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Profile of Down Syndrome–associated malignancies: Epidemiology, clinical features and therapeutic aspects

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Abstract

Down syndrome (DS) is a congenital chromosomal abnormality caused by the presence of all or part of a third copy of chromosome 21 (+21). DS is frequently complicated by congenital heart or digestive tract diseases at birth. DS patients are prone to infections and have mental retardation, with dementia such as Alzheimer's disease showing in later life. Furthermore, malignancies with specific characteristics are also highly reported in DS patients compared with non-DS patients. Therefore, DS is believed to be a cancer predisposition syndrome due to the chromosomal instability. Acute myeloid leukemia (AML) and especially acute megakaryoblastic leukemia (AMKL) by French-American-British (FAB) classification are the most frequent hematological malignancies in DS patients, occurring at a rate that is 500 times higher than that in non-DS patients. Interestingly, transient abnormal myelopoiesis (TAM) is observed in approximately 10% of DS neonates with *GATA1* mutations, and most TAM patients are asymptomatic and show spontaneous regression; however, about 10%–20% of TAM cases are fatal because of complications such as fetal effusion, liver fibrosis, and other complications.

Acute lymphoblastic leukemia (ALL) is also associated with DS, occurring at a rate that is 20 times higher than that in non-DS patients. Furthermore, the prognosis of

DS-ALL patients is poorer than that of non-DS-ALL patients. A recent genetic analysis revealed that more than half of DS-ALL cases have a mutation in the CRLF2–JAK pathway, indicating that JAK inhibitors might have a limited effect for DS-ALL patients.

Notably, solid tumors such as neuroblastoma, Wilms tumor, and brain tumor, which are frequently observed in non-DS children, are rarely reported in DS children. The reason remains unknown, but it may be because of the triplication of the Down syndrome critical region 1 (DSCR1) gene on chromosome 21. In adult patients with DS, the expected age-adjusted incidence rates of solid tumors are low compared with age-matched euploid cohorts for most cancers except for testicular cancer. Although the average life expectancy of patients with DS will increase with advances in healthcare, the detailed health problems including cancer rates in older DS patients remain unknown. Therefore, these issues will be needed to be addressed in future studies.

Keywords:

Down syndrome; acute myeloid leukemia; acute megakaryoblastic leukemia; transient abnormal myelopoiesis; acute lymphoblastic leukemia; solid tumor; cancer predisposition syndrome; GATA1; Down syndrome critical region 1

1. Background

Down syndrome (DS) is a congenital chromosomal abnormality caused by trisomy 21 (+21) and is frequently complicated by infection, congenital heart or digestive tract diseases, mental retardation, and developmental delay [1, 2]. Individuals with DS are more likely to be diagnosed (10–30 times) with hematological malignancies than non-DS individuals, and therefore DS is believed to be a cancer predisposition syndrome due to the chromosomal instability caused by +21 [3-6].

A high frequency of acute myeloid leukemia (AML), especially acute megakaryoblastic leukemia (AMKL), has been observed in DS patients with mutations in *GATA1*, which encodes the GATA1 transcription factor [7-9]. AMKL occurs at a rate of 500 times higher in DS patients than non-DS patients. *GATA1* mutations have also been found in patients with transient abnormal myelopoiesis (TAM) [10, 11]. However, the risk of acute lymphoblastic leukemia (ALL) is 20-fold greater in DS patients than non-DS patients. Approximately 10% of DS neonates also show TAM/transient myeloproliferative disorder (TMD)/transient leukemia. After regression of TAM, about 20%–30% of DS patients develop AMKL. The morphology and immunophenotypes of TAM and AMKL are similar and the same *GATA1* mutation has been observed in both TAM and AMKL blasts [11, 12]. However, the prognosis of AMKL with DS seems to

be better than that of AMKL without DS, even with reduced-intensity chemotherapeutic regimens [13, 14]. More than half of DS-ALL patients have high expression of type I cytokine receptor, *CRLF2*, with *P2RY8–CRLF2* fusion genes and alterations in *JAK* [15-17]. The reason underlying the high frequency of leukemia in DS patients remains unknown, but the extra copy of 21 may affect leukemogenesis [5]. Notably, several genes on chromosome 21 such as *Runt-related transcription factor 1* (*RUNX1)*, *erythroblast transformation-specific* (*ETS*), and *ETS-related gene* (*ERG)* are believed to affect leukemogenesis [18-23] Furthermore, immunological disturbances such as decreased maturation of T/B cells and NK cell dysfunction in DS may affect leukemogenesis [24-26]. However, solid tumors such as neuroblastoma, hepatoblastoma, and brain tumors are rarely reported in DS [27-30]. The mechanism underlying the specific tumor spectrum in DS has been unclear. Notably, the Down syndrome candidate region 1 (*DSCR1*) gene on chromosome 21 encodes a protein that suppresses vascular endothelial growth factor (VEGF)-mediated angiogenic signaling by the calcineurin pathway [31]. Attenuation of calcineurin activity by DSCR1 dramatically diminishes angiogenesis, which plays a crucial role in the proliferation and expansion of solid tumors.

In this article, DS-related malignancies including hematological malignancies and

solid tumors are reviewed.

2. DS-related myeloid disorders

DS is complicated by AML, especially AMKL, according to the French-American-British (FAB) classification [32-35]. In AMKL, immature megakaryoblasts spontaneously proliferate, resulting in neutropenia, anemia, and thrombocytopenia. Hence, the 2016 revision to the World Health Organization (WHO) classification included a distinct category of myeloid leukemia related to DS (ML-DS) [36]. In the process of TAM [37], TMD [38], or transient leukemia [39], morphologically similar blasts proliferate in DS neonates and exist in the peripheral blood (PB) for 3–4 months. Furthermore, some DS patients continuously show anemia or thrombocytopenia even after the disappearance of the TAM blasts, at which point a diagnosis of myelodysplastic syndrome (MDS) is made.

2-1. TAM in DS patients

Approximately 10% of DS neonates present with TAM. The majority of TAM patients show spontaneous regression of TAM blasts within 3-4 months, and thus the prognosis seems to be better even without therapy. After spontaneous remission, around

20%–30% of TAM patients develop AMKL (M7 by FAB classification) within 5 years after birth. This type of AML is very rarely reported in adults (<1%) and occurs in 15% of children [40-43]. However, approximately 10%–20% of TAM cases are complicated by fetal hydrops and irreversible liver fibrosis, which results in liver failure and coagulopathy [44-49]. Unfortunately, the prognosis of these fetal TAM patients is quite poor. A prospective study by the Children's Cancer Group (COG) revealed that approximately 10% of early deaths occurred in TAM patients [50]. TAM develops *in utero* because of the presence of mutant *GATA1* [9, 51]. Thus, TAM is sometimes complicated by severe fetal hydrops and pleural and abdominal effusion [45, 49, 52, 53]. Unknown stillbirth of DS might sometimes be because of TAM [46].

2-1-1. Clinical features of TAM, asymptomatic to severe

The clinical symptoms of TAM patients range from asymptomatic to severe. The most common physical finding in TAM patients is hepatosplenomegaly. *In utero*, TAM blasts start to proliferate in the liver and spleen [46]. After birth, the main location of hematopoiesis changes from extramedullary organs such as the liver or spleen to an intramedullary location, the BM niche (Figure 1).

In contrast to asymptomatic TAM patients, severe TAM patients show marked hepatosplenomegaly, pleural or abdominal effusion, multiple organ failure, and

coagulopathy such as disseminated intravascular coagulation. Delayed-onset hyperbilirubinemia is a sign of progressive liver fibrosis that can result in fatal liver failure, even after the disappearance of TAM blasts [45, 47]. This is the main cause of the early deaths of TAM patients within 6 months of birth. This liver failure step seems to be irreversible, and thus novel treatment approaches are required to halt the progression of liver failure.

2-1-2. Laboratory findings of TAM patients

The diagnosis of TAM is relatively straightforward because characteristic leukocytosis and blasts exhibiting typical morphology called bulla or bleb are found in the PB of DS neonates. The white blood cell (WBC) count in PB is sometimes increased by more than 100×10^9 cells/L, resulting in leukocytosis. The immunophenotype of TAM blasts is similar to that of AMKL blasts, with positivity for stem cell markers (CD34, CD117), myeloid markers (CD13, CD33), and megakaryocytic lineage markers (CD41 or CD61). However, some TAM blasts in PB were detected at less than 5% accompanied by a normal WBC count. Furthermore, a relatively small percentage of blasts are found in the bone marrow (BM) of TAM patients [50, 59, 60]. Therefore, BM aspiration or biopsy is not recommended for the diagnosis of TAM. Asymptomatic DS neonates may be accidentally diagnosed with TAM due to the detection of blasts in their

PB with or without the presence of leukocytosis.

2-1-3-1. Pathogenesis of TAM: *GATA1* mutations

GATA1 is located on chromosome X and encodes a transcription factor that regulates the maturation of erythroid and megakaryocyte lineages, which produce red blood cells or platelets. Almost all DS-TAM patients have *GATA1* mutations, and these mutations are frequently found in exons 2 and 3 (Figure 2) [10, 11]. These mutations lead to the expression of a truncated GATA1 protein, GATA1s, which lacks the N-terminal transcription activity and results in different gene expression profiles [42, 61, 62]. *GATA1* mutations are found in approximately 10% in DS patients. However, some DS neonates are not diagnosed with TAM but as having *GATA1* mutations. Surprisingly, Roberts et al. reported that 195 of 200 (97.5%) DS neonates had circulating TAM blasts. *GATA1* mutations were found in 17 of 200 DS neonates (8.5%) by Sanger sequence/denaturing high performance liquid chromatography and all with blasts >10%. Low abundance of *GATA1* mutated clones was detected by targeted next generation sequence (NGS) in 18 of 88 (20.4%) DS neonates without *GATA1* mutations by standard detection methods. These cases are known as “silent TAM” [40]. Therefore, 35 of 200 (17.5%) DS neonates had *GATA1* mutations and blasts >10% at diagnosis of TAM, demonstrating an important clinical point of TAM patients. If these highly

sensitive NGS methods were combined, *GATA1* mutations would be identified in approximately 20% of DS neonates. Using single cell analysis for DS neonates, more patients with *GATA1* mutation would be identified. Conversely, Terui et al. reported *GATA1* mutations in 56% of genomic DNA samples from BM and 71% cDNA samples from PB as detected by Sanger sequencing; the study showed that 89% of TAM neonates have *GATA1* mutations. Furthermore, targeted NGS detected *GATA1* mutations in 90% of TAM neonates. In total, *GATA1* mutations were detected in 98% of DS-TAM patients using a combined approach of Sanger sequencing and NGS [63]. Therefore, the detection method of *GATA1* mutations is an important consideration in future studies.

2-1-3-2. Pathogenesis of TAM: uniparental disomy of chromosome 21

The usual frequency of uniparental disomy due to the chromosomal non-disjunction in meiosis is estimated at 1:3 in paternal origin vs. maternal origin in DS non-TAM patients. The precise data in DS-TAM patients remains unknown, but the mapping of a possible gene for TAM at 21q11.2 was reported in 1991 [64, 65]. Furthermore, several genes including *DSCR1* on partial chromosome 21 could cause the myeloproliferation in TAM [23, 66, 67]. Takahashi et al. found a 10-Mb amplification of 21q22.12–21q22.3 by SNP array and revealed that dual specificity tyrosine-phosphorylation-regulated kinase 1A (*DYRK1A*), *ERG*, and *ETS* but not *RUNX1* are candidate genes for the

genesis of TAM [23]. In particular, *DYRK1A* promoted megakaryoblastic leukemia in a murine model of DS [4, 68].

A recent study in induced pluripotent stem cells revealed that trisomy 21 alone could affect myeloproliferation, and thus *GATA1* mutations are insufficient for the proliferation of TAM blasts [69]. Furthermore, a NGS study conducted in a large number of TAM patients revealed that only *GATA1* mutations were detected in these patients [70, 71]. Therefore, *GATA1* mutations and an extra copy of several genes including *DYRK1A* on chromosome 21 cooperate with TAM genesis. Future studies might reveal the gene(s) on chromosome 21 responsible for TAM genesis.

2-1-3-3. Pathogenesis of TAM: the role of the microenvironment in DS fetal liver and BM

The most defining feature of TAM compared with other hematological malignancies seems to be the origin of TAM blast proliferation. TAM develops *in utero*, and thus the main site of development is the fetal liver and spleen, which is called extramedullar hematopoiesis [9, 12, 72]. The direct evidence for TAM blasts in the fetal liver was observed in some autopsy cases [44]. The fetal liver is likely to provide the necessary microenvironment for driving and/or maintaining abnormal hematopoiesis in DS, but the factors responsible for maintaining or proliferating TAM blasts are not fully

understood. Miyauchi and Kawaguchi reported that stromal cells of the fetal liver, but not fetal BM, potently supported the proliferation of TAM blast progenitors, mainly through humoral factors such as granulocyte macrophage-colony stimulating factor (GM-CSF) through co-culture experiments. Therefore, fetal liver stromal cells provide a pivotal hematopoietic microenvironment for TAM blasts, and GM-CSF produced by fetal liver stromal cells may have an important role in the pathogenesis of TAM [72].

There is no strict evidence of the existence of leukemia stem cells (LSCs) in TAM. In the field of AML, LSC concept seems to be common and LSCs themselves have some genetic abnormalities [73]. However, the same mutant *GATA1* clone proliferates and develops into AMKL after the spontaneous regression of TAM blasts. Therefore, LSCs or progenitor cells with *GATA1* mutations could start to proliferate in the BM microenvironment within 5 years after birth. However, the precise mechanism of TAM colonization to initiate proliferation in the BM microenvironment remains unknown.

2-1-3-4. Pathogenesis of TAM: the role of inflammation

Several reports suggested that abnormal cytokine levels are present in TAM patients, including transforming growth factor (TGF)-β, interferon (IFN)-γ, interleukin (IL)-1β, and IL-6 [51, 53-56, 58, 72]. Our previous data suggested that lethal TAM cases are frequently complicated by uncontrolled pro-inflammatory cytokinemia, especially

highly elevated IL-1β, TNF-α, and IFN-γ [51, 55]. TAM blasts produce TGF-β, which is correlated with liver fibrosis [54]. Furthermore, some reports suggested that pro-inflammatory cytokinemia has already developed *in utero* and is sustained even after the regression of TAM blasts, which means that some abnormal cytokines were also maintained by the immunological bias of DS [24-26]. The levels of inflammatory cytokines with TAM were different from those without TAM. The precise mechanism of spontaneous regression of TAM blasts remains unknown, but these inflammatory cytokines might affect the spontaneous regression of TAM. Another explanation is that TAM blasts lose the support from the stroma cells of the fetal liver [72]. Miyauchi et al also reported that TAM blasts could differentiate into basophil/mast cells and megakaryocyte lineages *in vitro* [57]. Therefore, spontaneous regression might alternatively indicate the differentiation of TAM blasts. Another report suggested that chemokine levels or monocyte chemoattractant protein-1 (MCP-1) predicts the progress of liver failure of TAM patients [58, 74].

2-1-4. Treatment of TAM patients

Most TAM patients are usually asymptomatic and do not need chemotherapy because the blasts will spontaneously regress within 3–4 months. Nonetheless, symptomatic fetal TAM cases such as those with multi-organ failure, hyperleukocytosis

(WBC > 100×10^9/L), hepatosplenomegaly, hydrops fetalis, pleural or cardiac effusions, renal failure, and coagulopathy with bleeding should be considered for treatment. Exchange transfusion (ET) is effective in reducing TAM blasts, especially hyperleukocytosis, although it is not effective for other complications. Low dose cytosine arabinoside (LDCA) is the most preferable chemotherapeutic regimen for TAM patients; there was a non-significant trend towards improved survival (80±6% vs. 67±7%, p=0.1) in symptomatic TAM patients compared with a historical control. Furthermore, there was no apparent reduction in the cumulative incidence of DS-related myeloid leukemia (19±6% vs. 22±4%, p=0.95) [75]. Hydrops fetalis is a lethal clinical condition in DS neonates. In our previous study, three TAM neonates with hydrops fetalis were successfully treated with ET followed by LDCA [49]. The cases received LDCA after ET and all three remain alive to date. Liver failure is the biggest problem for fetal TAM patients, and the majority of TAM patients who suffer from severe irreversible liver failure will die. A recent report suggested the possibility of liver transplantation for fetal liver failure in TAM patients [76].

2-1-5. Who will develop AMKL in later life?

Approximately 20%–30% of TAM patients develop AMKL within 5 years after birth, but some patients will develop AML later in life. Kanezaki et al. revealed that

GATA1 mutations (with GATA1s expression) were significantly associated with a risk of progression to ML-DS [61]. However, DS patients who do not have a history of TAM could also develop AMKL in later life. An important question is whether ML-DS is always preceded by TAM [77]. There is the possibility that minor clones with *GATA1* mutations already exist during the neonatal period of these patients; these patients are referred to as “silent TAM” patients [78]. Alternatively, more minor clones such as LSCs might exist in the neonatal period. Saida et al. presented a xenograft model of TAM, which revealed that genetically heterogeneous subclones with varying leukemia-initiating potential already exist in the neonatal TAM phase, and ML-DS may develop from a pool of such minor clones through clonal selection [79].

There is currently no specific biomarker to predict AMKL development. However, *GATA1* mutations may be useful to detect minimal residual disease. If NGS technology can provide highly sensitive detection of a mutant *GATA1* clone, it will be a good monitoring method for the development of AMKL. Therefore, continuous observation is needed for TAM patients for at least 5 years after birth, even after the spontaneous regression of TAM. Flasinski et al. reported that LDCA treatment helped to reduce TMD-related mortality compared with the historical control but was insufficient in preventing progression to ML-DS [75]. Further studies are required to examine

strategies to prevent leukemogenesis.

2-2. MDS in DS patients

Despite the spontaneous regression of TAM blasts, DS patients sometimes show continuous anemia or thrombocytopenia with or without blasts. This is known as MDS in DS patients and frequently requires the same chemotherapy as DS-AMKL [80].

Mast et al. published a large study on DS-MDS (n=60) and DS-AML (n=103) and found that dysplastic change was frequently observed in megakaryocyte and erythroid lineages with reticulin fibrosis but infrequent in myeloid lineage in both DS-MDS and DS-AML. Patients with DS-MDS and DS-AML demonstrated similar rates of 5-year event-free survival (EFS) (MDS, 92%±7%; AML, 88%±6%) and overall survival (OS) (MDS, 95%±6%; AML, 90%±6%) [81]. Therefore, the only criterion to distinguish MDS and AML was blast percentage [MDS, mean 11% (range 2%–17%); AMKL 42% (range 20%–90%)].

2-3. DS-related myeloid malignancy

Most DS-related myeloid malignancies are AMKL (M7 by FAB classification; ML-DS by WHO classification); other types of AML were also infrequently reported

especially in patients older than 4 years old [81]. In general, <1% of adult AML is AMKL and 15% of pediatric AML is AMKL [35]. The prognosis is quite different from that of DS-AMKL (ML-DS) and non-DS-AMKL (non-ML-DS). A Japanese nationwide prospective study of DS-AMKL reported that the 3-year EFS and OS rates were 83.3%±4.4% and 87.5%±3.9%, respectively [82]. An international retrospective study of non-DS-AMKL reported that the 5-year EFS and OS were 43.7%±2.7% and 49.0%±2.7%, respectively [83]. the German Berlin-Frankfurt-Miinster (BFM) data achieved an improved 5-year OS (AML-BFM 04; 70±6% vs. AML-BFM 98; 45±8%, P log rank = 0.041) [84]. A recent molecular study revealed that the *CBFA2T3-GLIS2* chimera in non-DS-AMKL subgroup showed poor prognosis [35, 85, 86]. According to the specific chimera, the prognosis was clearly different in non-DS-AMKL [87].

Approximately 20%–30% of TAM patients develop AMKL and the identical *GATA1* mutation is found in both TAM and AMKL blasts, as previously described.

2-3-1. Molecular background of ML-DS development

GATA1 mutations are essential but insufficient for the development of AMKL. A previous study amplified the segment in the critical DS region on chromosome 21 between DS and euploid AML-M0, which excludes *RUNX1*, *ERG*, and *ETS* [88]. Recent exome sequencing studies of ML-DS revealed a high frequency of mutations in

cohesins, CCCTC-binding factor (CTCF), or other chromatin regulators [70]. Other mutations, believed to enhance growth and proliferation, occur in genes in signaling pathways, such as RAS and the thrombopoietin receptor MPL, or downstream JAK-STAT signaling [70]. Notably, these additional genetic events occur within 5 years after birth, and therefore ML-DS seems to be a good model to understand leukemogenesis [77].

2-3-2. Diagnosis of ML-DS

The morphology and immunophenotypes of blasts of ML-DS are typical and are similar to those of TAM, with erythroid and megakaryoblastic lineages. The immunophenotype of AMKL blasts is similar to that of TAM blasts and is positive for stem cell markers (CD34, CD117), myeloid markers (CD13, CD33), and megakaryocytic lineage markers (CD41 or CD61). BM aspiration is frequently unsuccessful because of myelofibrosis or due to a dry tap; therefore, blast counts are often underestimated at below 20% of nucleated cells, which does not satisfy the definition of AML. Therefore, the diagnosis of ML-DS is not dependent on blast counts. Furthermore, additional chromosome abnormalities are frequently acquired in AMKL blasts, but the common translocations associated with non-DS-AML are rare [89].

2-3-3. Treatment of ML-DS

DS-AMKL blasts showed a high sensitivity to cytarabine (CA) [90, 91]. Several decades ago, the same intensity chemotherapeutic regimen for non-DS patients was applied for ML-DS, but the clinical outcome was worse because early death related to severe infection or regimen-related toxicities was frequently observed. Thus, chemotherapeutic regimens at reduced intensities were used for ML-DS. Kojima et al. reported that remission induction chemotherapy consisting of daunorubicin (25 mg/m^2/d for 2 days), CA (100 mg/m^2/d for 7 days), and etoposide (VP16, 150 mg/m^2/d for 3 days) showed a relatively good prognosis [92]. Kudo et al. reported that 70 of 72 (97.2%) patients with ML-DS treated with remission induction chemotherapy consisting of pirarubicin (25 mg/m^2/d for 2 days), CA (100 mg/m^2/d for 7 days), and VP16 (150 mg/m^2/d for 3 days) achieved complete remission with an estimated 4-year EFS rate of 83±9% [93]. The authors concluded that a less intensive chemotherapeutic regimen produces excellent outcomes in standard-risk ML-DS patients, and thus this specific regimen was applied for ML-DS in a nationwide study in Japan. The clinical outcome was superior, and a low relapse rate was also observed. Hence, a less intensive chemotherapeutic regimen produces excellent outcomes in ML-DS. The international ML-DS 2006 trial and COG trial A2971 also supported this concept [94, 95].

Notably, among ML-DS cases, some patients cannot receive reduced intensity

chemotherapeutic regimens because of other complications such as heart failure. Furthermore, relapsed ML-DS still shows poor prognosis. Relapse is the main cause of death of survivors of ML-DS. Hematopoietic stem cell transplantation (HSCT) is a very limited option for relapsed ML-DS. Therefore, future studies might be needed to identify new targeted therapies for relapsed ML-DS.

2-3-4. DS-ML other than AMKL

The common subtype of AML that occurs in non-DS patients (non-AMKL) also occurs in DS patients and is especially predominant in patients older than 4 years old. DS-AML in patients older than 4 years old is not associated with *GATA1* mutation and good prognosis like DS-AMKL [96].

3. DS-related lymphoid malignancy

3-1. Epidemiology, clinical, and laboratory features of DS-ALL

DS-ALL is uncommon compared with DS-ML; however, DS-ALL occurs at a 20-fold greater incidence than non-DS-ALL [27]. Several studies of children with DS-ALL showed an inferior outcome compared with non-DS patients [101-103]. Event-free (56% vs 74%; $P < .001$) and disease-free (55% vs 73%; $P < .001$) survival at 10 years was significantly lower in the standard-risk DS-ALL population compared with

non DS-ALL, but not in high-risk DS-ALL population [101]. An international retrospective study revealed that the major immunophenotype is precursor B cell ALL, and T cell ALL is rare [97]. Furthermore, normal karyotype was dominant (40.3%) and high hyperdiploid was infrequent in DS-ALL.

3-2. Genetic background of DS-ALL

Unlike ML-DS, in which a specific and critical disease-associated mutation *GATA1* has been identified, the genetic background of DS-ALL is quite heterogeneous. Common genetic events such as *BCR-ABL1*, *MLL* rearrangement, and *ETV6-RUNX1* are infrequent in DS-ALL compared with non-DS-ALL. However, more than half of DS-ALL patients have alterations in the CRLF2-JAK2 pathway, such as increased expression of CRLF2 and activating mutations JAK2 [15-17, 97]. These cases are considered Philadelphia chromosome-like ALL [41, 98]. In total, 50% of DS-ALL patients had more than one deletion in B-cell development genes: *PAX5* (12%), *VPREB1* (18%), and *IKZF1* (35%). JAK2 was mutated in 15% of patients, and genomic CRLF2 rearrangements were observed in 62% [99]. Outcome was significantly worse in patients with IKZF1 deletions (6-year EFS 45%±16% vs. 95%±4%; P = 0.002), which was confirmed in the validation cohort (6-year EFS 21%±12% vs. 58%±11%; P = 0.002). IKZF1 deletion was a strong independent predictor for outcome (hazard ratio

EFS 3.05; P = 0.001). Neither CRLF2 nor JAK2 were predictors for worse prognosis. The authors suggested that IKZF1 deletions may be used for risk-group stratification in DS-ALL [99].

Integrative genomic analysis of 25 matched diagnosis-remission and -relapse DS-ALLs revealed that CRLF2 rearrangements are early events during DS-ALL evolution and generally stable between diagnoses and relapse [100]. Secondary activating signaling events in the JAK-STAT/RAS pathway were ubiquitous but highly redundant between diagnosis and relapse, suggesting that this signaling is essential but that no specific mutations are “relapse driving.” Furthermore, activated JAK2 may be naturally suppressed in 25% of CRLF2-positive DS-ALLs by loss-of-function aberrations in USP9X, a deubiquitinase previously shown to stabilize activated phosphorylated JAK2. Therefore, the authors concluded that the therapeutic effect of JAK specific inhibitors may be limited [100].

3-3. Clinical outcome of DS-ALL

In general, the prognosis of DS-ALL is worse compared with that of non-DS-ALL [101-103]. Apart from ML-DS, there has been no specific study protocol for DS-ALL, and therefore the treatment protocol for non-DS-ALL was applied to DS-ALL in each study protocol. The EFS or OS of DS-ALL was 10%–20% lower than that of

non-DS-ALL [101-103]. The reasons postulated for this are that DS-ALL has a higher relapse rate, a higher induction failure rate, and a higher death rate due to severe complications. DS patients commonly incur severe infections after chemotherapy that could result in death [104, 105]. Another explanation is the relatively low frequency of favorable cytogenetic risk groups such as t(12;21) in DS-ALL [106].

In the international Ponte di Lengo study previously mentioned, DS-ALL patients had a higher 8-year cumulative incidence of relapse (26%±2% vs. 15%±1%, $P < 0.001$) and 2-year treatment-related mortality (TRM) (7%±1% vs. 2.0%±<1%, $P < 0.0001$) than non-DS patients, resulting in lower 8-year EFS (64%±2% vs. 81%±2%, $P < 0.0001$) and OS (74%±2% vs. 89%±1%, $P < 0.0001$) [97]. Relapse is the main contributor to poorer survival in DS-ALL; infection-associated TRM was increased in all protocol elements, unrelated to treatment phase or regimen.

ETV6-RUNX1 conferred an excellent prognosis and high hyperdiploidy with trisomy of chromosomes 4 and 10 was associated with a very low cumulative incidence of relapse [97]. The authors suggested that these patients, comprising 12% of DS-ALL, may be eligible for future treatment reduction to reduce TRM and can be treated according to the same risk-stratified algorithms as non-DS patients in the collaborative study group protocols [97]. Interestingly, the authors identified a clinically favorable

prognostic subgroup of DS-ALL patients, characterized by age < 6 years and WBC $<10\times10^9$/L.

Until now, some DS-ALL patients could not continue or complete the ALL-therapeutic regimen, and partial reductions in chemotherapeutic drugs were needed because of treatment toxicities. However, the reduction in chemotherapeutic drugs resulted in increased relapse and death rates [102]. The intensified treatment was not tolerable for DS patients, and the reduced intensity of chemotherapy such as that for ML-DS will not benefit DS-ALL.

The recent Dana-Farber Cancer Institute ALL consortium protocols 00-001 and 05-001 showed similar clinical outcomes of DS-ALL patients to non-DS-ALL despite a high rate of mucositis [107]. A recent COG study revealed the excellent long-term survival of DS children with standard risk ALL. The ten-year EFS rates for DS patients randomized to intravenous methotrexate (MTX) vs. oral MTX were 94.4% vs. 81.5%, respectively [108]. Furthermore, there were no increases in hepatic toxicity, systemic infections, or treatment-related deaths in DS-ALL patients.

Another ALL-BFM report suggested MTX toxicity in DS-ALL [109]. Higher MTX plasma levels were associated with increased toxicity, and therefore the authors concluded that a dose reduction of the first MTX course reduced severe toxicities

without increasing the risk of relapse.

3-4. Relapsed DS-ALL

Increased deaths and treatment-related mortality are the main barriers for the successful outcome of relapsed DS-ALL therapy [110]. Recently, relapsed DS-ALL patients were treated with clofarabine therapy or HSCT. Meissner et al. reported that relapse, and not regimen-related toxicity, was the main cause of death in DS-ALL patients who received HSCT [111]. These findings were confirmed by a recent study [112].

Several new therapeutic approaches have been used for DS-ALL such as blinatumomab [113] and inotuzumab-ozogamicin (IO) [114]. Blinatumomab is an anti-CD19 bispecific T-cell engager antibody construct that shows a good response in minimal residual disease-positive non-DS-ALL. IO is a humanized anti-CD22 monoclonal antibody conjugated to calicheamicin. IO has sub-nanomolar binding affinity and is rapidly internalized into cells that express CD22 to deliver the conjugated calicheamicin. Calicheamicin binds to the minor groove of DNA and induces double-strand cleavage with subsequent apoptosis. However, its severe adverse effects included cytokine release syndrome and sinusoidal obstruction syndrome/veno-occlusive disease in non-DS-ALL. For DS-ALL, the reduced

myelosuppression by both drugs is preferable, but there is only one case report to date. Further larger studies are needed to define the effectiveness of both drugs for DS-ALL. Chimeric antigen receptor T-cell therapy will be also applicable to DS-ALL patients.

4. DS-related solid tumors

4-1. DS-related solid tumors in children

Solid tumors in children such as neuroblastoma, Wilms tumor, and brain tumors that are common in euploid children are rarely reported in DS children [27]. A previous analysis of 6724 patients with neuroblastoma reported from 11 European countries identified no cases of neuroblastoma among children with DS [115]. The National Wilms Tumor Study registry reviewed 5854 Wilms tumor cases and did not identify any kidney tumors in children with DS [116]. Only retinoblastoma, an occult tumor, might have an association with DS [27, 117]. No report has suggested these tumors could occur *in utero*.

4-2. DS-related solid tumors in adults

In adult DS patients, solid tumors are also uncommon, and most types have significantly lower than expected age-adjusted incidence rates [28]. For example, the standardized incidence ratios (SIRs) of breast cancer in DS patients compared with

age-matched euploid cohorts were 0 and 0.4 from two studies [27, 118]. Hasle et al. reported that the overall risk of solid tumors was decreased (SIR 0.45; 95% CI 0.34–0.59), especially in patients aged 50 years or older (SIR 0.27; 95% CI 0.16–0.43), with significantly lower risks of lung cancer (SIR 0.10; 95% CI 0.00–0.56), breast cancer (SIR 0.16; 95% CI 0.03–0.47), and cervical cancer (SIR 0.0; 95% CI 0.00–0.77). Testicular cancer was the only solid tumor with an increased SIR (2.9; 95% CI 1.6–4.8) [28].

Data from US death certificates from 1983 to 1997 revealed that malignant neoplasms other than leukemia were listed on death certificates of people with DS less than one-tenth as often as expected [119]. A strikingly low standardized mortality odds ratios for malignancy was associated with DS at all ages, in both sexes, and for all common tumor types except leukemia and testicular cancer. In data of autopsied cases operated by the Japanese Society of Pathology from 1974 to 2000, 104 cases with malignant disorders (61 male, 42 female and one case with unrecorded sex), including 87 cases with hematopoietic malignancies (83.7%) and 17 cases with solid tumors (16.3%), were identified [120]. The 17 solid tumors identified included three hepatocellular carcinomas, three extrahepatic cholangiocarcinomas, two gallbladder adenocarcinomas, three brain tumors, and three seminomas, and the most frequent age

range of patients with solid tumors was 40–50 years old.

Testicular tumors are frequently found in DS patients but the underlying reason is unclear. High incidence of cryptorchidism [121], high serum level of follicle-stimulating hormone or luteinizing hormone [122], or Ets-2, an oncogene on chromosome 21 [123], and maturation delay of germ cells with trisomy 21 might result in increased risk of testicular cancer [124].

4-3. The contribution of trisomy 21 in solid tumors

Solid tumors in DS seem to be rare in children and adults, but the reason is unclear. One explanation may be that the decreased immunosurveillance enables cancer cells to survive and proliferate due to the decreased efficiency of T cells, B cells, and NK cells in DS patients [26]. Such immunodeficiencies in DS are the cause of the high incidence of infection in these patients and might contribute to leukemogenesis but not solid tumor growth.

Another explanation is that the attenuation of calcineurin activity by *DSCR1*, together with another chromosome 21 gene *DYRK1A*, may be sufficient to markedly diminish angiogenesis [31]. Therefore, suppression of tumor angiogenesis by an additional copy of *DSCR1* contributes to the reduced cancer incidence in individuals with DS and the calcineurin pathway in the tumor vasculature might be a potential

target for cancer treatment. These observations were confirmed in a murine lung tumor model [125]. Rethore et al. proposed that for women with DS, breast cancer screening is not recommended, but annual clinical monitoring should be conducted, with the option to perform ultrasound or MRI examinations in suspect cases. For cervical cancer, screening could be proposed for women who are sexually active, beginning at 25 years of age. Annual surveillance for testicular cancer via palpation by a health professional is preferable from ages 15 to 45 [30].

DS was believed to be a model of progeria (accelerated aging) or immunosenescence [126]. Aging is characterized by a chronic, low-grade, and sterile inflammation, called “inflammaging,” which has been directly associated with several age-related conditions [127]. Individuals with DS have increased spontaneous circulating levels of pro-inflammatory cytokines, such as TNF-α, IFN-γ, IL-6 and IL-1β [128]. The chronic pro-inflammatory state observed in patients with DS is likely to greatly contribute to neurodegeneration. Inflammation is considered as an important contributor to neurodegenerative disorders, such as Alzheimer’s disease, and is a critical component of tumor progression [129, 130]. However, there was no specific data to support that immunosenescence, inflammaging, or inflammation contributes to increased solid tumors in DS.

Advances in healthcare have improved survival in DS patients over the last 60 years [131]. The mean life expectancy at age 12 years has increased to approximately 60 years [132]. DS is associated with a high risk of stroke in patients of all ages [133]. Ischemic stroke risk in DS appears to be mostly driven by cardioembolic risk. The greater risk of hemorrhagic stroke and lower risk of coronary events in DS males [133]. Furthermore, majority of death reason in older DS patients was respiratory infections. The detailed health problems in older DS patients including the prevalence of cancer remain unknown and will need to be clarified in future studies.

5. Conclusion

DS is a cancer predisposition syndrome, especially for leukemia in children and testicular cancer in adults. In children with DS, most cases related to myeloid malignancies were AMKL and TAM preceded most AMKL cases. The prognosis of DS-AMKL has improved over several decades; however, the prognosis of DS-ALL remains poor. Solid tumors have been rarely reported in children with DS. The expected age-adjusted incidence rates of solid tumors in adult patients with DS compared with age-matched euploid cohorts was low for most cancers except for testicular cancer. Thus, the cancers associated with DS have a unique cancer profile, cancer

predisposition & cancer evasion. Further study might help elucidate the unique contribution of +21 to oncogenesis.

Abbreviations:

Down syndrome (DS), acute myeloid leukemia (AML), acute megakaryoblastic leukemia (AMKL), bone marrow (BM), peripheral blood (PB), French American British (FAB), World Health Organization (WHO), transient abnormal myelopoiesis (TAM), transient abnormal myelopoiesis (TMD), transforming growth factor (TGF), interferon (IFN), interleukin (IL), cytarabine (CA), hematopoietic stem cell transplantation (HSCT), acute lymphoblastic leukemia (ALL), Down syndrome critical region-1 (DSCR 1), event-free survival (EFS), overall survival (OS), methotrexate (MTX)

Declarations

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References

[1] Roizen NJ, Patterson D. Down's syndrome. Lancet. 2003;361:1281-9.
[2] Weijerman ME, de Winter JP. Clinical practice. The care of children with Down syndrome. Eur J Pediatr. 2010;169:1445-52.
[3] Bruwier A. Chantrain CF. Hematological disorders and leukemia in children with Down syndrome. Eur J Pediatr. 2012;171:1301-7.
[4] Malinge S, Bliss-Moreau M, Kirsammer G, Diebold L, Chlon T, Gurbuxani S. et al. Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. J Clin Invest. 2012;122: 948-62.
[5] Nižetić D, Groet J. Tumorigenesis in Down's syndrome: big lessons from a small chromosome. Nat Rev Cancer. 2012;12:721-32.
[6] Porter CC. Germ line mutations associated with leukemias. Hematology Am Soc Hematol Educ Program. 2016:302-308.
[7] Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32:148-52.
[8] Rainis L, Toki T, Pimanda JE, Rosenthal E, Machol K, Strehl S, et al. The proto-oncogene ERG in megakaryoblastic leukemias. Cancer Res. 2005;65:7596-602.
[9] Ahmed M, Sternberg A, Hall G, Thomas A, Smith O, O'Marcaigh A, et al. Natural history of GATA1 mutations in Down syndrome. Blood. 2004;103:2480-9.
[10] Xu G, Nagano M, Kanezaki R, Toki T, Hayashi Y, Taketani T, et al. Frequent mutations in the GATA-1 gene in the transient myeloproliferative disorder of Down syndrome. Blood. 2003;102:2960-8.
[11] Hitzler JK, Cheung JLiY, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood.

2003;101:4301-4.
[12] Shimada A, Maruyama K, Shitara T, Kato M, Cho K, Kobayashi T, et al. Proinflammatory cytokinemia associated with transient myeloproliferative disorder in down syndrome. Biol Neonate 2004;85:167-72.
[13] de Rooij JD, Branstetter C, Ma J, Li Y, Walsh MP, Cheng J, et al. Pediatric non-Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. Nat Genet. 2017;49:451-6.
[14] Hara Y, Shiba N, Ohki K, Tabuchi K, Yamato G, Park MJ, et al. Prognostic impact of specific molecular profiles in pediatric acute megakaryoblastic leukemia in non-Down syndrome. Genes Chromosomes Cancer. 2017;56:394-404.
[15] Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41:1243-6.
[16] Hertzberg L, Vendramini E, Ganmore I, Cazzaniga G, Schmitz M, Chalker J, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood. 2010;115:1006-17.
[17] Hanada I, Terui K, Ikeda F, Toki T, Kanezaki R, Sato T, et al. Gene alterations involving the CRLF2-JAK pathway and recurrent gene deletions in Down syndrome-associated acute lymphoblastic leukemia in Japan. Genes Chromosomes Cancer. 2014;53:902-10.
[18] Rainis L, Bercovich D, Strehl S, Teigler-Schlegel A, Stark B, Trka J, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood. 2003;102:981-6.
[19] Ge Y, LaFiura KM, Dombkowski AA, Chen Q, Payton SG, Buck SA, et al. The role of the proto-oncogene ETS2 in acute megakaryocytic leukemia biology and therapy. Leukemia. 2008;22:521-9.
[20] Ng AP, Hyland CD, Metcalf D, Carmichael CL, Loughran SJ, Di Rago L, et al. Trisomy of Erg is required for myeloproliferation in a mouse model of Down syndrome. Blood. 2010;115:3966-9.
[21] De Vita S, Canzonetta C, Mulligan C, Delom F, Groet J, Baldo C, et al. Trisomic dose of several chromosome 21 genes perturbs haematopoietic stem and progenitor cell differentiation in Down's syndrome. Oncogene. 2010;29:6102-14.
[22] Ng AP, Hu Y, Metcalf D, Hyland CD, Ierino H, Phipson B, et al. Early lineage priming by trisomy of Erg leads to myeloproliferation in a Down syndrome model. PLoS Genet. 2015;11:e1005211.

[23] Takahashi T, Inoue A, Yoshimoto J, Kanamitsu K, Taki T, Imada M, et al. Transient myeloproliferative disorder with partial trisomy 21. Pediatr Blood Cancer. 2015;62: 2021-4.
[24] Verstegen RH, Kusters MA, Gemen EF, De Vries E. Down syndrome B-lymphocyte subpopulations, intrinsic defect or decreased T-lymphocyte help. Pediatr Res. 2010;67:563-9.
[25] Ram G. Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol. 2011;164:9-16.
[26] Satgé D. Seidel MG. The Pattern of Malignancies in Down Syndrome and Its Potential Context With the Immune System. Front Immunol. 2018;9:3058.
[27] Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355:165-9.
[28] Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2:429-36.
[29] Dey N, Krie A, Klein J, Williams K, McMillan A, Elsey R, et al. Down's Syndrome and Triple Negative Breast Cancer: A Rare Occurrence of Distinctive Clinical Relationship. Int J Mol Sci. 2017;18:pii: E1218.
[30] Rethoré MO, Rouëssé J. Satgé D. Cancer screening in adults with down syndrome, a proposal. Eur J Med Genet. 2019;103:783.
[31] Baek KH, Zaslavsky A, Lynch RC, Britt C, Okada Y, Siarey RJ, et al. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. Nature. 2009;459:1126-30.
[32] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33:451-8.
[33] Jaffe ES, Harris NL, Stein H, Vardiman, J. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC press, Lyon; 2001.
[34] Swerdlow SH, Campo E, Harris NL, Elaine SJ, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC press, Lyon. 2008.
[35] Gruber TA. Downing JR. The biology of pediatric acute megakaryoblastic leukemia. Blood. 2015;126:943-9.
[36] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-405.

[37] Kurahashi H, Hara J, Yumura-Yagi K, Murayama N, Inoue M, Ishihara S, et al. Monoclonal nature of transient abnormal myelopoiesis in Down's syndrome. Blood. 1991;77:1161-3.
[38] Hayashi Y, Eguchi M, Sugita K, Nakazawa S, Sato T, Kojima S, et al. Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down's syndrome. Blood. 1988;72:15-23.
[39] Brodeur GM, Dahl GV, Williams DL, Tipton RE, Kalwinsky DK. Transient leukemoid reaction and trisomy 21 mosaicism in a phenotypically normal newborn. Blood. 1980;55:691-3.
[40] Roberts I, Alford K, Hall G, Juban G, Richmond H, Norton A, et al. GATA1-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. Blood. 2013;122:3908-17.
[41] Roberts I. Izraeli S. Haematopoietic development and leukaemia in Down syndrome. Br J Haematol. 2014;167:587-99.
[42] Saida S. Evolution of myeloid leukemia in children with Down syndrome. Int J Hematol. 2016;103:365-72.
[43] Watanabe K. Recent advances in the understanding of transient abnormal myelopoiesis in Down syndrome. Pediatr Int. 2019;61:222-9.
[44] Miyauchi J, Ito Y, Kawano T, Tsunematsu Y, Shimizu K. Unusual diffuse liver fibrosis accompanying transient myeloproliferative disorder in Down's syndrome: a report of four autopsy cases and proposal of a hypothesis. Blood. 1992;80:1521-7.
[45] Dormann S, Krüger M, Hentschel R, Rasenack R, Strahm B, Kontny U. et al. Life-threatening complications of transient abnormal myelopoiesis in neonates with Down syndrome. Eur J Pediatr. 2004;163:374-7.
[46] Ishigaki H, Miyauchi J, Yokoe A, Nakayama M, Yanagi T, Taga T, et al. Expression of megakaryocytic and myeloid markers in blasts of transient abnormal myelopoiesis in a stillbirth with Down syndrome: report of histopathological findings of an autopsy case. Hum Pathol. 2011;42:141-5.
[47] Park MJ, Sotomatsu M, Ohki K, Arai K, Maruyama K, Kobayashi T, et al. Liver disease is frequently observed in Down syndrome patients with transient abnormal myelopoiesis. Int J Hematol. 2014;99:154-61.
[48] Traisrisilp K, Charoenkwan P, Tongprasert F, Srisupundit K, Tongsong T. Hemodynamic assessment of hydrops foetalis secondary to transient myeloproliferative disorder associated with foetal Down syndrome: A case report and literature review. J Obstet Gynaecol. 2016;36:861-4.

[49] Okamura T, Washio Y, Yoshimoto J, Tani K, Tsukahara H, Shimada A. Exchange Transfusion and Cytarabine for Transient Abnormal Myelopoiesis in Hydrops Fetalis. Acta Med Okayama. 2019;73:181-8.
[50] Gamis AS, Alonzo TA, Gerbing RB, Hilden JM, Sorrell AD, Sharma M, et al. Natural history of transient myeloproliferative disorder clinicallydiagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood. 2011;118:6752-9.
[51] Shimada A, Xu G, Toki T, Kimura H, Hayashi Y, Ito E. Fetal origin of the GATA1 mutation in identical twins with transient myeloproliferative disorder and acute megakaryoblastic leukemia accompanying Down syndrome. Blood. 2004;103:366.
[52] Tamblyn JA, Norton A, Spurgeon L, Donovan V, Bedford Russell A, Bonnici J, et al. Prenatal therapy in transient abnormal myelopoiesis: a systematic review. Arch Dis Child Fetal Neonatal Ed. 2016;101:F67-71.
[53] Shitara Y, Takahashi N, Aoki Y, Kato M, Nishimura R, Tsuchida S, et al. Cytokine Profiles in Pericardial Effusion in a Down Syndrome Infant with Transient Abnormal Myelopoiesis. Tohoku J Exp Med. 2017;241:149-53.
[54] Hattori H, Matsuzaki A, Suminoe A, Ihara K, Nakayama H, Hara T. High expression of platelet-derived growth factor and transforming growth factor-beta 1 in blast cells from patients with Down Syndrome suffering from transient myeloproliferative disorder and organ fibrosis. Br J Haematol. 2001;115:472-5.
[55] Shimada A, Hayashi Y, Ogasawara M, Park MJ, Katoh M, Minakami H, et al. Pro-inflammatory cytokinemia is frequently found in Down syndrome patients with hematological disorders. Leuk Res. 2007;31:1199-203.
[56] Ogawa J, Kanegane H, Tsuneyama K, Kanezaki R, Futatani T, Nomura K, et al. Platelet-derived growth factor may be associated with fibrosis in a Down syndrome patient with transient myeloproliferative disorder. Eur J Haematol. 2008;81:58-64.
[57] Miyauchi J, Ito Y, Tsukamoto K, Takahashi H, Ishikura K, Sugita K, et al. Blasts in transient leukaemia in neonates with Down syndrome differentiate into basophil/mast-cell and megakaryocyte lineages in vitro in association with down-regulation of truncated form of GATA1. Br J Haematol. 2010;148:898-909.
[58] Kobayashi K, Yoshioka T, Miyauchi J, Nakazawa A, Yamazaki S, Ono H, et al. Monocyte Chemoattractant Protein-1 (MCP-1) as a Potential Therapeutic Target and a Noninvasive Biomarker of Liver Fibrosis Associated With Transient Myeloproliferative Disorder in Down Syndrome. J Pediatr Hematol Oncol. 2017;39:e285-e9.
[59] Massey GV, Zipursky A, Chang MN, Doyle JJ, Nasim S, Taub JW, et al. A

prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood. 2006;107:4606-13.

[60] Klusmann JH, Creutzig U, Zimmermann M, Dworzak M, Jorch N, Langebrake C, et al. Treatment and prognostic impact oftransient leukemia in neonates with Down syndrome. Blood. 2008;111:2991-8.

[61] Kanezaki R, Toki T, Terui K, Xu G, Wang R, Shimada A, et al. Down syndrome and GATA1 mutations in transient abnormal myeloproliferative disorder: mutation classes correlate with progression to myeloid leukemia. Blood. 2010;116:4631-8.

[62] Roberts I, Izraeli S. Haematopoietic development and leukaemia in Down syndrome. Br J Haematol. 2014;167:587-99.

[63] Terui K, Toki T, Taga T, Iwamoto S, Miyamura T, Hasegawa D, et al. Highly sensitive detection of GATA1 mutations in patients with myeloid leukemia associated with Down syndrome by combining Sanger and targeted next generation sequencing. Genes Chromosomes Cancer. 2020;59:160-7.

[64] Mikkelsen M, Poulsen H, Nielsen KG. Incidence, survival, and mortality in Down syndrome in Denmark. Am J Med Genet Suppl. 1990;7:75-8.

[65] Niikawa N, Deng HX, Abe K, Harada N, Okada T, Tsuchiya H, et al. Possible mapping of the gene for transient myeloproliferative syndrome at 21q11.2. Hum Genet. 1991;87:561-6.

[66] Korbel JO, Tirosh-Wagner T, Urban AE, Chen XN, Kasowski M, Dai L, et al. The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. Proc Natl Acad Sci U S A. 2009;106:12031-6.

[67] Pelleri MC, Cicchini E, Locatelli C, Vitale L, Caracausi M, Piovesan A, et al. Systematic reanalysis of partial trisomy 21 cases with or without Down syndrome suggests a small region on 21q22.13 as critical to the phenotype. Hum Mol Genet. 2016;25:2525-2538.

[68] Birger Y, Izraeli S. DYRK1A in Down syndrome: an oncogene or tumor suppressor? J Clin Invest. 2012;122:807-10.

[69] Maclean GA, Menne TF, Guo G, Sanchez DJ, Park IH, Daley GQ, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci U S A. 2012;109:17567-72.

[70] Yoshida K, Toki T, Okuno Y, Kanezaki R, Shiraishi Y, Sato-Otsubo A, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45:1293-9.

[71] Nikolaev SI, Santoni F, Vannier A, Falconnet E, Giarin E, Basso G, et al. Exome sequencing identifies putative drivers of progression of transient myeloproliferative disorder to AMKL in infants with Down syndrome. Blood. 2013;122:554-61.
[72] Miyauchi J, Kawaguchi H. Fetal liver stromal cells support blast growth in transient abnormal myelopoiesis in Down syndrome through GM-CSF. J Cell Biochem. 2014;115:1176-86.
[73] Hanekamp D, Cloos J, Schuurhuis GJ. Leukemic stem cells: identification and clinical application. Int J Hematol. 2017;105:549-57.
[74] Kinjo T, Inoue H, Kusuda T, Fujiyoshi J, Ochiai M, Takahata Y, et al. Chemokine levels predict progressive liver disease in Down syndrome patients with transient abnormal myelopoiesis. Pediatr Neonatol. 2019;60:382-8.
[75] Flasinski M, Scheibke K, Zimmermann M, Creutzig U, Reinhardt K, Verwer F, et al. Low-dose cytarabine to prevent myeloid leukemia in children with Down syndrome: TMD Prevention 2007 study. Blood Adv. 2018;2:1532-40.
[76] Yasuoka K, Inoue H, Tanaka K, Fujiyoshi J, Matsushita Y, Ochiai M, et al. Successful Liver Transplantation for Transient Abnormal Myelopoiesis-Associated Liver Failure. Neonatology. 2017;112:159-62.
[77] Roy A, Roberts I, Vyas P. Biology and management of transient abnormal myelopoiesis (TAM) in children with Down syndrome. Semin Fetal Neonatal Med. 2012;17:196-201.
[78] Roberts I, O'Connor D, Roy A, Cowan G, Vyas P. The impact of trisomy 21 on foetal haematopoiesis. Blood Cells Mol Dis. 2013;51:277-81.
[79] Saida S, Watanabe K, Sato-Otsubo A, Terui K, Yoshida K, Okuno Y, et al. Clonal selection in xenografted TAM recapitulates the evolutionary process of myeloid leukemia in Down syndrome. Blood. 2013;121:4377-87.
[80] Mast KJ, Taub JW, Alonzo TA, Gamis AS, Mosse CA, Mathew P, et al. Pathologic Features of Down Syndrome Myelodysplastic Syndrome and Acute Myeloid Leukemia: A Report From the Children's Oncology Group Protocol AAML0431. Arch Pathol Lab Med. 2020;144:466-72.
[81] Mast KJ., et al Pathologic Features of Down Syndrome Myelodysplastic Syndrome and Acute Myeloid Leukemia Arch Pathol Lab Med 2020;144:446-472.
[82] Taga T, Watanabe T, Tomizawa D, Kudo K, Terui K, Moritake H, et al. Preserved High Probability of Overall Survival with Significant Reduction of Chemotherapy for Myeloid Leukemia in Down Syndrome: A Nationwide Prospective Study in Japan. Pediatr Blood Cancer. 2016;63:248-54.
[83] Inaba H, Zhou Y, Abla O, Adachi S, Auvrignon A, Beverloo HB, et al.

Heterogeneous cytogenetic subgroups and outcomes in childhood acute megakaryoblastic leukemia: a retrospective international study. Blood. 2015;126:1575-84.

[84] Schweitzer J, Zimmermann M, Rasche M, von Neuhoff C, Creutzig U, Dworzak M, et al. Improved outcome of pediatric patients with acute megakaryoblastic leukemia in the AML-BFM 04 trial. Ann Hematol. 2015;94:1327-36.

[85] Hahn AW, Li B, Prouet P, Giri S, Pathak R, Martin MG. Acute megakaryocytic leukemia: What have we learned. Blood Rev. 2016;30:49-53.

[86] Masetti R, Bertuccio SN, Pession A, Locatelli F. CBFA2T3-GLIS2-positive acute myeloid leukaemia. A peculiar paediatric entity. Br J Haematol. 2019;184:337-47.

[87] de Rooij JD, Branstetter C, Ma J, Li Y, Walsh MP, Cheng J, et al. Pediatric non-Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. Nat Genet. 2017;49:451-6.

[88] Canzonetta C, Hoischen A, Giarin E, Basso G, Veltman JA, Nacheva E, et al. Amplified segment in the 'Down syndrome critical region' on HSA21 shared between Down syndrome and euploid AML-M0 excludes RUNX1, ERG and ETS2. Br J Haematol. 2012;157:197-200.

[89] Forestier E, Izraeli S, Beverloo B, Haas O, Pession A, Michalová K, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111:1575-83.

[90] Taub JW, Matherly LH, Stout ML, Buck SA, Gurney JG, Ravindranath Y. Enhanced metabolism of 1-beta-D-arabinofuranosylcytosine in Down syndrome cells: a contributing factor to the superior event free survival of Down syndrome children with acute myeloid leukemia. Blood. 1996;87:3395-403.

[91] Taub JW, Ge Y. Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer. 2005;44:33-9.

[92] Kojima S, Sako M, Kato K, Hosoi G, Sato T, Ohara A, et al. An effective chemotherapeutic regimen for acute myeloid leukemia and myelodysplastic syndrome in children with Down's syndrome. Leukemia. 2000;14:786-91.

[93] Kudo K, Kojima S, Tabuchi K, Yabe H, Tawa A, Imaizumi M, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2007;25:5442-7.

[94] Sorrell AD, Alonzo TA, Hilden JM, Gerbing RB, Loew TW, Hathaway L, et al. Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology

Group trial A2971: a report from the Children's Oncology Group. Cancer. 2012;118:4806-14.

[95] Uffmann M, Rasche M, Zimmermann M, von Neuhoff C, Creutzig U, Dworzak M, et al. Therapy reduction in patients with Down syndrome and myeloid leukemia: the international ML-DS 2006 trial. Blood. 2017;129:3314-21.

[96] Hasle H, et al. Myeloid leukemia in children 4 years or older with Down syndrome often lacks GATA1 mtation and cytogenetics and risl of relapse are more akin to sporadic AML. Leukemia. 2008; 22:1428-30.

[97] Buitenkamp TD, Izraeli S, Zimmermann M, Forestier E, Heerema NA, van den Heuvel-Eibrink MM, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood. 2014;123:70-7.

[98] Pui CH, Roberts KG, Yang JJ, Mullighan CG. Philadelphia Chromosome-like Acute Lymphoblastic Leukemia. Clin Lymphoma Myeloma Leuk. 2017;17:464-70.

[99] Buitenkamp TD, Pieters R, Gallimore NE, van der Veer A, Meijerink JP, Beverloo HB, et al. Outcome in children with Down's syndrome and acute lymphoblastic leukemia: role of IKZF1 deletions and CRLF2 aberrations. Leukemia. 2012;26: 2204-11.

[100] Schwartzman O, Savino AM, Gombert M, Palmi C, Cario G, Schrappe M, et al. Suppressors and activators of JAK-STAT signaling at diagnosis and relapse of acute lymphoblastic leukemia in Down syndrome. Proc Natl Acad Sci U S A. 2017;114:E4030-9.

[101] Whitlock JA, Sather HN, Gaynon P, Robison LL, Wells RJ, Trigg M, et al. Clinical characteristics and outcome of children with Down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2005;106:4043-9.

[102] Goto H, Inukai T, Inoue H, Ogawa C, Fukushima T, Yabe M, et al. Acute lymphoblastic leukemia and Down syndrome: the collaborative study of the Tokyo Children's Cancer Study Group and the Kyushu Yamaguchi Children's Cancer Study Group. Int J Hematol. 2011;93:192-198.

[103] Pennella CL, Rossi JG, Baialardo EM, Alonso CN, Guitter MR, Sánchez La Rosa CG, et al. Acute lymphoblastic leukemia in children with Down syndrome: Comparative analysis versus patients without Down syndrome. Arch Argent Pediatr. 2018;116:e500-e507.

[104] Rabin KR, Smith J, Kozinetz CA. Myelosuppression and infectious complications in children with Down syndrome and acute lymphoblastic leukemia.

Pediatr Blood Cancer. 2012;58:633-5.
[105] O'Connor D, Bate J, Wade R, Clack R, Dhir S, Hough R, et al. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. Blood. 2014;124:1056-61.
[106] Lanza C, Volpe G, Basso G, Gottardi E, Perfetto F, Cilli V, et al. The common TEL/AML1 rearrangement does not represent a frequent event in acute lymphoblastic leukaemia occuring in children with Down syndrome. Leukemia. 1997;11:820-1.
[107] Athale UH, Puligandla M, Stevenson KE, Asselin B, Clavell LA, Cole PD, et al. Outcome of children and adolescents with Down syndrome treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium protocols 00-001 and 05-001. Pediatr Blood Cancer. 2018;65:e27256.
[108] Matloub Y, Rabin KR, Ji L, Devidas M, Hitzler J, Xu X, et al. Excellent long-term survival of children with Down syndrome and standard-risk ALL: a report from the Children's Oncology Group. Blood Adv. 2019;3:1647-56.
[109] Kroll M, Kaupat-Bleckmann K, Mörickel A, Altenl J, Schewel DM, Stanullal M, et al. Methotrexate-associated toxicity in children with Down syndrome and acute lymphoblastic leukemia during consolidation therapy with high dose methotrexate according to ALL-BFM treatment regimen. Haematologica. 2020;105:1013-20.
[110] Meyr F, Escherich G, Mann G, Klingebiel T, Kulozik A, Rossig C, et al. Outcomes of treatment for relapsed acute lymphoblastic leukaemia in children with Down syndrome. Br J Haematol. 2013;162:98-106.
[111] Meissner B, Borkhardt A, Dilloo D, Fuchs D, Friedrich W, Handgretinger R, et al. Relapse, not regimen-related toxicity, was the major cause of treatment failure in 11 children with Down syndrome undergoing haematopoietic stem cell transplantation for acute leukaemia. Bone Marrow Transplant. 2007;40:945-9.
[112] Vonasek J, Asdahl P, Heyman M, Källén K, Hasle H. Late mortality and morbidity among long-term leukemia survivors with Down syndrome: A nationwide population-based cohort study. Pediatr Blood Cancer. 2018;65:e27249.
[113] Wadhwa A, Kutny MA, Xavier AC. Blinatumomab activity in a patient with Down syndrome B-precursor acute lymphoblastic leukemia. Pediatr Blood Cancer. 2018;65(2).
[114] Murillo L, Dapena JL, Velasco P, de Heredia CD. Use of inotuzumab-ozogamicin in a child with Down syndrome and refractory B-cell precursor acute lymphoblastic leukemia. Pediatr Blood Cancer. 2019;66:e27562.

[115] Satgé D, Sasco AJ, Carlsen NL, Stiller CA, Rubie H, Hero B, et al. A lack of neuroblastoma in Down syndrome: a study from 11 European countries. Cancer Res. 1998;58: 448-52.

[116] JM Olson, A Hamilton and NE Breslow Med Pediatr Oncol, 1995;24:305-9.

[117] Satgè D, Schorderet DF, Balmer A, Beck-Popovic M, Addor MC, Beckmann JS, et al. Association Down syndrome-retinoblastoma: a new observation. Ophthalmic Genet. 2005;26:151-2.

[118] Patja K, Pukkala E, Sund R, Iivanainen M, Kaski M. Cancer incidence of personswith Down syndrome in Finland: a population-based study. Int J Cancer. 2006;118:1769-72.

[119] Yang Q, Rasmussen SA, Friedman JM. Motality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. The Lancet 2002;359:1019-25.

[120] Ehara H, Ohno K, Ito H. Benign and malignant tumors in Down syndrome: Analysis of the 1514 autopsied cases in Japan. Pediatr Int 2011;53:72-77.

[121] Miki M, Ohtake N, Hasumi M, Ohi M, Moriyama S. Seminoma associated with bilateral cryptorchidism in Down's syndrome: a case report. Int J Urol. 1999;6:377-80.

[122] Satgé D, Sasco AJ, Curé H, Leduc B, Sommelet D, Vekemans MJ. An excess of testicular germ cell tumors in Down's syndrome: three case reports and a review of the literature. Cancer. 1997;80:929-35.

[123] Maroulakou IG, Papas TS, Green JE. Differential expression of ets-1 and ets-2 proto-oncogenes during murine embryogenesis. Oncogene. 1994;9:1551-65.

[124] Cools M, Honecker F, Stoop H, Veltman JD, de Krijger RR, Steyerberg E, et al. Maturation delay of germ cells in fetuses with trisomy 21 results in increased risk for the development of testicular germ cell tumors. Hum Pathol. 2006;37:101-11.

[125] Shin J, Lee JC, Baek KH. A single extra copy of Dscr1 improves survival of mice developing spontaneous lung tumors through suppression of tumor angiogenesis. Cancer Lett. 2014;342:70-81.

[126] Gensous N, Bacalini MG, Franceschi C, Garagnani P. Down syndrome, accelerated aging and immunosenescence. Semin Immunopathol. 2020 in press.

[127] Franceschi C, Bonafè M, Valensin S, Olivieri F, de Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–254.

[128] Trotta MB, Serro Azul JB, Wajngarten M, Fonseca SG, Goldberg AC, Kalil

JE. Inflammatory and Immunological parameters in adults with Down syndrome. Immun Ageing. 2011;8:4.

[129] Gensous N, Bacalini MG, Franceschi C, Garagnani P. Down syndrome, accelerated aging and immunosenescence. Semin Immunopathol. 2020 in press.

[130] Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.

[131] Glasson EJ, Jacques A, Wong K et al Improved survival in Down syndrome over the last 60 years and the impact of perinatal factors in recent decades. J Pediatr 2016;169:214–20.

[132] Englund, A., Jonsson, B., Zander, C. S., Gustafsson, J. Anneren, G. Changes in mortality and causes of death in the Swedish Down syndrome population. Am. J. Med. Genet. A 2013;161A:642–9.

[133] Sobey CG, Judkins CP, Sundararajan V, Phan TG, Drummond GR, Srikanth VK. Risk of Major Cardiovascular Events in People with Down Syndrome. PLoS One. 2015;10:e0137093.

Figure Legends

Figure 1. Suspected myeloid leukemogenesis mechanism of Down syndrome (DS). Transient abnormal myelopoiesis (TAM) and acute megakaryoblastic leukemia (AMKL) are characteristic to DS. Proliferation of TAM is initiated in fetal liver after acquired +21 and *GATA1* mutation. The majority of DS-TAM shows spontaneous regression, but about 10% of DS-TAM cases develop AMKL within 3–4 years after birth.

Figure 2. Predicted structure of GATA1 protein. *GATA1* mutation is frequently found in DS-TAM and AMKL patients, and this mutation causes a truncated form of GATA1 (GATA1s).

Figure 2