 8 and loss of 12p, their functional outcome remains unresolved. In fact, these common, cytogenetically unbalanced changes may not be “reducible” to one or a few genes gained or lost. Larger gene dosage effects are perhaps more likely but unfortunately less amenable for analysis.

TCR Translocations and Inversions

In the beginning of the 1980s, it was shown that some avian leucosis viruses could induce tumors in chicken by integrating close to, and thus deregulating, "cellular oncogenes"; this became known as "oncogenesis by promoter insertion" (Hayward et al., 1981). It was subsequently hypothesized that also human neoplasms could result from genetic transpositions, such as translocations, leading to aberrant expression of genes located at the chromosome breakpoints (Klein, 1981). Evidence in support of this soon emerged from studies of Burkitt lymphoma revealing that the *MYC* gene at 8q24.21 was deregulated as a consequence of being illegitimately recombined with the *IGH*, *IGK*, and *IGL* loci at 14q32.33, 2p11.2, and 22q11.22, respectively (Leder et al., 1983). A few years later, aberrant expression of oncogenes due to illegitimate rearrangements with the *TCR* genes was also identified in T-ALL (Erikson et al., 1986).

Translocations and inversions involving the *TCR* genes are now recognized as the oncogenic hallmark of T-ALL; they are present in close to 30% of all cases as ascertained by FISH and PCR analyses, with almost 50% going undetected by chromosome banding analysis (Le Noir et al., 2012). The *TCR* loci most often illegitimately recombined with target genes are *TRD* and *TRB*, i.e., translocations and inversions involving 14q11 and 7q34, respectively. In contrast, *TRA* is seldom involved and aberrations affecting *TRG* are very rare (Cauwelier et al., 2006; Le Noir et al., 2012; Sugimoto et al., 2014).

By characterizing *TCR* rearrangements several partner genes have been identified and proved to be important for T-ALL oncogenesis (Erikson et al., 1986; Ellisen et al., 1991; Fitzgerald et al., 1991), as further discussed below. The formation of *TCR* aberrations are facilitated by the somatic *TCR* recombination process that is active in all developing thymocytes (Fig. 2) and dependent on the induction of multiple double strand breaks (DSBs) by the recombination-activating RAG proteins. If there are foreign DSBs, occasionally also

recombinase-mediated (Le Noir et al., 2012), present in the vicinity during this process, the repair system may misalign the strands, resulting in illegitimate recombinations.

Pathogenetic Outcome

Most illegitimate *TCR* recombinations generated probably do not afford any advantages to the cell and are hence not selected for – thus, they will go undetected. If, on the other hand, DSBs occur close to a gene with transforming enhancing capability, it may be placed under the control of *TCR* enhancers or promoters, resulting in its overexpression and conferring a selective advantage to the cell (Graux et al., 2006). However, not all *TCR* translocations exert their influence in this way. For example, Dik et al. (2007) showed that removal of a negative regulatory element from the *LMO2* locus is the main mechanism underlying the activation of this gene by the t(11;14)(p13;q11). Furthermore, *TCR* translocations may actually, albeit rarely, instead lead to silencing of a gene, as shown for *BCL11B* and *LEF1*; these two genes are otherwise relatively often targeted by deletions or inactivating mutations in T-ALL (Gutierrez et al., 2010b, 2011; Le Noir et al., 2012; Ehrlich et al., 2014).

Approximately 30 genes are now known to recombine illegitimately with *TCR* loci in T-ALL (Table 3). The gene categories mainly targeted are class II basic helix-loop-helix (bHLH) and homeobox transcription factor genes and LIM-domain-only (LMO) genes that modulate transcriptional complexes by protein-protein interactions. More rarely, other types of gene are implicated, such as those coding for cyclins, kinases, and receptors. Thus, the functional outcome of most *TCR* translocations is deregulated transcription with ensuing aberrant gene expression. Indeed, cases with certain *TCR* partner genes, such as *HOXA*, *LMO1/2*, *TAL1*, *TLX1*, and *TLX3*, represent T-ALL subgroups that are characterized by distinct gene expression patterns (Ferrando et al., 2002; Van Vlierberghe et al., 2008b,c).

It should be emphasized that some of the genes targeted may be deregulated by mechanisms other than *TCR* rearrangements, for example *TAL1* by interstitial 1p33 deletions, insertions, disruption of looping chromosomal structures, and long-range chromatin interactions (Janssen et al., 1993; Mansour et al., 2014; Patel et al., 2014; Navarro et al., 2015; Hnisz et al., 2016), *LMO2* by 11p12-13 deletions (Van Vlierberghe et al., 2006), *TLX3* by a translocation to *BCL11B* (MacLeod et al., 2003), *MYB* by gene duplication (Clappier et al., 2007), *HOXA* by the fusion proteins KMT2A-MLLT1, PICALM-MLLT10, and SET-NUP214 (Soulier et al., 2005; Van Vlierberghe et al., 2008c), *MYC* by non-*TCR* rearrangements and focal duplications of the NOTCH1-driven *MYC* enhancer (Herranz et al., 2014; La Starza et al., 2014), and *NOTCH1* by activating mutations (Weng et al., 2004). Thus, a rare *TCR* partner may well play a greater role in T-ALL development than surmised based on the incidence of the translocation alone.

Fusion Genes

More than 75 fusion genes generated through various types of abnormality, mainly translocations but also deletions, insertions, and episome formation, have so far been reported in T-ALL (Table 4). Half of these encode factors involved in transcriptional regulation, thus recapitulating the “altered gene expression” theme of *TCR* translocations and inversions. A smaller fraction (14/76; 18%) codes for tyrosine kinases, several of which are candidates for targeted therapy.

Some of the fusion genes, for example those involving *ABL1*, *ETV6*, and *KMT2A*, were identified through molecular genetic characterization of cytogenetically detectable chromosomes changes or abnormalities identified by FISH screening for rearrangements of these genes. In the last few years, however, NGS analyses have revealed a plethora of novel fusion genes. In fact, half of the fusion genes listed in Table 4 were detected by whole-

genome and transcriptome sequencing. Many of these fusions are out-of-frame, resulting in loss of function (Zhang et al., 2012; Atak et al., 2013). Furthermore, they often co-occur in individual cases. For example, there was an average of 5.5 fusion events per patient sample in the study by Atak et al. (2013). Considering that all NGS-identified fusions have, as of yet, only been reported in single cases, their pathogenetic and clinical impact remains to be elucidated. It will be an arduous, almost Herculean, task to characterize functionally all new gene fusions discovered by NGS; some of them may well turn out to be innocent bystanders or, rather, passengers. In fact, this may be the case for the vast majority of tumor-associated fusion genes identified by NGS to date (Mertens et al., 2015).

Submicroscopic Copy Number Alterations and Uniparental Isodisomies

In the "old days", cytogenetically cryptic genomic imbalances in T-ALL were mainly identified by targeted interphase FISH analyses focusing on only a few loci/genes, such as 9p21/*CDKN2A* and 1p33/*STIL-TAL1*. Thus, our knowledge about the pattern of copy number abnormalities (CNAs) in T-ALL was not only limited but also heavily biased. However, this all changed with the advent of SNP-A (Mullighan et al., 2007), which enabled genome-wide detection of CNAs as well as of uniparental isodisomies (UPIDs).

SNP-A analyses of larger T-ALL series have revealed that 30-40% harbor segmental UPIDs (sUPIDs) and that most carry CNAs, with a mean of 3-7 CNAs per case (Mullighan et al., 2007, 2008; Yu et al., 2011; La Starza et al., 2013; Karrman et al., 2015). In contrast, chromothripsis, i.e., tens to hundreds of clustered CNAs that oscillate between two copy number states and that most likely occur during a single cellular catastrophe (Stephens et al., 2011; Cai et al., 2014), seems to be quite rare in T-ALL, with the possible exception of ETP ALL (Zhang et al., 2012; Karrman et al., 2015).

Deletions

The vast majority (>75%) of CNAs in T-ALL are deletions, many of which possibly RAG-mediated (Raschke et al., 2005; Mendes et al., 2014), that typically target tumor suppressor genes, most frequently (>70% of cases) *CDKN2A*, with a concurrent *CDKN2B* deletion in a slightly lower frequency (Mullighan et al., 2008; Yu et al., 2011; Zhang et al., 2012; Karrman et al., 2015). Other relatively common deletions involve the transcriptional repressor *BCL11B* (at 14q32.2), the transcription factor *LEF1* (4q25), the tumor suppressors *PTEN* (10q23.31), and *WT1* (11p13), the tyrosine phosphatase *PTPN2* (18p11.21), and genes involved in epigenetic regulation, such as those coding for the histone modifiers EED (11q14.2), EZH2 (7q36.1), PHF6 (Xq26.2), and SUZ12 (17q11.2) (Balgobind et al., 2008; Gutierrez et al., 2009, 2010b, 2011; Tosello et al., 2009; Kleppe et al., 2010; Van Vlierberghe et al., 2010; Ntziachristos et al., 2012; Zhang et al., 2012). Many of these, with the notable exception of *CDKN2A/B*, are also relatively often inactivated through mutations, with or without coexisting deletions of the other allele, in T-ALL (Table 5).

The pathogenetic impact of deletions is not always loss of gene function, however. Instead, they may actually lead to activation of target genes. A prime example of this is the interstitial 90 kb deletion in 1p33 that places *TAL1* under the control of the promoter of the neighboring gene *STIL*, which is highly expressed in T-cells; this promoter swapping results in overexpression of *TAL1* (Janssen et al., 1993; Ferrando et al., 2002; Yu et al., 2011). Furthermore, cryptic deletions of variable size in 11p12-13 have been shown to remove a negative regulatory element located 3 kb upstream of *LMO2*, leading to elevated expression of this gene (Van Vlierberghe et al., 2006, 2008a). Yet another outcome of a deletion in T-ALL is a fusion of the two genes located in the breakpoints, as exemplified by the *SET-NUP214* chimera that is generated by an approximately 3 Mb deletion between 9q34.q11 (where *SET* is located) and 9q34.q13 (*NUP214*) (Van Vlierberghe et al., 2008c).

Deletions involving the *TCR* loci, corresponding to monoclonal, legitimate *TCR* rearrangements, are, in our experience, found by SNP-A analyses in more than 90% of T-ALL cases. Although these deletions are not pathogenetically important, they may nevertheless provide important information in the sense that they are a valuable marker for the presence of leukemic cells in the investigated sample; this is useful in a diagnostic setting when SNP-A analysis is used to identify genomic imbalances in T-ALL.

Gains

Only a few recurrent submicroscopic chromosomal gains have been identified in T-ALL. One example is the rearrangement between *NUP214* and *ABL1* at 9q34 that is amplified on episomes, or occasionally in homogeneously staining regions, in ~5% of T-ALL cases (Graux et al., 2009). The *NUP214-ABL1* fusion codes for a constitutively active tyrosine kinase and although the clinical experience is limited some T-ALL patients with this chimeric gene have responded well to treatment with tyrosine kinase inhibitors (Quintás-Cardama et al., 2008; Clarke et al., 2011; Crombet et al., 2012). *MYB* (6q23.3) is gained in approximately 10% of cases, in most instances as a focal tandem duplication involving only the *MYB* locus; this results in a strong and sustained expression of the gene (Clappier et al., 2007; Lahortiga et al., 2007). An alternative mechanism for deregulated *MYB* expression is the *TRB* translocation t(6;7)(q23;q34), seen in a small percentage of T-ALL cases (Table 3).

Uniparental isodisomy

Copy number neutral stretches of loss of heterozygosity can arise through different mechanisms: i) homozygosity by descent, ii) meiotic UPID, and iii) mitotic UPID. Constitutional homozygosity by descent is common in all populations and is increased in frequency and length when there is close kinship between the parents (Kirin et al., 2010). A

UPID, which may be either constitutional or acquired, is generated when both alleles are from the same parental origin and identical, hence giving rise to homozygosity (Engel, 1980). Meiotic missegregation (meiosis II error) can result in UPIDs associated with different syndromes, sometimes with an increased risk of cancer (Lapunzina and Monk, 2011). That somatically acquired mitotic UPIDs are involved in cancer development was first described in 1983 when it was shown that sUPIDs of 13q could constitute the "second hit", according to Knudson's two-hit hypothesis, leading to homozygosity of the germline *RB1* mutation in retinoblastoma (Cavenee et al., 1983). With the advent of the SNP-A technology, it became apparent that sUPIDs are present in a wide variety of neoplastic disorders and often associated with duplications of mutations or deletions of tumor-associated genes, rendering these changes homozygous (Makishima and Maciejewski, 2011).

Whole chromosome UPIDs are very rare (<1%) in T-ALL (Kawamata et al., 2008; Karrman et al., 2015) in contrast to pediatric BCP ALL where such UPIDs are found in 5-10% of cases (Kawamata et al., 2008; Lundin et al., 2016). One reason for this frequency discrepancy could be the fact that whole chromosome UPIDs are particularly common in aneuploid malignancies (Lundin et al., 2016) and that T-ALL cases only rarely are aneuploid (Fig. 3A). In contrast, sUPIDs are present in approximately one-third of cases (Kawamata et al., 2008; Karrman et al., 2015); the corresponding frequency in BCP ALL is 10-15% (Kawamata et al., 2008; Lundin et al., 2016). Most sUPIDs in T-ALL involve 9p and seem specifically to target *CDKN2A*. In fact, Karrman et al. (2015) reported that all sUPID9p in pediatric T-ALL were associated with homozygous *CDKN2A* deletions and that a heterozygous *CDKN2A* deletion had occurred prior to the sUPID formation in all instances. To date, only a few other recurrent sUPIDs, involving the chromosome segments 4q13.3qter, 10q21.3qter, and 17q12qter, have been identified in T-ALL (Mullighan et al., 2007; Szczepański et al., 2011; La Starza et al., 2013; Karrman et al., 2015). The tumor suppressor

gene *PTEN* at 10q, encoding a phosphatase that is negatively controlled by NOTCH1 and that is often inactivated through deletions or mutations in T-ALL (Table 5) (Palomero et al., 2007), was homozygously deleted in one case with sUPID10q (Mullighan et al., 2007) and could hence be a possible target of this abnormality. However, *PTEN* was neither deleted nor mutated in another T-ALL with sUPID10q (Karrman et al., 2015). The genes targeted by the sUPIDs of 4q and 17q also remain to be identified. NGS analyses of T-ALL cases with sUPIDs could of course resolve this issue. However, the functional outcome may instead be loss or gain of imprinted loci, as is the case in some constitutional disorders (Lapunzina and Monk, 2011). If so, one would expect that either the maternal or paternal allele will be retained in all cases with the same sUPID. This is easily analyzed if parental DNA is available, but methylation analyses of known imprinted loci located within the sUPIDs could also provide important information in this regard. However, and quite surprisingly, the possibility of imprinting effects of acquired UPIDs in leukemia has received little attention to date.

Gene Mutations

A large number of genes have been shown to be mutated in T-ALL, albeit at relatively low frequencies, equivalent to minor hills in the cancer genetic landscape (Vogelstein et al., 2013). However, the mutation frequency of one gene would definitely represent a mountain (to keep the metaphor) namely *NOTCH1*.

The first indication that *NOTCH1* could be pathogenetically important in T-ALL came in the early 1990s when the t(7;9)(q34;q34) was cloned; this rare translocation and its variant t(9;14)(q34;q11) result in overexpression of a truncated, constitutively activated form of NOTCH1 (Ellisen et al., 1991; Suzuki et al., 2009). However, the pivotal role of this gene in T-ALL became apparent first when activating *NOTCH1* mutations were found in more than

50% of cases (Weng et al., 2004) (Table 5). NOTCH1, which is crucial for T-cell fate and differentiation, is a transmembrane heterodimeric receptor composed of two subunits – one extracellular and one transmembrane/intracellular – that interact via the heterodimerization domain (HD) (Koch and Radtke, 2011). The binding of ligands, such as Delta-like 1, 3, and 4 and Jagged 1 and 2, to the extracellular unit induces conformational changes that lead to cleavage of NOTCH1. This results in the release of the intracellular domain that then translocates into the nucleus where it associates with DNA-binding proteins and cofactors, constituting a complex that acts as a transcriptional activator. NOTCH1 regulates the expression of several genes, mainly those involved in proliferation, metabolism, and cell cycling, such as *MYC*, *HES1* (affecting the PI3K-AKT-mTOR signaling pathway), and *CCND3* (Paganin and Ferrando, 2011; Tosello and Ferrando, 2013). Phosphorylation of the PEST domain of NOTCH1 and recruitment of FBXW7, a ubiquitin protein ligase, induce NOTCH1 degradation. *NOTCH1* mutations cluster in the HD, resulting in ligand-independent activation, and in the PEST domain, stabilizing NOTCH1 by impairing degradation by FBXW7. The latter effect can also be accomplished by inactivating *FBXW7* mutations, found in ~15% of T-ALL (Table 5). Approximately 20% of cases harbor dual HD/PEST or HD/*FBXW7* mutations; these co-existing mutations work in synergy, optimizing the NOTCH1 signaling (Paganin and Ferrando, 2011; Tosello and Ferrando, 2013). The frequent aberrant NOTCH1 signaling in T-ALL has obviously made this pathway an attractive target for therapy, although with limited success so far (Roti and Stegmaier, 2014).

In recent years, the mutational landscape of T-ALL has been radically re-defined by the use of NGS. Although the emerging picture is still unclear, the genes implicated can nevertheless often be allocated to specific biological processes, such as signaling (*DNM2*, *IL7R*, *JAK1*, *JAK3*, *NRAS*, and *PTEN*), transcription (*BCL11B*, *LEF1,* and *WT1*), epigenetic modification (*CREBBP*, *EED*, *EZH2*, *PHF6*, and *SUZ12*), and hematopoiesis (*ETV6, IKZF1*,

and *RUNX1*) (Table 5) (Zhang et al., 2012; Atak et al., 2013; Karrman et al., 2015; Vicente et al., 2015). The mutational spectra differ to some extent among subclasses of T-ALL as defined by expression of certain transcription factor genes: activating mutations in *IL7R*, *JAK1*, and *JAK3* are particularly common in *HOXA*- and *TLX3*-positive cases (Vicente et al., 2015), inactivating mutations in *PHF6* are associated with *TLX1*- and *TLX3*-positivity (Van Vlierberghe et al., 2010), and *WT1* mutations are more frequent in cases expressing *TLX1*, *TLX3*, or *HOXA* (Tosello et al., 2009). The types of gene mutated in "conventional" T-ALL and in ETP ALL also differ somewhat, with those involved in hematopoiesis, cytokine or RAS signaling, and histone modification more often being targeted in ETP ALL, as mentioned to above (Zhang et al., 2012).

EPIGENETICS OF T-ALL

It has been known since the early 1980s that also epigenetic abnormalities contribute to tumorigenesis by perturbing gene expression, not associated with alterations of the DNA sequences coding for the deregulated genes in question (Feinberg and Tycko, 2004). There are several underlying mechanisms for switching genes on and off, including changes in DNA methylation, post-translational histone modifications, and altered expression patterns of non-coding RNAs (Jiang et al., 2013). Although genetics and epigenetics reflect two distinct mechanisms, it should be emphasized that there is a complex interplay between epigenetic and genetic aberrations, where the former may well be caused by the latter. In fact, many of the genes deleted and/or mutated in T-ALL code for factors involved in epigenetic regulation (Table 5). Indeed, it has been reported that more than 50% of pediatric T-ALL cases harbor mutations in such regulators (Huether et al., 2014). Thus, T-ALL is very much an epigenetic disease.

DNA methylation of CpG islands, mediated by members of the DNA methyltransferase (DNMT) family, is strongly associated with gene silencing (Peirs et al., 2015). Hence, identification of hypermethylated promoters may indicate the presence of tumor suppressor genes. In T-ALL, suppressors thus identified include the ATP-binding cassette sub-family G member 2 (*ABCG2* at 4q22.1), cyclin-dependent kinase inhibitor 1A (*CDKN1A*; 6p21.2), cyclin-dependent kinase inhibitor 2B (*CDKN2B*; 9p21.3), CCAAT/enhancer binding protein alpha (*CEBPA*; 19q13.11), paired box 5 (*PAX5*; 9p13.2), and spleen tyrosine kinase (*SYK*; 9q22.2) (Van der Meulen et al., 2014).

There are several types of post-translational histone modification – acetylation, methylation, phosphorylation, ubiquitination, sumoylation biotination, citrullination, poly-ADP-ribosylation, and N-glycosylation (Wang et al., 2016). So far, most studies of T-ALL have focused on methylation and acetylation. The polycomb repressive complex 2 (PRC2), which consists of EZH2, EED, SUZ12, and RBBP4 and effectuates histone methylation and gene silencing, is targeted by loss-of-function mutations and deletions in a substantial proportion of T-ALL cases, in particular ETP ALL. Similarly, the PHF6 complex, associated with a closed chromatin configuration, is inactivated by *PHF6* mutations/deletions in approximately 20% of T-ALL cases (Van Vlierberghe et al., 2010; Zhang et al., 2012) (Table 5). Conversely, histone acetylation, which is linked to transcriptional active regions, is targeted by mutations in *EP300* and *CREBBP*, coding for acetyltransferases (Zhang et al., 2012; Vicente et al., 2015). Thus, several gene mutations/deletions in T-ALL result in deregulated gene expression by modifying histone methylation or acetylation.

MicroRNAs are short single-stranded non-coding RNA molecules that interfere with mRNA, negatively affecting protein translation, and that can function as both tumor suppressors and oncogenes, depending on their target genes (Mets et al., 2014, 2015). For example, onco-miRNAs, such as miR19b, miR20a, miR26a, miR92, miR128-3p, and

miR223, suppress a network of tumor suppressor genes in T-ALL, including *BIM*, *IKZF1*, *FBXW7*, *PHF6*, and *PTEN* (Mavrakis et al., 2011; Mets et al., 2014). Thus, not only deletions, inactivating mutations, and DNA hypermethylation but also microRNAs target tumor suppressor genes in T-ALL. Conversely, some miRNAs, e.g., miR223, are regulated by T-ALL oncogenes, such as *NOTCH1* and *TAL1* (Mansour et al., 2013; Kumar et al., 2014), and inactivation of tumor suppressor miRNAs, such as miR29, miR31, miR155, miR193-3p, and miR200, promotes leukemogenesis by activating the oncogenes *HBPI*, *MCL1*, and *MYB* (Sanghvi et al., 2014; Mets et al., 2015). Other types of non-coding RNA, such as long non-coding RNAs (lncRNAs), have also been implicated in T-ALL (Wallaert et al., 2016). For example, the lncRNAs *LUNAR1* and *NALT*, both of which regulated by NOTCH1, function as transcriptional regulators of IGF1R and NOTCH signaling, respectively (Durinck et al., 2014; Trimarchi et al., 2014; Wang et al., 2015). In conclusion, the epigenetic landscape of T-ALL is definitely intricate and compounded.

TUMOR EVOLUTION

Initiating Event and Cell of Origin

An inherent problem in cancer research is the simple fact that we are not present to witness the birth of a tumor cell – or as stated by Theodor Boveri already in 1914: it is impossible to observe a neoplasm *in statu nascendi* (Boveri, 1914; Harris, 2008). Thus, any evidence for certain mutations being “initiating events” in T-ALL is circumstantial and definitely open to interpretation. This notwithstanding, it has been shown that certain genetic aberrations have the potential to give rise to pre-leukemic clones that have been ascribed several characteristics: 1) the ability to differentiate into several lineages, indicating a hematopoietic stem cell phenotype; 2) the capacity, after acquisition of additional mutations, to evolve into leukemia-initiating cells; 3) a propensity to survive chemotherapy, hence acting

as a potential reservoir for relapses; and 4) to remain at remission as well as to exist in samples from healthy individuals (Shlush et al., 2014). Of all the genetic aberrations in T-ALL, which would fit this "job description"? Oncogenes deregulated by *TCR* translocations, such as *TAL1*, *LMO1/2*, *TLX1/3*, and *HOXA*, have been suggested to possess pre-leukemic potentials (Tremblay and Curtis, 2014) and, furthermore, mouse models have substantiated that *LMO2* expression induces aberrant self-renewal in committed T-cells, hence generating a pre-leukemic clone (McCormack et al., 2010). *NOTCH1* activation has also been implicated as a potential mechanism to generate a pre-leukemic or leukemia-initiating phenotype (Armstrong et al., 2009; Blackburn et al., 2012). However, there are several examples of T-ALL cases harboring *NOTCH1* mutations only in subclones and/or being positive for such mutations at diagnosis but negative at relapse; taken together, this shows that at least some *NOTCH1* mutations are secondary events (Mansour et al., 2007; Clappier et al., 2011; Karrman et al., 2015). In conclusion, available evidence indicates that illegitimate *TCR* rearrangements are the main candidates for initiating events, while gene mutations and deletions are secondary and important for clonal evolution and overt leukemia.

The cell of origin is also contentious. T-ALL-initiating cells, identified through serial mouse transplantation assays, could be tracked by analyzing the original clonal *TCR* rearrangement present in the initial diagnostic sample, indicating an origin in a committed T-cell progenitor rather than in the hematopoietic stem cell compartment (Armstrong et al., 2009). In addition, it has been shown that the majority of *TCR* translocations arise in thymocytes negative for CD1a, CD4, and CD8. However, many T-ALL cases display immunophenotypic features corresponding to a later differentiation arrest, at the cortical CD1a-positive stage. Hence, the oncogene activation and the differentiation block seem to be uncoupled, which perhaps is not surprising considering that additional aberrations are needed for leukemic transformation (Le Noir et al., 2012). It should also be emphasized that different

subtypes of T-ALL may well arise in distinct progenitor cells. Indeed, it has been shown that the cell of origin influences which genetic events that may drive transformation (Berquam-Vrieze et al., 2011).

The Road to Relapse

Four different types of relapse have been identified by analyses of paired diagnostic/relapse T-ALL samples, based on genetic relationships with the major clone at diagnosis: 1) identical clones; 2) clonal evolution; 3) evolution from an ancestral clone; and 4) genetically “distinct” leukemias (Mullighan et al., 2008; Clappier et al., 2011; Szczepański et al., 2011; Tzoneva et al., 2013; Karrman et al., 2015). The most common road to relapse appears to be evolution from a preleukemic clone that often is present as a minor cell population at diagnosis and that is resistant to the therapy given. Hence, most relapse clones are characterized by loss of some abnormalities found at diagnosis and gain of a few additional aberrations; for example, *NT5C2*, *SMARCA4*, *SUZ12*, and *WHSC1* mutations are enriched at relapse (Tzoneva et al., 2013; Kunz et al., 2015). Relapses are nevertheless remarkably genomically stable, something that strongly argues against chromosomal instability playing a major role during disease progression (Tosello et al., 2009).

CLINICAL, GENETIC, AND EPIGENETIC FEATURES AND PROGNOSIS

Considering that the outcome of T-ALL is quite dismal, at least compared with BCP ALL, and that patients who relapse respond poorly to reinduction therapy, several studies have tried to identify prognostically important factors – clinical, genetic as well as epigenetic – that could be used for risk stratification at the time of diagnosis or early on during treatment. However, many such factors “are called, but few are chosen” (Table 6).

Clinical Features

Age, gender, and WBC count at diagnosis have not been clearly, or repeatedly, shown to influence the outcome (Table 6). As regards maturational stages, such as T-I and T-II versus T-III and T-IV, ETP ALL, and absence of biallelic *TRG* rearrangements, the more immature subtypes tend to be associated with an inferior prognosis; however, the data are limited and also somewhat conflicting. Thus, in all practice, clinical and immunophenotypic features at the time of diagnosis are not used for treatment decisions. In contrast, treatment response, as measured by MRD analyses at different time points, is definitely a strong prognostic indicator (Table 6) and is hence used in current treatment protocols to modify subsequent therapy.

Genetic Features

As seen in Table 6, cytogenetic features do not play a prognostic role in T-ALL, nor do types of TCR translocation/inversion. Furthermore, data on fusion genes are either contradictory or based on very few analyzed cases; thus, the presence of certain fusion genes should not, as of yet, influence clinical decision making, at least not as a single marker for prognostication.

The clinical implications of the majority of deletions and mutations are also unknown. This is most probably because most are quite infrequent and that hence large patient cohorts are required to ascertain their effect on outcome, in particular as independent risk factors. As for the genes deleted and/or mutated that have been analyzed from a prognostic perspective, most have been reported not to confer any significant impact, i.e., deletions of *CDKN2A*, deletions/inactivating mutations of *BCL11B*, *LEF1*, *PHF6*, *PTEN*, and *WT1*, and activating mutations of *NRAS* (Table 6). The mutations that have been most extensively investigated in T-ALL are undoubtedly those affecting the *NOTCH1* gene. Several studies have identified a

favorable early therapy response in cases with *NOTCH1* mutations (Table 6), something that, however, only could be translated into improved outcome in a few studies (Kox et al., 2010; Zuurbier et al., 2010). This could of course reflect differences in therapy among treatment protocols. Jenkinson et al. (2013) reported a significantly improved overall survival of pediatric T-ALL cases with coexisting *FBXW7* and *NOTCH1* mutations, but that could not be confirmed in a Swedish T-ALL cohort (Fogelstrand et al., 2014). Finally, a meta-analysis of 711 pediatric T-ALL patients did not find any correlation between the presence of *NOTCH1* mutations and event-free survival (Ma and Wu, 2012).

Epigenetic Features

Only a few studies have focused on the prognostic impact of epigenetic changes in pediatric T-ALL, reporting, for example, that high levels of miR-16 and miR-221 are associated with shorter survival. This has, however, not been seen in all studies and, furthermore, these studies were based on quite a low number of patients, which often included both pediatric and adult patients, precluding any firm conclusions as to the prognostic role of miRs in childhood T-ALL (Kaddar et al., 2009; Gimenes-Teixeira et al., 2013; Xi et al., 2013). Data on promoters that are differentially methylated in T-ALL have been applied to define CpG island methylator phenotypes, delineating cases as either hypermethylated or hypomethylated (Borssén et al., 2013, 2016), with hypomethylated cases having a significantly worse event-free and overall survival in one of the studies (Borssén et al., 2013). Obviously, this needs to be addressed in larger, prospective cohorts.

CONCLUSION

During the last decade, a vast amount of data has been forthcoming as regards the genetic and epigenetic genetic blueprint of T-ALL. The tasks now are to integrate these

genetic and epigenetic data into an all-encompassing (and preferably) testable theory on the pathogenesis of T-ALL and to translate them into clinical use for better risk stratification and improved outcome.

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Figure legends

Figure 1. (**A**) The normal T-cell differentiation process. Early thymic progenitors enter the thymus through vessels in the corticomedullary junction. During their thymic circuit, they undergo distinct developmental stages, characterized by somatic rearrangemants of the T-cell receptor (*TCR*) genes, resulting in surface expression of TCR molecules, most commonly TCRαβ. As the maturing T-cells move towards the cortex, *TRB* rearrangements are initiated. Expression of pre-TCR takes place in the outmost periphery of the cortex. T-cells that pass the so called β selection will subsequently express the final TCRαβ at the cell surface. Cortical thymic epithelial cells express the individual's unique antigen-presenting proteins together with peptides. Only T-cells with TCRs that bind to a presented peptide with an appropriate affinity will survive, a process called positive selection. Surviving T-cells then migrate towards the central medulla where they are exposed to epithelial cells expressing self-antigen complexes. At this stage, negative selection leads to destruction of cells that bind with high affinity to self-antigens; this process reduces autoreactivity. Hence, stringent criteria are applied to ensure sensitivity and specificity of the newly assembled TCRs, effectuated by both positive and negative selection. Only some 5% of the thymocytes generated in the thymus leave as mature T-cells. (**B**) The malignant intrathymic process. Stepwise accumulation of genetic alterations in early T-cells results in self-renewal capacity, differentiation block, and enhanced proliferation. Some of the more common T-ALL-associated genetic aberrations that contribute to the development of a malignant clone are listed to the left (CNA, copy number abnormality; sUPID, segmental uniparental isodisomy). The malignant T-lymphoblasts expand in the thymus and spill over into the peripheral circulation and invade other lymphatic and non-lympathic organs. (**C**) Normal T-cell circulation. Hematopoietic stem cells residing in the bone marrow give rise to thymus-seeding progenitor cells that migrate to the thymus, thereby sustaining thymopoiesis. The most immature cells entering the thymus are called early thymic progenitors (see **A**). The mature T-cells exit from the thymus and circulate in the

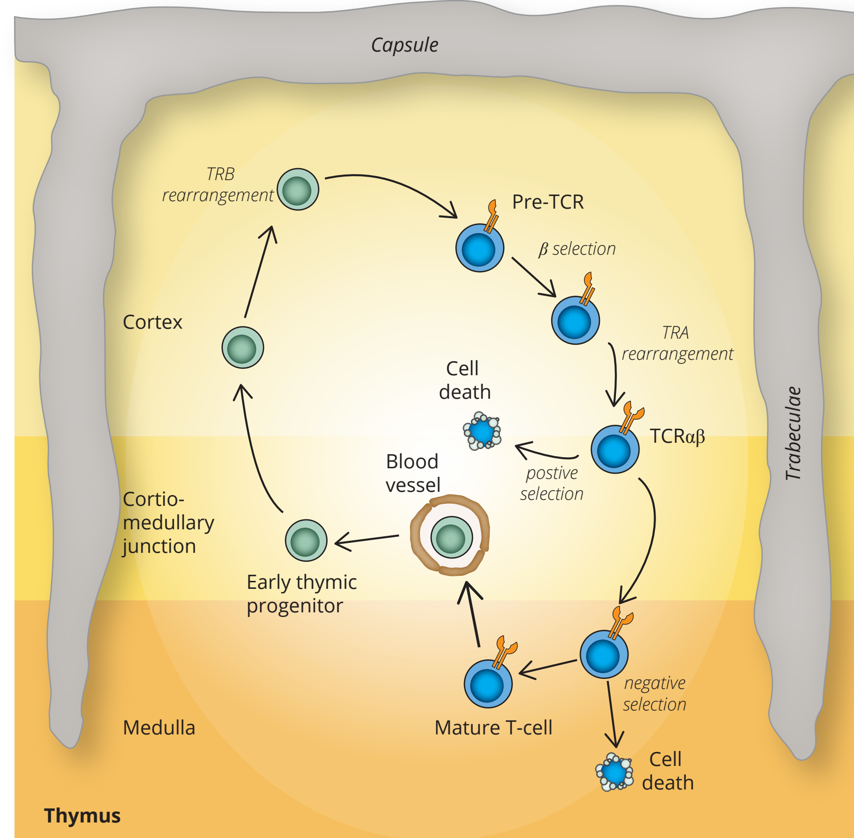
blood as well as through different lymphatic and non-lymphatic organs, some of which are shown here. (**D**) Circulation of malignant T-lymphoblasts. If genetic aberrations accumulate in an early T-cell (see **B**), the maturation process is disrupted, with ensuing proliferation of the malignant clone. The malignant T-lymphoblasts exit the thymus, overflow the peripheral circulation, and invade lymphoid and non-lymphoid organs such as the spleen, lymph nodes, bone marrow, and central nervous system. The crowding of malignant cells in these sites results in various T-ALL-associated symptoms and signs, such as suppressed bone marrow function, leukocytosis, neurological abnormalities, and respiratory distress.

Figure 2. T-cell receptor (TCR) structures are assembled from multiple separate gene segments. The *TCR* genes *TRB*, *TRA,* and *TRD* (located within the *TRA* locus) consist of several different segments, denoted variable (V), diversity (D), joining (J), and constant (C); the numbers of the different segments were ascertained from Atlas of Genetics and Cytogenetics in Oncology and Haematology (http://atlasgeneticsoncology.org/). *TRG* is not graphically depicted but is organized as the other *TCR* genes. In the process of *TRA* recombination, *TRD* will be excised due to its intragenic position. The combinatorial possibilities created by V(D)J recombination are astonishing and are a cornerstone in our immune defense. If a DNA double strand break is introduced during *TCR* recombination and misaligned to a double strand break in the vicinity of a proto-oncogene, the resulting *TCR* translocation may have an oncogenic potential.

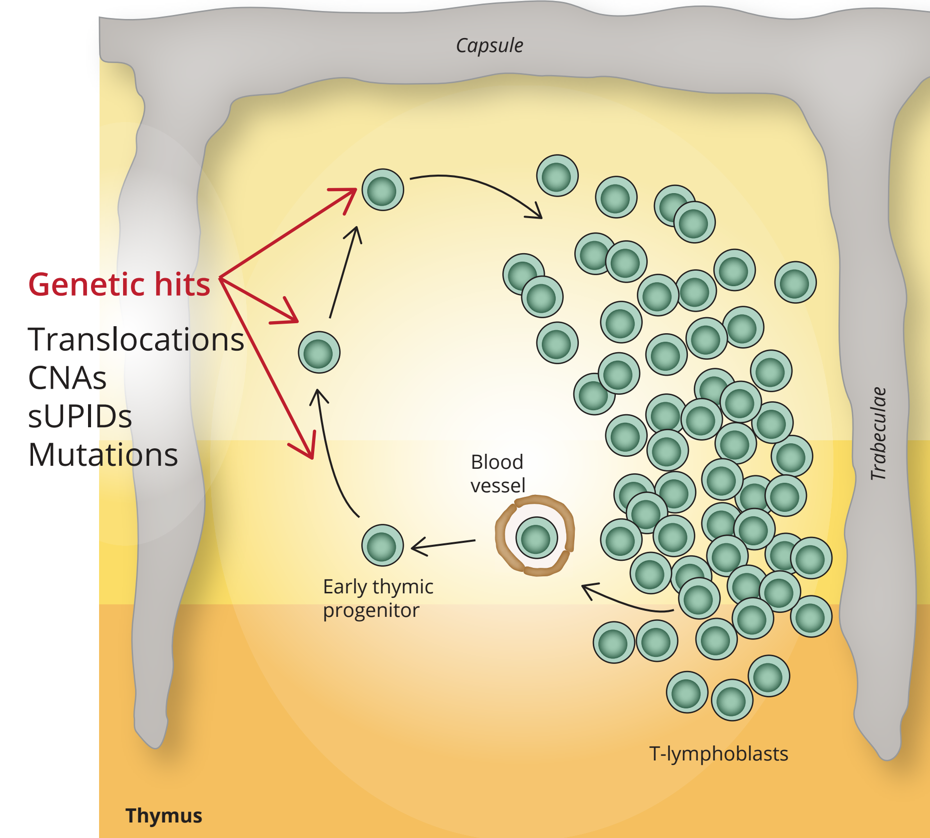
Figure 3. (**A**) Distribution of modal chromosome numbers in karyotypically abnormal pediatric T-ALL (http://cgap.nci.nih.gov/Chromosomes/Mitelman). The vast majority (~70%) of cases displays a pseudodiploid karyotype, i.e., 46 chromosomes, with most remaining cases having modal chromosome numbers of 45 or 47-49. (**B**) Distribution of number of

abnormalities in pediatric T-ALL (http://cgap.nci.nih.gov/Chromosomes/Mitelman). Approximately half of all chromosomally abnormal cases display only one aberration, whereas two, three, and four changes are found in ~25%, ~10%, and ~8%, respectively.

A



B



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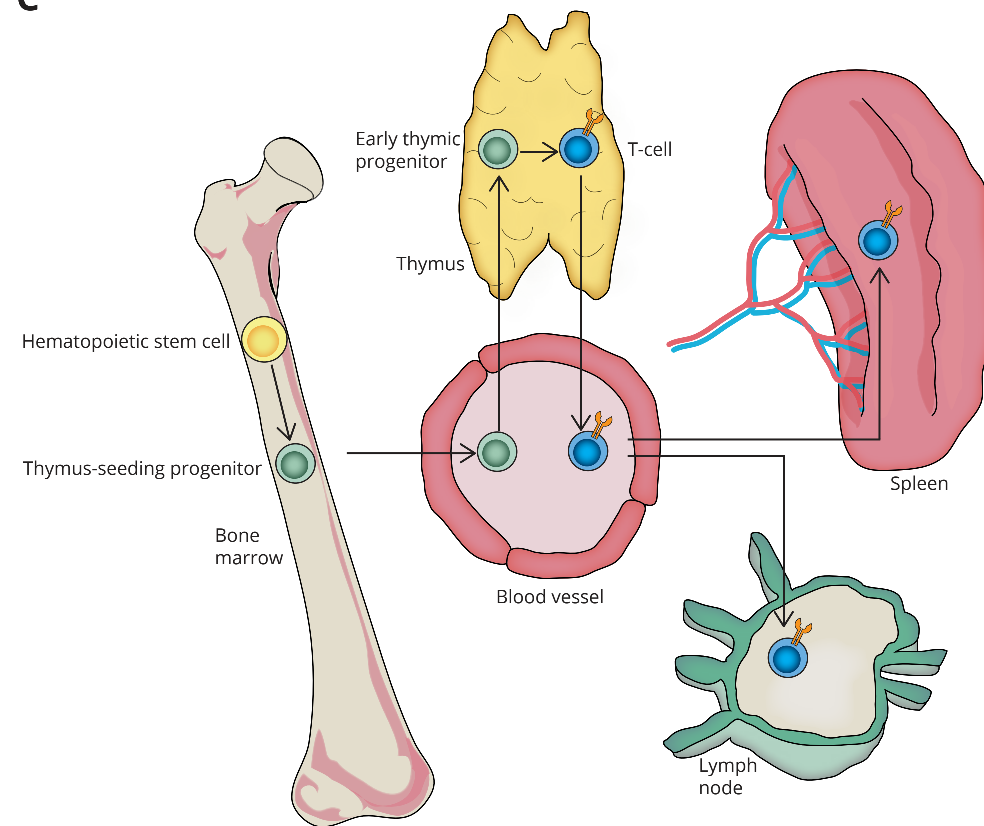

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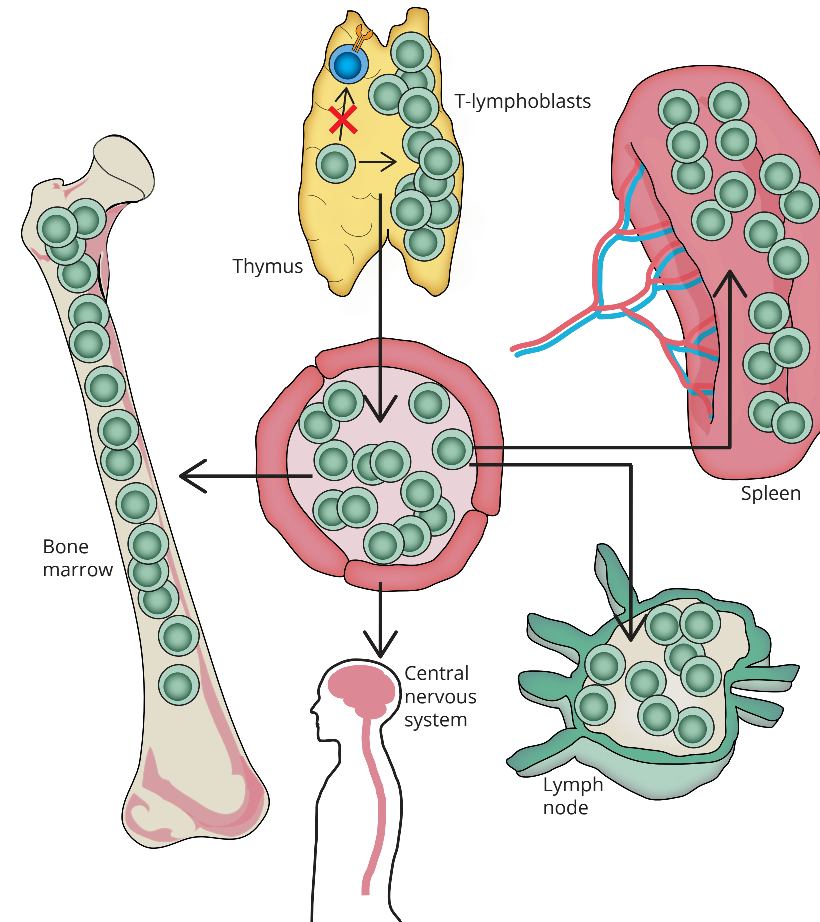
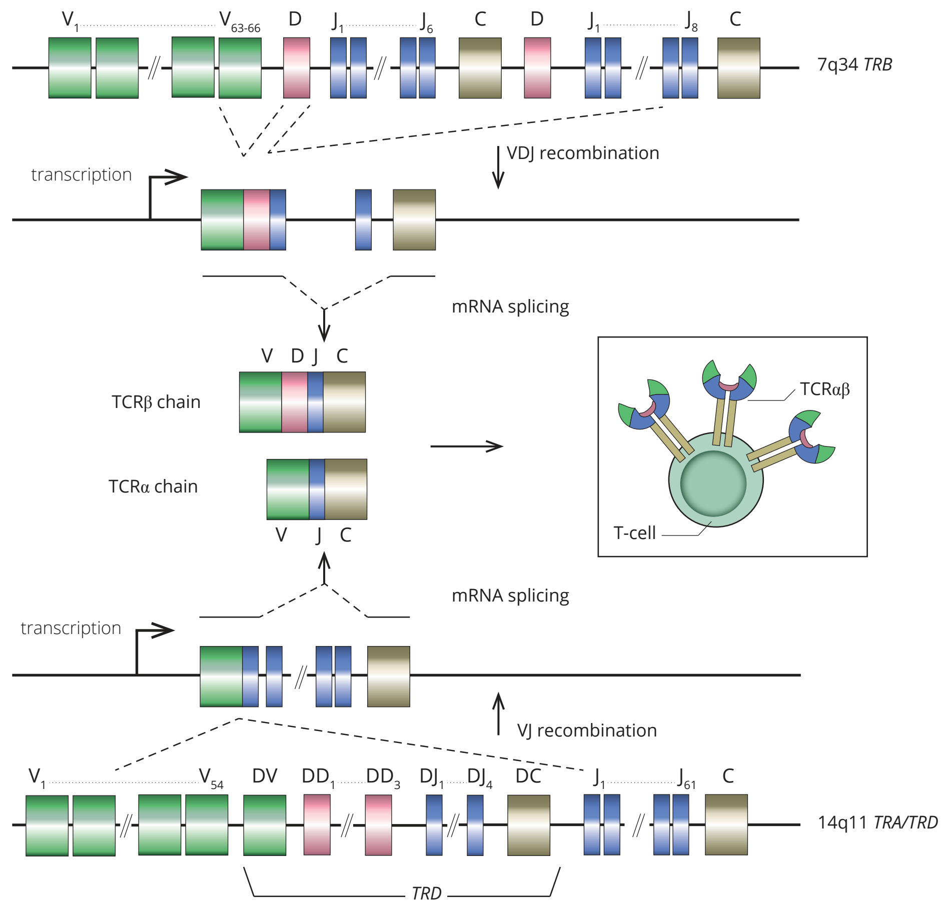




V1
V63-66
D
J1
J6
C
D
J1
J8
C
7q34 TRB
VDJ recombination
transcription
mRNA splicing
V D J C
TCRβ chain
TCRα chain
V J C
TCRαβ
T-cell
mRNA splicing
transcription
VJ recombination
V1
V54
DV
DD1
DD3
DJ1
DJ4
DC
J1
J61
C
14q11 TRA/TRD
TRD

TABLE 1. Outcome for Children, Adolescents, and Younger Adults with T-ALL

Treatment protocol	No. of patients	Age group (years)	Inclusion period	5-year pEFS (SE)	5-year pOS (SE)	Study
AIEOP-BFM ALL 2000 and AIEOP ALL R2006	359	1-17	2000-2009	0.70 (0.02)	0.77 (0.02)	D'Angiò et al. 2015
CCG-1900	522	<21	1996-2002	0.73 (0.02)	0.80 (0.02)	Gaynon et al. 2010
COG ALL clinical trials	459	0-22	2000-2005		0.82 (0.02)	Hunger et al. 2012
DCOG ALL-9	90	1-18	1997-2004	0.72 (0.05)		Veerman et al. 2009
NOPHO ALL-2000	115	1.0-<15	2002-2007	0.64 (0.05)	0.72 (0.0)	Schmiegelow et al. 2010
Total XV Study	76	1-18	2000-2007	0.78 (0.08)	0.88 (0.06)	Pui et al. 2009
UKALL 2003	187[a]	1-24	2003-2011	0.85 (0.05)	0.91 (0.04)	Patrick et al. 2014

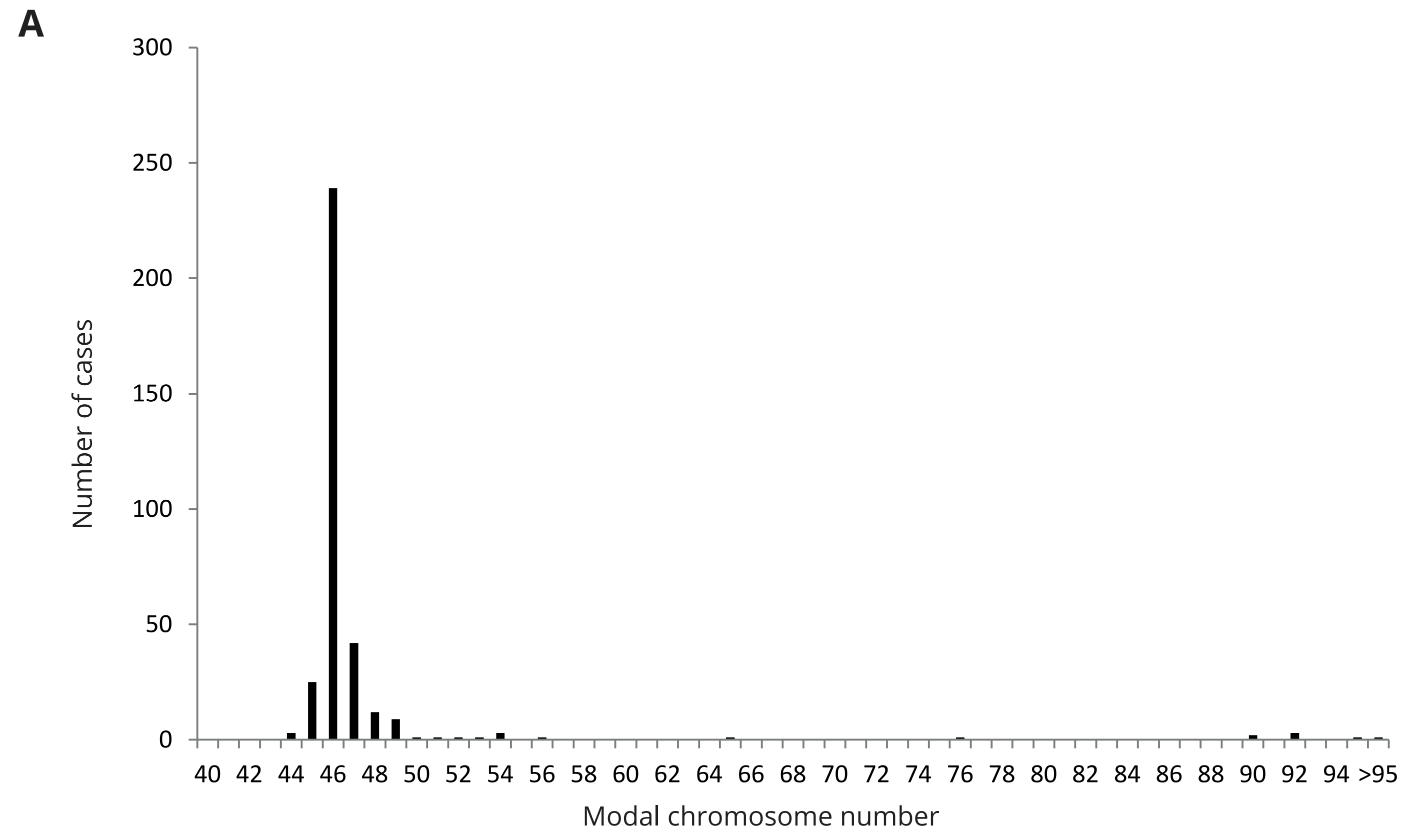
pEFS, probability of event-free survival; pOS, probability of overall survival, SE, standard error.

[a]Does not include probable/definite early T-cell precursor ALL cases.

TABLE 2. Frequencies of Karyotypically Abnormal T-ALL Cases

Frequency	Age group (years)	No. of patients	Study
48%	<18	249	Karrman et al. 2009a
52%	14-52	60	Vitale et al. 2006
57%	1-<22	143	Bash et al. 1993
57%	1-<21	354	Schneider et al. 2000
57%	0-17	111	Cavé et al. 2004
61%	<21	169	Heerema et al. 1998
62%	1-<18	69	van Grotel et al. 2006
72%	2-<18	57	Raimondi et al. 1988
72%	15-59	204	Marks et al. 2009
77%	4-78	65	Park et al. 2014

A



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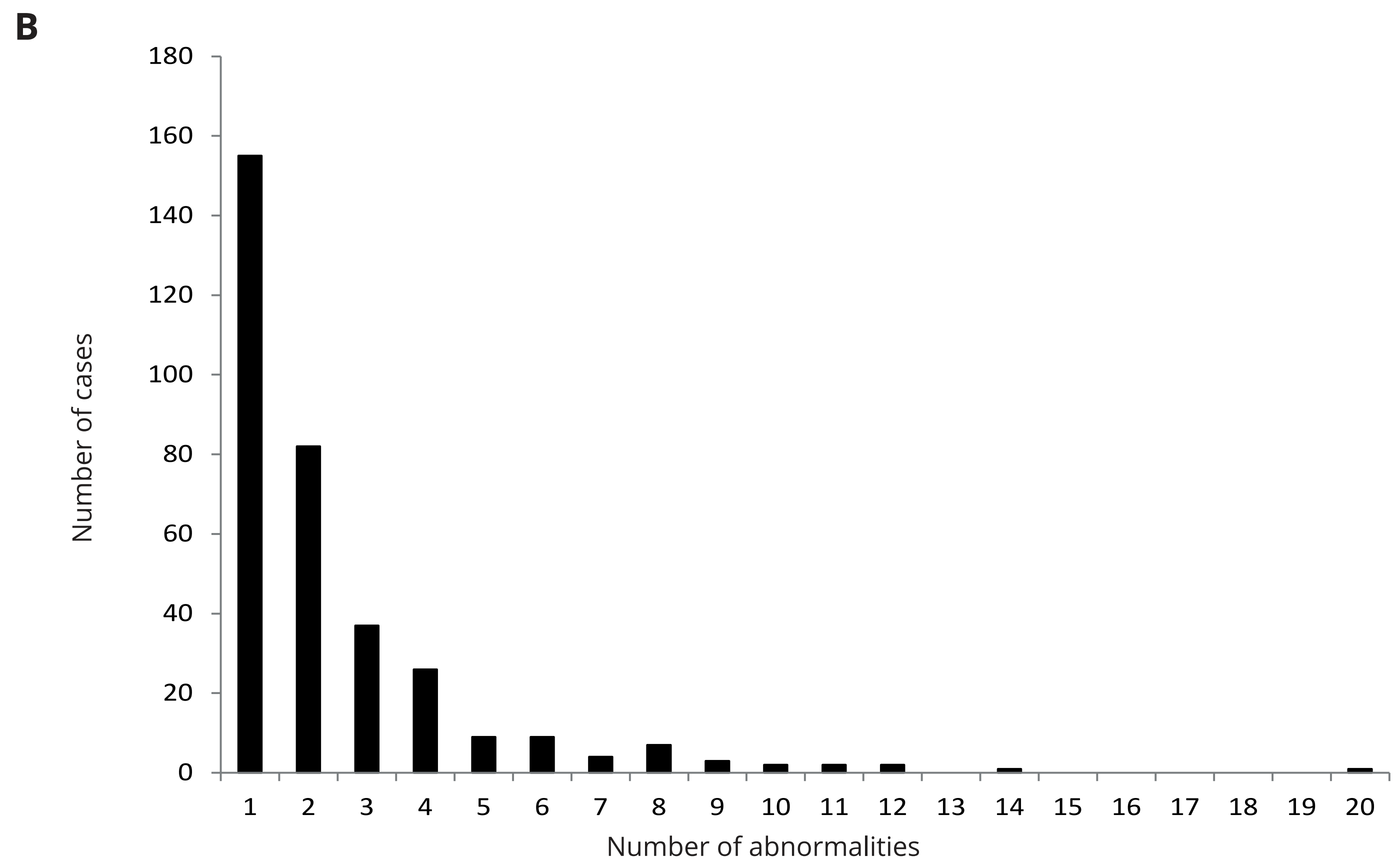

TABLE 3. Gene Partners in *TCR* Rearrangements in T-ALL

Gene[a] (location)	Function of the target protein[a]	Frequency (%)[b]	Study
Recurrently involved			
CCND2 (12p13)	Cyclin family	<1	Clappier et al. 2006
HOXA9/10/11 (7p15)	Homeobox transcription factor	5	Speleman et al. 2005
IRS4 (Xq22)	Cytoplasmic protein	<1	Karrman et al. 2009b
LCK (1p35)	Protein tyrosine kinase	<1	Tycko et al. 1991
LMO1 (11p15)	Transcriptional regulator	1-2	McGuire et al. 1989
LMO2 (11p13)	Transcriptional regulator	3-12	Royer-Pokora et al. 1991
LYL1 (19p13)	Basic helix-loop-helix transcription factor	<1	Mellentin et al. 1989
MYB (6q23)	Transcription factor	3	Clappier et al. 2007
MYC (8q24)	Transcription factor	1-3	Hayashi et al. 1986
NOTCH1 (9q34)	Transcriptional regulator	<1	Ellisen et al. 1991
RIC3 (11p15)	Chaperone protein	<1	Atak et al. 2013
TAL1 (1p33)	Basic helix-loop-helix transcription factor	3-6	Finger et al. 1989
TAL2 (9q31)	Basic helix-loop-helix transcription factor	<1	Xia et al. 1991
TCL1A (14q32)	Coactivator of the cell survival kinase AKT	<1	Sugimoto et al. 2014
TLX1 (10q24)	Homeobox-containing transcription factor	5-10	Kagan et al. 1989
TLX3 (5q35)	Homeobox-containing transcription factor	<1	Hansen-Hagge et al. 2002
Single cases only			

BCL11B (14q32), *GNAQ* (9q21), *IL2RB* (22q12), *IL7R* (5p13), *LEF1* (4q25), *NKX2-4* (20p11), *NKX2-5* (5q35), *OLIG2* (21q22), *PLAG1* (8q12), *PVT1* (8q24), *SFTA3* (14q13), and *TLX1NB* (10q24) (Kasai et al., 1992; Wang et al., 2000; Przybylski et al., 2005, 2006; Le Noir et al., 2012; Atak et al., 2013)

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[a]Based on RefSeq summary (http://www.ncbi.nlm.nih.gov/refseq/) and UniProt (http://www.uniprot.org/uniprot/).

[b]Based on Graux et al. (2006), Van Vlierberghe et al. (2008b), Van Vlierberghe and Ferrando (2012), La Starza et al. (2014), and Mitelman et al. (2016).

TABLE 4. Fusion Genes in T-ALL

Fusion gene	Rearrangement[a]	Molecular functions of the proteins involved[b]	Frequency (%)[c]	Study
Recurrent				
BCL11B-NKX2-5	t(5;14)(q35;q32)	Transcriptional repressor/transcription factor	<1	Nagel et al. 2003
BCL11B-TLX3	t(5;14)(q35;q32)	Transcriptional repressor/transcription factor	20	Bernard et al. 2001
BCR-ABL1	t(9;22)(q34;q11)	Serine threonine kinase/tyrosine kinase	<1	Fizzotti et al. 1994
DDX3X-MLLT10	t(X;10)(p11;p12)	RNA helicase/transcription factor	<1	Brandimarte et al. 2013
ETV6-ABL1	t(9;12)(q34;p13)	Transcription factor/tyrosine kinase	<1	Zaliova et al. 2016
ETV6-JAK2	t(9;12)(p24;p13)	Transcription factor/tyrosine kinase	<1	Lacronique et al. 1997
ETV6-NCOA2	t(8;12)(q13;p13)	Transcription factor/transcriptional coactivator	<1	Zhou et al. 2012
KMT2A-FOXO4	t(X;11)(q13;q23)	Transcriptional coactivator/transcription factor	<1	Borkhardt et al. 1997
KMT2A-MLLT1	t(11;19)(q23;p13)	Transcriptional coactivator/transcription regulation	<1	Corral et al. 1993
KMT2A-MLLT4	t(6;11)(q27;q23)	Transcriptional coactivator/signaling transduction	<1	Tanabe et al. 1996
NUP98-RAP1GDS1	t(4;11)(q23;p15)	Nuclear pore complex/GTPase activator activity	<1	Hussey et al. 1999
NUP214-ABL1	r(9)(q34q34)	Nuclear pore complex/tyrosine kinase	5	Graux et al. 2004
PICALM-MLLT10	t(10;11)(p12;q14)	Assembly protein/transcription factor	5	Dreyling et al. 1998
SET-NUP214	del(9)(q34q34)	Histone binding/nuclear pore complex	3	Van Vlierberghe et al. 2008c
STIL-TAL1	del(1)(p33p33)	Cytoplasmic protein/developmental protein	12	Bernard et al. 1991
SQSTM1-NUP214	t(5;9)(q35;q34)	Scaffold protein/nuclear pore complex	<1	Zhang et al. 2012
Single cases only				
ASCC1-MAFG, *B4GALT3-TPM3*, *BCR-FGFR1*, *BCR-PDGFRA*, *CEP85L-PDGFRB*, *CDK6-RPL35P4*, *CDK6-TLX3*, *CDKN2A-IFNWP19*, *CLINT1-MEF2C*, *CTNNA3-ARHGAP21*, *CUX1-FGFR1*, *DPY19L1-HOXA11*, *EEF1G-OOEP*, *EML1-ABL1*, *ETV6-ABL2*, *ETV6-ARNT*, *ETV6-INO80D*, *FAM133B-CDK6*, *FIP1L1-*				

PDGFRA, *FUS-SET*, *GREB1-E2F6*, *GUK1-ARF1*, *HNRNPH1-MLLT10*, *HOXA11-AS/CDK6*, *IKZF1-ABCA13*, *KANSL1-ARL17A*, *KCNK17-KIF6*, *KMT2A-CASC5*, *KMT2A-CREBBP*, *KMT2A-TET1*, *L3MBTL3-PTPRK*, *MBNL1-LMO2*, *MGAT4A-CXCR4*, *MGAT5-GPR39*, *MIR550A1-ZNRF2*, *MYB-AHI1*, *NAP1L1-MLLT10*, *NDST2-RUNX1*, *NUP98-ADD3*, *NUP98-CCDC28A*, *NUP98-PSIP1*, *NUP98-SETBP1*, *OAZ1-RNF126*, *PAGE2B-ALAS2*, *PCMTD1-PRKAR1B*, *RUNX1-AFF3*, *RUNX1-EVX1*, *RUNX1-FGA7*, *SENP6-NKAIN2*, *SGCA-PPP1R9B*, *SSBP2-FER*, *STAG2-LMO2*, *STAT5B-STAT3*, *TCTA-TAL1*, *TPM3-JAK2*, *UGCG-PVT1*, *XPO1-MLLT10*, *ZEB1-BMI1*, *ZMYM2-FGFR1*, and *ZNF219-HNRNPC* (Aplan et al., 1995; Rowley et al., 1997; Griesinger et al., 2002; Tagawa et al., 2002; Kuefer et al., 2003; Lahortiga et al., 2003; Mikhail et al., 2004; Su et al., 2004; De Keersmaecker et al., 2005; Romana et al., 2006; Etienne et al., 2007; Panagopoulos et al., 2007; Chinen et al., 2008; Otsubo et al., 2010; Chen et al., 2011; Kim et al., 2011; Wasag et al., 2011; Chmielecki et al., 2012; Zhang et al., 2012; Atak et al., 2013; Brandimarte et al., 2013; Giacomini et al., 2013; Ittel et al., 2013; Bond et al., 2014; Yigit et al., 2015).

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[a]Many of the rearrangements are based on the chromosomal locations of the genes involved and have not been observed cytogenetically; instead they are inferred from molecular genetic analyses, such as next generation sequencing. Thus, some of the "translocations" may well be other types of abnormality, such as inversions, deletions, and insertions.

[b]Based on RefSeq summary (http://www.ncbi.nlm.nih.gov/refseq/) and UniProt (http://www.uniprot.org/uniprot/).

[c]Based on Graux et al. (2006), Van Vlierberghe et al. (2008b), Van Vlierberghe and Ferrando (2012), and Mitelman et al. (2016).

TABLE 5. Genes Frequently Deleted and/or Mutated in T-ALL

Gene (location)	Frequency (%)	Deletions/mutations	Function[a]	Study
CDKN2A (9p21.3)	70	Deletion	Cell cycle regulation	Yu et al. 2011; Karrman et al. 2015
CDKN2B (9p21.3)	60	Deletion	Cell cycle regulation	Karrman et al. 2015; Vicente et al. 2015
NOTCH1 (9q34.3)	60	Activating mutation	Transcriptional regulator	Weng et al. 2004; Zuurbier et al. 2010
PHF6 (Xq26.2)	20	Deletion/inactivating mutation	Chromatin remodelling	Van Vlierberghe et al. 2010; Vicente et al. 2015
PTEN (10q23.31)	20	Deletion/inactivating mutation	Tumor supressor	Gutierrez et al. 2009; Jenkinson et al. 2016
FBXW7 (4q31.3)	15	Inactivating mutation	Protein degradation	Kox et al. 2010; Zuurbier et al. 2010
WT1 (11p13)	15	Deletion/innactivating mutation	Transcription factor	Tosello et al. 2009; Vicente et al. 2015
LEF1 (4q25)	10-15	Deletion/innactivating mutation	Transcription factor	Gutierrez et al. 2010b; Vicente et al. 2015
BCL11B (14q32.2)	10	Deletion/innactivating mutation	Transcriptional repressor	Gutierrez et al. 2011; Vicente et al. 2015
DNM2 (19p13.2)	10	Inactivating mutation	Signaling transduction	Zhang et al. 2012; Vicente et al. 2015
EZH2 (7q36.1)	10	Deletion/innactivating mutation	Chromatin remodelling	Zhang et al. 2012; Vicente et al. 2015
IL7R (5p13.2)	10	Activating mutation	Receptor	Zenatti et al. 2011; Vicente et al. 2015
ETV6 (12p13.2)	5-15	Deletion/innactivating mutation	Transcription factor	Zhang et al. 2012; Vicente et al. 2015
EED (11q14.2)	5-10	Deletion/innactivating mutation	Chromatin remodelling	Zhang et al. 2012; Vicente et al. 2015
NRAS (1p13.2)	5-10	Activating mutation	Signaling transduction	Van Vlierberghe et al. 2011; Zhang et al. 2012
RUNX1 (21q22.12)	5-10	Inactivating mutation	Transcription factor	Zhang et al. 2012; Vicente et al. 2015
SUZ12 (17q11.2)	5-10	Deletion/innactivating mutation	Chromatin remodelling	Zhang et al. 2012; Vicente et al. 2015
CREBBP (16p13.3)	5	Deletion/innactivating mutation	Chromatin remodelling	Karrman et al. 2015; Vicente et al. 2015
ECT2L (6q24.1)	5	Inactivating mutation	Unknown	Zhang et al. 2012; Vicente et al. 2015
JAK1 (1p31.3)	5	Activating mutation	Signaling transduction	Karrman et al. 2015; Vicente et al. 2015
JAK3 (19p13.11)	5	Activating mutation	Signaling transduction	Zhang et al. 2012; Karrman et al. 2015

PTPN2 (18p11.21)	5	Deletion	Tumor supressor	Kleppe et al. 2010; Vicente et al. 2015
RB1 (13q14.2)	5	Deletion	Tumor supressor	Zhang et al. 2012; Karrman et al. 2015
RELN (7q22.1)	5	Inactivating mutation	Cell-cell interactions	Zhang et al. 2012; Vicente et al. 2015

[a]Based on RefSeq summary (http://www.ncbi.nlm.nih.gov/refseq/) and UniProt (http://www.uniprot.org/uniprot/).

TABLE 6. Clinical, Genetic, and Epigenetic Features and Prognosis of Pediatric T-ALL

Features	Prognostic impact[a]	Study
Clinical features		
Age (<10 vs ≥10 years)	None	van Grotel et al. 2008; Karrman et al. 2009a; Schrappe et al. 2011
Gender	None	van Grotel et al. 2008; Karrman et al. 2009a
WBC count		
<50 vs ≥50 x 10^9/l	None	van Grotel et al. 2008; Mansur et al. 2012
<149.5 vs ≥149.5 x 10^9/l	None	Ballerini et al. 2008
≥200 x 10^9/l	Poor/none	Karrman et al. 2009a; Hastings et al. 2015
<10 vs 10-49 vs 50-99 vs >100 x 10^9/l	None	Vaitkevičienė et al. 2011
Maturational stage		
Lack of biallelic *TRG* rearrangements	Poor/none	Gutierrez et al. 2010a; Yang et al. 2012; Farah et al. 2016
ETP ALL[b]	Poor/none	Coustan-Smith et al. 2009; Patrick et al. 2014; Conter et al. 2016
Pro- and pre-T-ALL (T-I and T-II)[c]	Poor/none	Schrappe et al. 2011; Patrick et al. 2014
MRD positivity at various time points	Poor	Willemse et al. 2002; Schrappe et al. 2011; Gao et al. 2014; Borssén et al. 2016
Cytogenetic features[d]		
Abnormal karyotype	None	Heerema et al. 1998; Karrman et al. 2009a
Deletion of 6q	None	Schneider et al. 2000; Karrman et al. 2009a; Remke et al. 2009
Trisomy 8	None	Schneider et al. 2000; Karrman et al. 2009a
Deletion of 11q or12p	Unknown	
TCR partners[d]		

LMO2	None	van Grotel et al. 2008
TLX1	None	van Grotel et al. 2008
HOXA9/10/11	Unknown	
Fusion genes[d]		
BCL11B-TLX3	Poor/none	Cavé et al. 2004; Ballerini et al. 2008; van Grotel et al. 2008
PICALM-MLLT10	Poor[e]	van Grotel et al. 2008
STIL-TAL1	Poor/none	Cavé et al. 2004; Ballerini et al. 2008; van Grotel et al. 2008; Mansur et al. 2012; D'Angiò et al. 2015
NUP214-ABL1	Unknown	
Gene mutations/deletions[d]		
BCL11B	None	Gutierrez et al. 2011
CDKN2A/B	None	Ramakers-van Woerden et al. 2001; Krieger et al. 2010; Kuchinskaya et al. 2011; Karrman et al. 2015
FBXW7	Poor/none	Kox et al. 2010; Mansur et al. 2012; Jenkinson et al. 2013; Yuan et al. 2015
LEF1	None	Gutierrez et al. 2010b
NOTCH1	Good/none	Breit et al. 2006; van Grotel et al. 2008; Kox et al. 2010; Zuurbier et al. 2010; Mansur et al. 2012; Jenkinson et al. 2013; Fogelstrand et al. 2014; Gao et al. 2014; Yuan et al. 2015
NRAS	None	Yuan et al. 2015; Jenkinson et al. 2016
PHF6	None	Yuan et al. 2015
PTEN	None	Mansur et al. 2012; Bandapalli et al. 2013; Jenkinson et al. 2016
WT1	None	Tosello et al. 2009
CREBBP, *DNM2*, *ECT2L*, *EED*, *ETV6*, *EZH2*, *IL7R*, *JAK1*, *JAK3*, *RB1*, *RELN*,	Unknown	

RUNX1, and *SUZ12*		
Epigenetic fatures		
Hypomethylated CpG island phenotype	Poor/none	Borssén et al. 2013, 2016

ETP ALL, early T-cell precursor acute lymphoblastic leukemia; HR, high risk; IR, intermediate risk; MRD, minimal residual disease; WBC, white blood cell.

[a]Statistically significant ($P<0.05$) impact on disease free survival, event free survival and/or overall survival.

[b]Defined as CD1a-/CD8-/CD5(+)/cCD3+/CD7+ and expression of the myeloid- or stem cell-associated markers CD13, CD33, CD34, CD117, HLA-DR, CD11b, and CD65.

[c]Double negative for CD4 and CD8.

[d]Only cytogenetic aberrations, *TCR* partners, fusion genes, and gene mutations/deletions reported in ≥5% of pediatric T-ALL cases are included.

[e]Based on only three cases.