

## Spontaneous Remission of Transient Leukemia in Down Syndrome: Extrinsic or Intrinsic Mechanism?

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### Abstract

Myeloid leukemias in childhood Down syndrome (DS) comprise a unique disease entity. Transient Leukemia (TL) in neonates with DS is a neoplastic disorder characterized by acute myeloid leukemia (AML)-like hematological abnormalities, which spontaneously resolves in several weeks or months. On the other hand, AML in young children with DS (AML-DS), which occurs several years later, usually after spontaneous remission of TL, does not resolve spontaneously and is a lethal disorder unless treated. These two types of myeloid leukemia in DS are a spectrum of disorders with common *GATA1* gene mutations and a background of trisomy 21, but arise in different organs at different developmental stages. TL is thought to arise in the fetal liver and is often accompanied by hepatic fibrosis in severe cases, whereas AML-DS arises in the postnatal BM and is often accompanied by myelofibrosis, with fibrosis of both organs being caused by a common mechanism through cytokines produced by leukemic blasts. The mechanism of the spontaneous remission of TL is unclear and two major hypotheses have been proposed: 1) a transition of major hematopoietic organs from the liver to the BM after birth might stop TL blast growth (extrinsic/environmental theory); and 2) the genetic mechanism controlling the switch from fetal- to adult-type haematopoiesis might trigger the end of TL blast growth (intrinsic/genetic theory).

**Keywords:** Down syndrome; Transient leukemia; Transient abnormal myelopoiesis (TAM); Transient myeloproliferative disorder (TMD); Spontaneous remission; Hematopoietic microenvironment; Acute myeloid leukemia (AML)

### Introduction

Down syndrome (DS) is a developmental anomaly well known for 10- to 20-fold higher risk of developing leukemia [1], although solid cancers are paradoxically infrequent compared with those in normal individuals. Myeloid leukemias in young children with DS have unique biological characteristics that are not seen in acute myeloid leukemia (AML) of either children without DS or adults. Data regarding the pathogenesis of myeloid leukemias in DS have been progressively accumulated in recent years and a multistep model of leukemogenesis has been proposed and widely accepted. This review concisely summarizes characteristic features of myeloid leukemias in children with DS, particularly focusing on transient leukemia (TL), including its prenatal origin and the role of the hematopoietic microenvironment in disease progression and/or regression, and discusses currently proposed hypotheses for the mechanism of spontaneous remission of TL.

### Myeloid leukemias in children with Down Syndrome (DS)

As in the case of children without DS, acute lymphoblastic leukemia (ALL) is the predominant type of leukemia in patients with DS aged 4 years or older. On the other hand, AML is as commonly seen as ALL in patients with DS less than 4 years of age [2]. Interestingly, acute megakaryoblastic leukemia (AMKL, or M7 in the FAB classification), a rare subtype of AML in non-DS patients, comprises the majority (about 70%) of AML in young children with DS (AML-DS) [3].

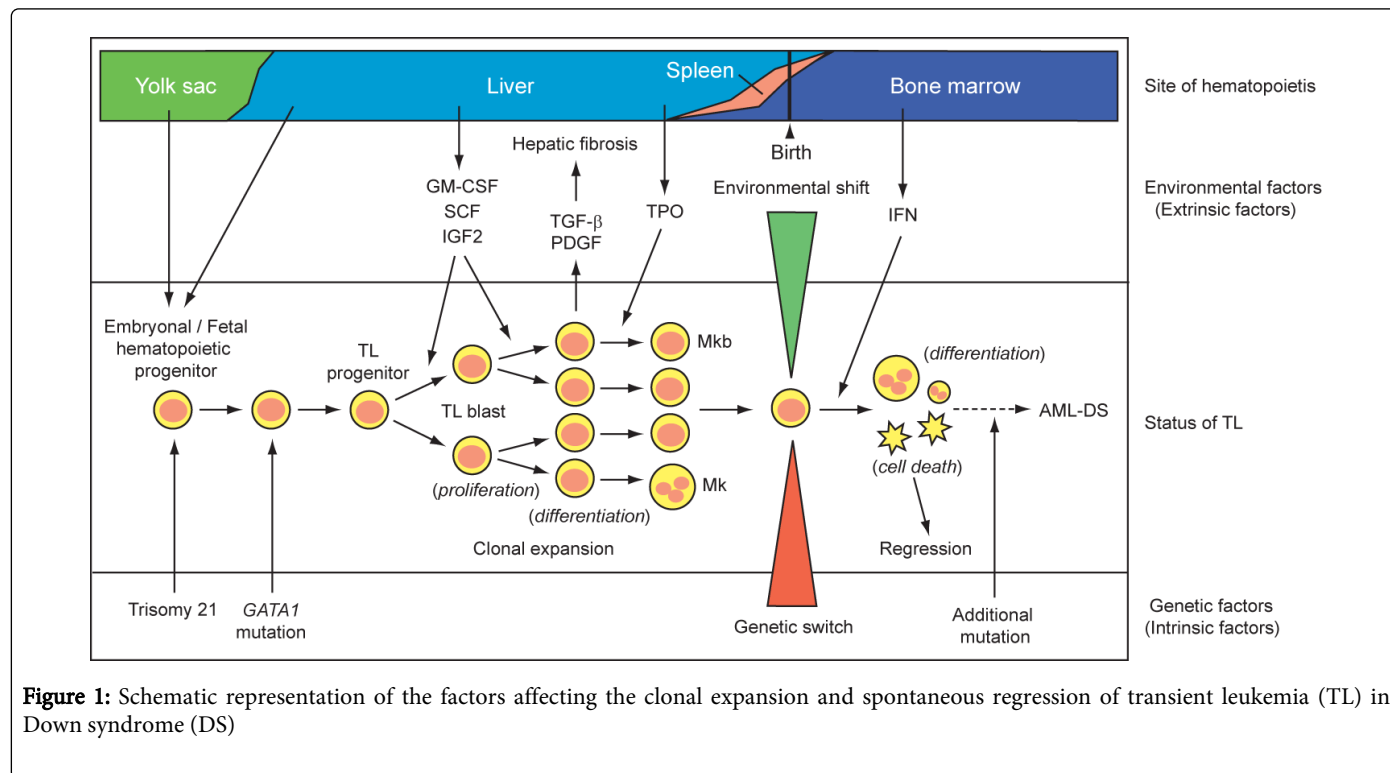
Furthermore, in neonates with DS, blasts that are indistinguishable from AML blasts may appear in the blood, but usually spontaneously disappear within several weeks or months with no or only minimal therapy. Because of this unique clinical course, it was controversial in the past whether this phenomenon is a leukemoid reaction due to ineffective hematopoiesis or a manifestation of real neoplasm, and a variety of names have been given to this disorder, such as TL, transient myeloproliferative disorder (TMD) and transient abnormal myelopoiesis (TAM). Since the blasts of TL and AML-DS exhibit very similar morphological as well as immunophenotypic characteristics and harbor common genetic abnormalities as described below, and AML-DS usually occurs several years after the spontaneous resolution of TL in 20-30% of patients [2,4], these disorders had been considered a disease entity and called “myeloid leukemias of Down syndrome”, which were later renamed “myeloid proliferations related to Down syndrome” in the current World Health Organization (WHO) Classification published in 2008 [2].

### Transient leukemia (TL) and multistep model of leukemogenesis

TL usually occurs in the neonatal period (median age at diagnosis, 7 days; range, 1-65 days)[5] in 4-10% of patients with DS[4,6]. Clinical manifestations in symptomatic cases include hepatosplenomegaly, effusions, bleeding and skin rash. Not infrequently, however, there are no signs of symptoms related to TL and the diagnosis is made as a result of a routine medical checkup or incidental blood examination performed because of another unrelated illness. The disease gradually disappears within the first 3 months of life (mean, 84 days) [5] in most cases without any therapy and the prognosis is generally good. However, severe life-threatening complications, such as hepatic dysfunction, cardiopulmonary disease, hyperleukocytosis,

hyperviscosity and hepatosplenomegaly, occur in approximately 15% of patients [7]. Fetal cases of TL, resulting in stillbirth, have also been reported, but the exact incidence of TL in utero is unclear because fetal

cases undergoing spontaneous resolution may be undetected and fatal cases may be missed unless autopsy is performed.



**Figure 1:** Schematic representation of the factors affecting the clonal expansion and spontaneous regression of transient leukemia (TL) in Down syndrome (DS)

Besides its transient nature, TL has more unique features as follows: 1) the blast ratio in the bone marrow (BM) is usually similar to, or lower than, that in the blood [3,5,8]. This phenomenon is quite unusual for ordinary AML, in which the BM is occupied by blasts when a large number of blasts appear in the blood; it is explained by the hypothesis that TL arises in the fetal liver (FL), but not the BM, as described below; 2) in autopsy cases of TL, characteristic diffuse sinusoidal fibrosis is often seen in the hepatic lobules [9], the reason for which can also be explained by the hypothesis mentioned above and will be discussed below; and 3) TL blasts have the capability of differentiating into mature blood cells of several lineages in vitro, including megakaryocyte, basophil/mast cell and eosinophil series [10,11]. This is also very unusual for AML blasts, in which morphological differentiation is largely limited.

Molecular analyses have demonstrated that TL blasts are monoclonal in origin [12,13], indicating that TL is a neoplastic disorder rather than a leukemoid reaction, although oligoclonal neoplastic populations can exist in some patients [14]. Because of the transient nature of TL, many investigators consider it as preleukemia that has not acquired the potential for perpetual progression. In TL, however, blasts indistinguishable from AML blasts exhibit overgrowth and tissue infiltration, which may lead to patients' death due to organ failure in severe cases. Thus, TL is a disorder distinct from myelodysplastic syndrome, a typical example of preleukemia in adults, and considered by others as a special form of leukemia [8], originating from prenatal cells with self-limiting growth potential. In this regard, TL appears to be similar to infantile neuroblastomas [15], which form tumor mass lesions with histology the same as that of fatal aggressive forms and can metastasize to neighbouring or even distant organs, leading to death of some patients, but usually spontaneously regress

and the majority of patients show favorable outcome even if metastasis has occurred.

More recent molecular studies have revealed that the *GATA1* gene, which encodes GATA1, one of the GATA family of zinc-finger transcription factors with the common DNA-binding site for the consensus nucleotide sequence (T/A) GATA (A/G), is mutated in an acquired manner exclusively in, and in nearly all cases of, TL and AML-DS [16-20]. A variety of *GATA1* mutations have been found, but almost all of these mutations result in uniform abnormalities at the protein level, namely, a lack of full-length GATA1 protein and exclusive expression of the short isoform of GATA1 protein, called GATA1s, lacking the N-terminal transactivation domain [16]. Although TL is a disorder of DS patients, it rarely occurs in phenotypically normal non-DS patients with trisomy 21 mosaicism, which is always present in blasts [21-23]. This fact indicates that trisomy 21 is also a prerequisite for leukemogenesis in TL. Although it has been shown that the *GATA1* mutation and trisomy 21 each alone can cause hyperproliferation of megakaryocytic progenitors (MkPs) in the FL, TL does not occur under the condition where only one of them is present. Therefore, acquired *GATA1* mutations in addition to constitutional or mosaic trisomy 21 are thought to play an essential role in leukemogenesis of TL, and postnatal additional mutations are required to cause transition from TL to AML-DS. This multistep model of myeloid leukemogenesis in DS is now widely accepted [1,3,4] (Figure 1).

### Prenatal origin and multiple clones of TL

There are plenty of data, although indirect, that show that the *GATA1* mutations occur in utero. For instance, since TL is usually

found in neonates and is a neoplastic disorder that takes time to evolve, it should arise in utero. Another example is that the same *GATA1* mutations have been detected in AML-DS blasts of two identical twins at older ages [19,24]. In this case, blasts in one of the twins with a *GATA1* mutation must have migrated to the other twin in utero through anastomosing blood vessels in the placenta. Other examples include that neonatal blood spots of AML-DS patients have been shown to harbor *GATA1* mutations not infrequently in multiple forms, including the same one as that of AML blasts and additional different ones, in single patients [25]. More than one form of *GATA1* mutation in an individual has also been detected in patients with TL [10,14]. These findings indicate that multiple forms of *GATA1* mutation can occur in utero, leading to multiple clones of leukemic cells in a single patient in both TL and AML-DS. A recent molecular analysis has further revealed that, in addition to 8.5% of DS neonates with *GATA1* mutations that are detected by Sanger sequencing/high-performance liquid chromatography, low-abundance *GATA1* mutant clones detected by targeted next-generation sequencing are present in about 20% of DS neonates [26]. Taken together, it is now clear that *GATA1* mutations occur at much higher frequency in DS fetuses than has ever been thought and that one or more clones of these *GATA1* mutant cells may evolve to TL in fetal/neonatal stages and later to AML-DS.

The prenatal origin of TL has also been suggested by experimental data on mice. Li et al. [27] created *GATA1*s knock-in mice, which exclusively produce *GATA1*s, and showed that dominant action of *GATA1*s leads to in vitro hyperproliferation of MkPs derived from yolk sac and early FL, but not postnatal BM, of the mice. These data indicate that embryonal/fetal MkPs are selectively sensitive to the effects of *GATA1*s and could be the target for leukemic transformation in TL, thereby accounting for the transient nature of TL (Figure 1).

## TL and hematopoietic microenvironment

### Hepatic fibrosis in TL

One of the characteristic features of AMKL is that it is very often, although not always, accompanied by myelofibrosis and it is thought that cytokines, including transforming growth factor  $\beta$  (TGF- $\beta$ ) and platelet-derived growth factor (PDGF), produced by leukemic megakaryoblasts stimulate fibroblasts in the BM and cause myelofibrosis [28]. Despite the fact that TL blasts exhibit a megakaryoblastic phenotype, similarly to AML-DS blasts, myelofibrosis is rare and instead liver fibrosis is often found in autopsy cases of TL [9]. The reason for this is thought to be that TL arises in the FL, where cytokines produced by TL blasts stimulate hepatic fibroblasts (or stellate cells) and cause liver fibrosis in a similar manner to that of myelofibrosis in AML-DS. In fact, the expression of TGF- $\beta$  and PDGF in TL blasts in the FL has been demonstrated [29,30]. Liver fibrosis seen in TL patients shows a unique form of diffuse sinusoidal fibrosis in hepatic lobules [9]. This fact is consistent with the autopsy findings of stillborns with TL that blasts proliferate in the hepatic lobules [31] as described below. The reason for the blast ratio of the BM being parallel to that of the peripheral blood can also be explained by the above hypothesis that TL originates from the FL. BM is usually the most important organ for leukemia cell proliferation, but this is not the case in TL, and the BM is only secondarily involved by disease progression in TL.

## TL blasts and hematopoietic growth factors

We and other investigators have demonstrated that the in vitro growth and differentiation of TL blasts are dependent on certain hematopoietic growth factors [10,32,33]. These include Interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF; alternatively called mast cell growth factor [MCGF]), thrombopoietin (TPO) and insulin-like growth factor 2 (IGF2). Most of these hematopoietic growth factors (GM-CSF, SCF, TPO and IGF2) have been shown to be produced by cells in the FL, including stromal cells [34] and hepatocytes/hepatoblasts [35,36]. Therefore, the FL can be an important microenvironment that provides conditions suitable for clonal expansion of TL blast progenitors and disease progression.

## In vivo growth and differentiation of TL blasts in fetal organs

Pathological observation of autopsy cases of TL stillborns and newborn babies immediately after birth has shown characteristic patterns of in vivo growth and differentiation of TL blasts in fetal organs: TL blasts and atypical megakaryocytes, which are highly likely to be their differentiated descendants, are present preferentially in certain organs, including the liver, spleen, heart and in the blood vessels of various organs [23,31,37] (literature data summarized in Table 1 in ref. 37). Among the fetal organs, the liver is most commonly involved in TL, with some cases being accompanied by hepatic fibrosis as in the postnatal cases of TL, indicating that FL is the main organ for the growth and differentiation of TL. TPO is an important hematopoietic growth factor that promotes megakaryopoiesis and the major source of TPO is known to be hepatocytes and hepatoblasts [35], which release it into the blood. Considering that TL blasts exhibit morphological and phenotypic characteristics of megakaryoblasts, have the potential to differentiate into mature cells, including megakaryocytes, and that TL blasts and atypical megakaryocytes are mainly located in the sinusoids of the FL and blood vessels of various organs [31,37], it seems plausible that TPO is involved in megakaryocytic differentiation of TL blasts.

## In vitro growth of TL blasts and FL stromal cells

Only limited data providing direct evidence of the functional relationship between the cells comprising the fetal hematopoietic microenvironment and TL blasts have been reported. We have recently analysed the in vitro effects of human FL and fetal BM (FBM) stromal cells on TL blasts using coculture and clonal culture methods [34]. The growth of TL blast progenitors was efficiently supported by FL, but not FBM, stromal cells in the absence of cell-to-cell contact. FL stromal cells produced hematopoietic growth factors and released them into the culture medium at high concentrations. Experiments with neutralizing antibodies have shown that, among the growth factors contained in the culture medium, GM-CSF is crucial for the growth of TL blast progenitors. These data are compatible with the in vivo observations described above, showing that FL is the main organ for TL blast growth, and support the hypothesis that TL arises in the FL.

The FL stromal cells that we used expressed nestin, CD146 and  $\alpha$ -smooth muscle actin, which are known to be markers of perivascular mesenchymal stem cells and hepatic stellate cells (Ito cells) [34]. These types of cell are known to support hematopoietic stem cells and hematopoiesis [38,39]. In addition, our FL stromal cells also expressed

cytokeratin 8, an epithelial antigen, indicating that these cells have the nature of unique cells in epithelial-mesenchymal transition (EMT) [40]. Such stromal cells with an EMT phenotype have been shown to be present in the FL and to support hepatic hematopoiesis [41].

### The mechanism of TL spontaneous remission

It is currently unclear why TL spontaneously resolves without any therapy. On the basis of previous clinical and research findings, two major hypotheses have been proposed [1,9,42] (Figure 1): 1) As described above, it has been shown that TL blasts are derived from hematopoietic progenitors in prenatal hematopoietic organs and that the growth of TL blasts is dependent on the microenvironment of the FL, not the BM. Since the major organ of hematopoiesis gradually shifts from the liver to the BM at late stage of prenatal life and hepatic hematopoiesis ceases shortly after birth, this may result in loss of the microenvironment necessary for TL blasts and arrest of their growth. It is also possible that there are unknown factors in the postnatal BM microenvironment that stop the proliferation of TL blasts. These scenarios are called the microenvironmental or “extrinsic” theory; and 2) Hematopoiesis occurs in several waves in different organs in ontogeny: it originates from “primitive erythropoiesis” in the yolk sac, then moves to the FL, where adult-type hematopoiesis (“definitive hematopoiesis”) begins, and further moves to the BM in the late fetal stage where it continues throughout life [43]. A good example of the developmental switch is seen in hemoglobin synthesis: several types of hemoglobin, a tetramer consisting of 2 pairs of globin subunit ( $\zeta$ ,  $\alpha$ ,  $\epsilon$ ,  $\gamma$ ,  $\delta$ ,  $\beta$ ), are successively produced from embryonal (HbE;  $\zeta 2\epsilon 2$ ) through fetal (HbF;  $\alpha 2\gamma 2$ ) to adult (HbA;  $\alpha 2\beta 2$ ) type in accordance with the hematopoietic site transition, and these hemoglobin subtype switches are mainly governed by a genetic mechanism and not determined by the site of erythropoiesis [44]. If TL blasts are derived from prenatal hematopoietic progenitors, they may already be destined to stop proliferation and/or die at an appropriate time according to the preset genetic program, even if they are neoplastic cells (genetic or “intrinsic” theory). Besides these hypotheses, another additional possibility is that the capability of TL blasts to differentiate into mature blood cells may also be involved in spontaneous remission of TL, irrespective of whether an extrinsic or intrinsic mechanism triggers the exertion of this ability.

Our recent *in vitro* experiment described above has shown that, although the growth of TL blasts is dependent on FL, but not FBM, stromal cells, it is also supported by KM101 cells, an adult human BM-derived stromal cell line [34]. This result is not consistent with the extrinsic hypothesis that TL blast growth is attenuated by a transition of major hematopoietic organs from the liver to the BM. It is unclear, however, whether neonatal BM stromal cells have full functional activity equivalent to that of adult BM stromal cells and the above hypothesis remains possible. The precise effects of the neonatal BM microenvironment on TL blasts should be elucidated to answer these questions.

Other recent data concerning spontaneous TL resolution are those of Woo et al. [45], who have demonstrated developmental stage-specific differences between MkPs in adult BM and those in early FL by showing that type I interferon (IFN)-inducible genes are upregulated in BM-MkPs versus FL-MkPs in wild-type mice and that this is still apparent in mice engineered exclusively to express GATA1s (“GATA1s mice” described above). In their experiment, hyperproliferation of FL-MkPs in GATA1s mice was inhibited by exogenously added IFN- $\alpha$ , indicating that the IFN- $\alpha$ / $\beta$  signaling

pathway may be poorly activated in the FL but contribute to the spontaneous resolution of hyperproliferation of GATA1s FL-MkPs in postnatal BM. Data supporting this notion have been provided by the authors in that adult BM-MkPs in compound *Ifnar1*<sup>-/-</sup>::GATA1s mice (made by breeding GATA1s mice with *Ifnar1*<sup>-/-</sup> mice) or those in GATA1s mice in the presence of neutralizing anti-IFN- $\alpha$  antibody partially reverted to a hyperproliferative state. These findings indicate that environmental factors, including IFNs, might play a role in the spontaneous resolution of TL.

### Conclusions

Spontaneous remission of cancers is a peculiar phenomenon, which is very often seen in infantile cancers, as described above, but also occasionally in adult cancers, including non-Hodgkin lymphomas (particularly low-grade lymphomas) [46-48]. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) has a well-known causal link with *Helicobacter pylori* and tumor regression may be achieved by eradication of the infection [49]. Other examples include cases of non-Hodgkin lymphomas, which regressed following the withdrawal of methotrexate in patients with rheumatoid arthritis [50]. The precise reason for the spontaneous regression of lymphomas is unknown and a variety of mechanisms have been proposed, including concomitant bacterial or viral infections, possibly mediated by endotoxin or interferon, respectively. Among the proposed hypotheses, the most probable cause has been attributed to an immunological mechanism [46,47]. Supporting this is the fact that low-grade, rather than high-grade, lymphomas are prone to regress spontaneously, which may indicate that the former are not full-blown cancers and more susceptible to immunoregulatory surveillance. Infection and withdrawal of immunosuppressants, such as methotrexate, can also activate the immune response. In contrast to adult cancers, spontaneous remission of infantile cancers with a prenatal cell origin, such as TL and neuroblastoma, occurs with a specific prenatal to postnatal genetic and environmental background, indicating that its mechanism should be distinct from those in adult cancers. Although it is currently unclear whether intrinsic and/or extrinsic factors play the primary role in the spontaneous remission of infantile cancers, there could be common fundamental mechanisms underlying these special types of cancer, along with different mechanistic bases for each cancer. The mechanism of spontaneous cancer remission has been, and will continue to be, an attractive research area because elucidation of the mechanism will lead to the development of novel therapeutic strategies for cancer patients involving both children and adults.

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