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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 Runx-related transcription factor 1 (RUNXI), erythroid 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 $100 \times 10^9$ 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: \textit{GATA1} mutations

\textit{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 \textit{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 \textit{GATA1} protein, \textit{GATA1}s, which lacks the N-terminal transcription activity and results in different gene expression profiles [42, 61, 62]. \textit{GATA1} mutations are found in approximately 10\% in DS patients. However, some DS neonates are not diagnosed with TAM but as having \textit{GATA1} mutations. Surprisingly, Roberts et al. reported that 195 of 200 (97.5\%) DS neonates had circulating TAM blasts. \textit{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 \textit{GATA1} mutated clones was detected by targeted next generation sequence (NGS) in 18 of 88 (20.4\%) DS neonates without \textit{GATA1} mutations by standard detection methods. These cases are known as “silent TAM” [40]. Therefore, 35 of 200 (17.5\%) DS neonates had \textit{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 > 100×10⁹/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 RUNXI, 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 < 10×10⁹/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
Shimada A. DS-related malignancies

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

Shimada A. DS-related malignancies


Shimada A. DS-related malignancies


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

GATA1 mRNA

GATA1s mRNA

GATA1 protein

GATA1s protein
Fetal liver

Hematopoietic progenitor cell

Trisomy 21 (1st hit)

GATA1 mutation (2nd hit)

TAM

Bone marrow

AMKL-DS

MDS

Additional genetic hit (3rd hit)