Review Article

Down syndrome and leukemia: insights into leukemogenesis and translational targets

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Abstract: Children with Down syndrome (DS) have a significantly increased risk of childhood leukemia, in particular acute megakaryoblastic leukemia (AMKL) and acute lymphoblastic leukemia (DS-ALL). A pre-leukemia, called transient myeloproliferative disorder (TMD), characterised by a GATA binding protein 1 (*GATA1*) mutation, affects up to 30% of newborns with DS. In most cases, the pre-leukemia regresses spontaneously, however one-quarter of these children will go on to develop AMKL or myelodysplastic syndrome (MDS). AMKL and MDS occurring in young children with DS and a *GATA1* somatic mutation are collectively termed myeloid leukemia of Down syndrome (ML-DS). This model represents an important multi-step process of leukemogenesis, and further study is required to identify therapeutic targets to potentially prevent development of leukemia. DS-ALL is a high-risk leukemia and mutations in the JAK-STAT pathway are frequently observed. JAK inhibitors may improve outcome for this type of leukemia. Genetic and epigenetic studies have revealed likely candidate drivers involved in development of ML-DS and DS-ALL. Overall this review aims to identify potential impacts of new research on how we manage children with DS, pre-leukemia and leukemia.

Keywords: (3-5)-MeSH headings; Down syndrome (DS); children; leukemia; transient myeloproliferative disorder (TMD) of Down syndrome; preleukemia

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Introduction

Children with Down syndrome (DS) have a significantly higher risk of developing leukemia in childhood as compared to children without DS (1), although curiously they have a lower risk of developing solid tumours (2). DS is defined by constitutional trisomy 21, which is the most common cytogenetic abnormality seen in live births, at a rate of 1/700 to 1/1,000 newborns (3,4).

The risk of developing acute megakaryoblastic leukemia (AMKL), which is a relatively rare subtype of acute myeloid leukemia (AML), is increased 500-fold in children with DS as compared to the general non-DS population; and risk of acute lymphoblastic leukemia (ALL) is 20-fold greater in children with DS (1).

Typically in childhood, ALL is significantly more common than AML. However in DS, the ratio of ALL to AML is 1.7 for children under 15 years of age. For the general population of non-DS children, the equivalent ratio is 6.5 (2).

In this review, we will focus on recent studies that have improved our understanding of leukemogenesis in DS, particularly myeloid leukemia of Down syndrome (ML-DS). We will also highlight important developments likely to translate into improved clinical treatment of ALL associated with DS (DS-ALL). Specifically we aim to identify potential impacts of new research on how we manage children with DS, pre-leukemia and leukemia.

Myeloid Leukemia of Down syndrome (ML-DS)

ML-DS includes acute megakaryoblastic leukemia

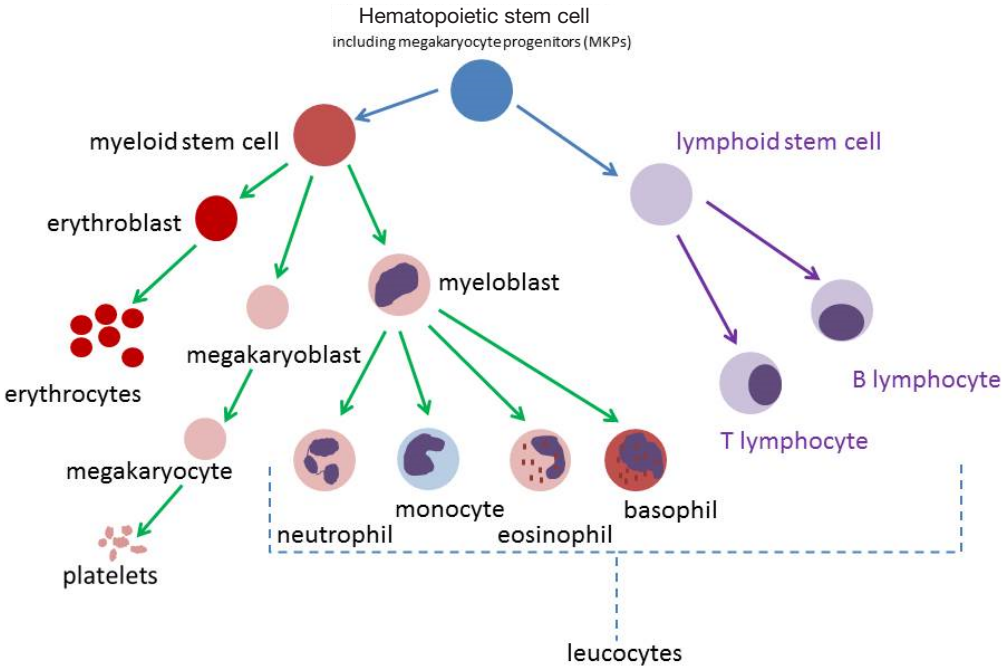

Figure 1 Myeloid and lymphoid stem cells. This figure describes in a simplified manner the broad division between myeloid and lymphoid lineages. A multipotent hematopoietic stem cell gives rise to a common myeloid progenitor, termed "myeloid stem cell" above; and a common lymphoid progenitor, termed "lymphoid stem cell". Of relevance to Down syndrome (DS), megakaryoblasts are derived from a myeloid stem cell. Transient myeloproliferative disorder (TMD), a pre-leukemia, and myeloid leukemia of Down syndrome (ML-DS) are both disorders that are due to abnormal megakaryoblasts. Lymphoblasts, seen in DS-acute lymphoblastic leukemia (DS-ALL) are derived from a lymphoid stem cell.

(DS-AMKL) and myelodysplastic syndrome (MDS) (5). MDS often precedes DS-AMKL. In this paper, we will refer to both entities (DS-AMKL and DS-related MDS) as "ML-DS".

DS-AMKL, a sub-type of AML, is characterised by an abnormal monomorphic population of circulating megakaryoblasts. AML refers to a broader category of blood cancers that are derived from myeloid precursors. Megakaryoblasts are derived from myeloid precursors (*Figure 1*). Normal megakaryoblasts will differentiate into megakaryocytes (MKs) (platelet-producing cells). Abnormal megakaryoblasts will overwhelm normal bone marrow production and reduce production of the other cell lines, such as leucocytes (including neutrophils) and erythrocytes.

ML-DS is characterised by transforming events that occur in the fetal and newborn period (6). There are two cytogenetic and genetic changes or "hits" that occur prenatally, that give rise to a pre-leukemic state, called transient myeloproliferative disorder (TMD). TMD is also referred to as transient abnormal myelopoiesis (TAM) or transient leukemia (TL). The first hit is the presence of trisomy 21, which leads to increased proliferation of megakaryocyte progenitors (MKPs) in the fetal liver. The subsequent transforming event is a mutation in GATA binding protein 1 (*GATA1*) which gives rise to TMD. The third and subsequent hits are as yet unknown (6).

Transient myeloproliferative disorder (TMD)

TMD can occur in DS; in children without DS but with acquired somatic trisomy 21 mutations; and also mosaic trisomy 21 patients (7-10). There may be other leukemogenic factors, as yet unknown; as hypothesised in a recent case report of a newborn with clinical TMD, who did not have trisomy 21 or a *GATA1* gene mutation in the leukemic blasts (7).

TMD occurs in at least 5-10% of newborns with DS, and is evidenced by presence of circulating megakaryoblasts, that are indistinguishable from blasts seen in ML-DS. Typical features of TMD include circulating peripheral megakaryoblasts with the immunophenotype CD33/38/117/34/7/56/36/71/42b (8), thrombocytopenia, variable presence of leucocytosis and

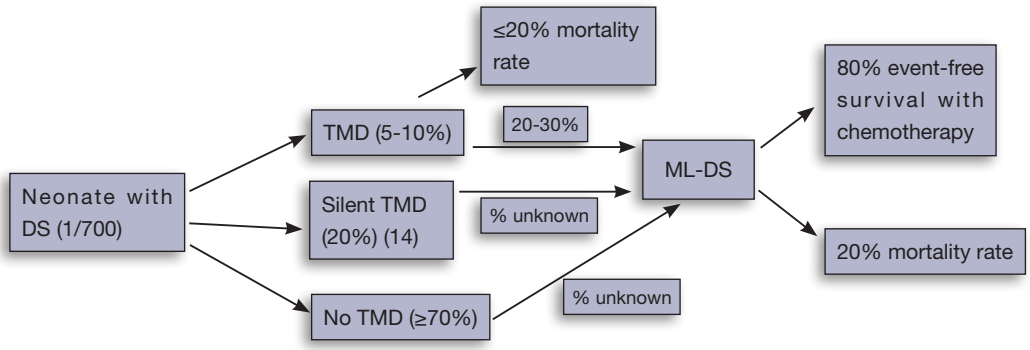

Figure 2 Natural history of TMD in children with Down syndrome (DS) and newly described diagnostic categories. The current incidence of DS is 1/700 to 1/1000 live births. Children who have blasts in the peripheral blood and *GATA1* gene mutation detected by conventional methods (Sanger Sequencing/Denaturing High Performance Liquid Chromatography) are termed "TMD" in our diagram above. Based on new findings (14), there is a subgroup of newborns with DS who do not have *GATA1* gene mutation detected by conventional methods, but who have a detectable *GATA1* gene mutation by next-generation sequencing. The proposed nomenclature for this group is "silent TAM" (14) or "silent TMD". The incidence of newborn DS with detectable *GATA1* gene mutation by conventional methods ("TMD") is up to 10%. The incidence of "silent TMD" could be up to 20% of newborns with DS. Approximately 20-30% of newborns with TMD will go on to develop ML-DS (myeloid leukemia of Down syndrome). The incidence of ML-DS in children with "silent TMD" or no prior TMD is unknown. ML-DS typically responds very well to chemotherapy. The event-free survival is approximately 80% for ML-DS.

anemia, hepatomegaly, splenomegaly, serous effusions and sometimes skin rash (9). Flow cytometry performed on peripheral blood can help characterise the lineage-specific markers present in the blast population. TMD can present *in utero* with severe hydrops fetalis.

Three prospective series found a median TMD diagnosis at 3-7 days postnatally and almost all cases had presented by age 2 months (8). Clinical features reflect transient abnormal megakaryopoiesis, which is thought to primarily originate in the liver (8,9). This is evidenced by the role of the fetal liver in hematopoiesis, and the generally lower presence of blasts in the bone marrow as compared to the peripheral blood (9).

Historically, TMD diagnoses have been made based on a full-blood count (FBC) being performed in a symptomatic DS newborn and the subsequent finding of megakaryoblasts in the peripheral blood. Currently there is no routine use of molecular testing of samples from TMD patients for *GATA1* mutations; and not all newborns with DS will have a FBC in the newborn period. This means that there is likely to be a population of newborns with DS who have subclinical TMD.

In the majority of cases, TMD will resolve spontaneously, usually within the first 3 months of life. Treatment may be required in the event of life-threatening symptoms from TMD (such as respiratory or liver impairment). In total, 85-90% of newborns with TMD will demonstrate resolution of TMD, either spontaneously or due to therapeutic intervention (11-13). Therapy for TMD, although not commonly given, consists of low dose cytarabine. There is a mortality rate of up to 20% for patients with TMD (8). Event-free survival rates are approximately 60%, due to death and leukemic relapse (11,13). Early death and poor event-free survival are both predicted by hyperleukocytosis, severe liver dysfunction, prematurity and failure of spontaneous TMD remission (11). Coagulopathy and renal failure are additional poor prognosticators (11).

In 20-30% of cases, newborns with a history of TMD will go on to develop ML-DS, within the first 2-4 years of life (8) (*Figure 2*). Overall, between 0.5-2% of children with DS will develop ML-DS (2,15). The lack of universal testing of newborn children with DS for TMD and *GATA1* mutations means that some newborns with DS and a significant risk of ML-DS in future, do not come to immediate clinical attention in the newborn period.

Unanswered questions in the field are: (I) What is the true incidence of subclinical (silent) TMD? (II) What is the clinical pattern of detectable *GATA1* mutations over the period between TMD and ML-DS or resolution? (III) How does the *GATA1* loss-of-function mutation contribute to the pre-leukemia, TMD, or the frank leukemia, ML-DS? (IV) Can children with DS who will progress to ML-DS be identified before frank leukemia develops; (V) Can progression from TMD to ML-DS be prevented?

The true incidence of silent TMD

Until recently, the concept of silent TMD was not well understood, however novel research findings have generated new hypotheses (14). Earlier studies found that the incidence of TMD in newborns with DS was between 3.8-6% (15,16). One of these studies used FBC results from all DS children within the first week of life (16), whilst another retrospectively analysed DS neonatal blood spots for *GATA1* mutations (15). Only those with a *GATA1* mutation at birth progressed to ML-DS (15). Many of these earlier studies were limited by sensitivity of PCR techniques that were available at the time (15).

In a recent population-based study of 200 neonates with DS, Roberts *et al*. have proposed that up to 30% of newborns with DS will have a detectable *GATA1* gene mutation, if both conventional and next-generation sequencing approaches are used (14). Next-generation sequencing of exon 2 of *GATA1* was performed on 104 patients. Newborns with a peripheral blast count of >10% and a detectable *GATA1* gene mutation by conventional (Sanger Sequencing/Denaturing High Performance Liquid Chromatography) techniques have been labelled as "TAM" or "TMD". Those newborns with a *GATA1* gene mutation detectable only by next-generation sequencing are termed "silent TAM" or "silent TMD". In this study all patients with silent TMD had a peripheral blast count of ≤10%. Newborns with peripheral blasts at birth, but no detectable *GATA1* mutation were categorised as "no TMD" (*Figure 2*).

Therefore, the true incidence of TMD (including asymptomatic or "silent TMD" cases) may lie between 10-31%, depending on definitions used (9,11-14). The clinical implications for those children with "silent TMD" is uncertain, however these children do have an increased risk of subsequent ML-DS (14). In the recent prospective UK cohort, 11% of newborns with *GATA1* mutations (including those with TMD and silent TMD) later developed ML-DS (14).

Currently however, *GATA1* gene mutation testing is not standard of care in newborns with DS. International guidelines recommend at least a FBC to be performed at least once before 1 month of age (17). Many institutions will perform a FBC at birth. Regular blood tests are recommended to monitor for systemic manifestations of DS, including yearly FBC for macrocytic anaemia and more frequent monitoring of thyroid function (17) .

Children who have documented TMD require monitoring for resolution of the disease, then more frequent hematological monitoring, due to their increased risk of ML-DS. In our own clinical practice, we perform a FBC and clinical review every 3 months for these children, up until age 4 years. This is consistent with international practice (8). After 4-5 years of age, the incidence of leukemia is significantly less (2).

Treatment and outcome for ML-DS

Children treated for ML-DS have a significantly higher disease-free survival (DFS) compared to other children treated for AML (DFS 88-89% compared to 42%, P<0.001) (18,19). Arguably this could be due to AML subtype but DS-AMKL also requires less intense therapy to achieve cure as compared to non-DS AMKL, indicating that children with ML-DS are more responsive to chemotherapy (18,19). A possible explanation for the chemosensitivity of ML-DS is an alteration in cytarabine drug metabolism (20,21) due to reduced cytidine deaminase gene expression in ML-DS (21) or increased expression of cystathionine β-synthase, which is encoded by chromosome 21 (20). Cytarabine is a key drug in successful therapy against ML-DS and AML.

Children with DS who develop AMKL beyond the age of 4 years old are thought to represent a different cohort of patients. Age >4 years old is a poor prognosticator, conferring a 5-year EFS of 33%, compared to 81% for DS children with myeloid leukemia aged <4 years old (19). Children who are older than 4 years of age, without *GATA1* mutations, are more likely to have similar cytogenetic aberrations to sporadic (non-DS) AML (22). These children are more likely to require more intensive therapy, as compared to children with ML-DS (22). In addition, DS patients with TMD have also been described who progress to ALL, although this is very rare; and occurs much less often than ML-DS (12,13).

Multi-step process of leukemogenesis in ML-DS: role of GATA1 loss-of-function mutation in the pre-leukemia, TMD, or the frank leukemia, ML-DS

The effect of trisomy 21 and *GATA1* mutation in promoting abnormal megakaryopoiesis has been recently clarified. The acquisition of trisomy 21 alone is the first hit, as trisomy 21 without *GATA1* mutation leads to altered myeloid progenitor self-renewal, altered lineage development (23,24) and increased clonogenicity of MKPs in human fetal livers (25).

Somatic *GATA1* mutation is identified as the "second

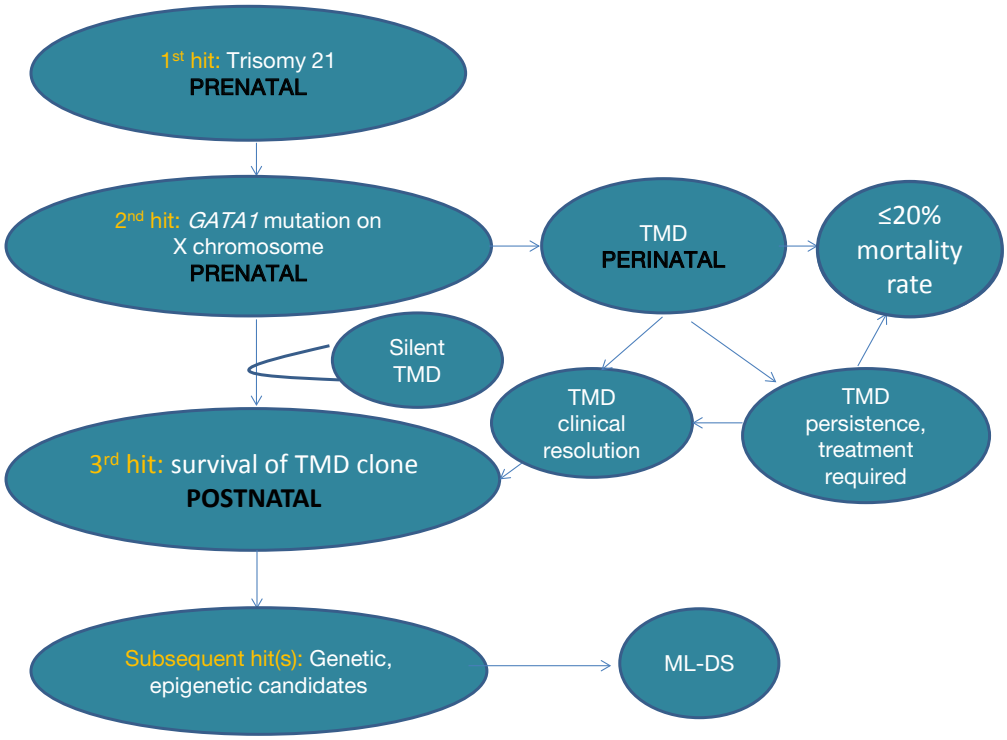

Figure 3 Schematic of "hits" involved in myeloid leukemogenesis in Down syndrome (DS). The first "hit", or genetic change, is trisomy 21. This occurs prenatally in the fetal liver, which is the site of definitive fetal hematopoiesis. The second hit is *GATA1* gene mutation. Transient myeloproliferative disorder (TMD) occurs as a result of the combination of trisomy 21 and *GATA1* mutation effects on megakaryocyte progenitors (MKPs). TMD may resolve spontaneously, or less commonly after use of low-dose chemotherapy, in approximately 85% of cases. In order to develop myeloid leukemia of Down syndrome (ML-DS), it is likely that a TMD clone persists due to an additional "hit"; and that this TMD clone is subject to further genetic and/or epigenetic leukemic drivers.

hit", and it is thought to block MK differentiation (26). The *GATA1* gene mutation has been identified in almost all cases of TMD and ML-DS. *GATA1* somatic mutation was first identified from a small series of ML-DS samples (26). *GATA1* is located on the X chromosome; and encodes a zinc finger-containing protein that is essential for normal erythropoiesis and megakaryopoiesis (26,27). *GATA1* gene mutation leads to sole production of a truncated GATA1 protein, called GATA1s. GATA1s lacks an amino-transactivation domain but retains both DNA-binding zinc fingers (26). The majority of mutations have been described in exon 2, with a minority in exon 3 or at the intronic boundary of exon 1 and 2. In 75% of cases these are insertions, deletions or duplications. Point mutations are described in 21% of cases (4). The presence of GATA1s is thought to impair *GATA1*-mediated regulation of other transcription factors, including GATA2, MYB, MYC and IKAROS family zinc finger 1 (IKZF1) in fetal MKs (28).

GATA1 mutations, in the absence of trisomy 21, have not been associated with leukemia. Instead, specific hematopoietic alterations due to *GATA1* mutation alone include cytopenias (26), Diamond-Blackfan anaemia (29) and trilineage bone marrow dysplasia in germline *GATA1* mutation (30). Therefore *GATA1* mutation represents the second hit, in the presence of trisomy 21.

Analysis of paired samples from the same patient has found the identical *GATA1* gene mutation in both the pre-leukemia (TMD) and leukemia (ML-DS) (11,27,31,32). It is highly likely that additional transforming events, or "hits", are involved in this leukemogenic process (6,33,34). Current knowledge regarding the ability to detect and quantify GATA1s or *GATA1* mutations from a clinical TMD episode through to either TMD resolution or evolution to ML-DS is limited. These additional 'hits" may be genetic and/or possibly epigenetic changes (*Figure 3*). In one study, 44% of ML-DS samples demonstrated additional genetic mutations, aside from trisomy 21 and *GATA1* mutation (33). Additional

"hits" may contribute to the survival of pre-leukemic cells in the postnatal environment and play a role in subsequent risk of leukemic transformation within the bone marrow compartment.

Modelling of DS and ML-DS

Several methods have been used to study clonal evolution and additional "hits". The most relevant studies to date have analysed primary patient samples including paired patient samples as well as mouse models of leukemogenesis; DS fetal tissue and induced pluripotent cells derived from fetal tissue. These are described in the next section.

Paired patient samples may provide an indication of clonal evolution, although clonal evolution is not always seen (11). Examples of clonal evolution, comparing TMD to ML-DS samples from the same patient, include additional genetic alterations such as trisomy 8 (27) and AML-associated changes such as der (3q), trisomy 19 and trisomy 11 (11). This indicates that there is abnormal persistence of a blast population, that subsequently gives rise to the definitive ML-DS state. This also implies that residual TMD clones could be detected by minimal residual disease (MRD) techniques such as polymerase chain reaction (PCR), flow cytometry, or next-generation sequencing. Use of semi-quantitative PCR has been recently reported for 2 patients (35). One patient had *GATA1* mutation analyses performed retrospectively after detection of the *GATA1* mutation in the ML-DS sample. This demonstrated presence of *GATA1* mutation from age 3 months (silent TMD) and persistence until ML-DS diagnosis. The other patient had TMD and reduction in the size of the *GATA1* mutated clone over subsequent months.

Several studies have attempted to define candidate genes, including genes on human chromosome 21, as potential effectors of leukemogenesis in DS. A summary of potential genes and drivers in DS-mediated leukemogenesis is listed in *Table 1*. Differing pathways for leukemogenesis may exist in DS-AMKL and non-DS-AMKL, as oncogene expression varies between the two entities (40,46).

Key candidate genes on chromosome 21 include *ERG*, *ETS2*, *RUNX1*, *GABPA* [reviewed in (4)], *BACH1* (8) and *DYRK1A* (40).

Members of the ETS gene family, *ERG*, *ETS2* and *FLI1* have been shown to contribute to dysregulation of megakaryopoiesis in fetal liver progenitors in *GATA1* mutant mice (37). *ERG* and *ETS2* have a proliferative effect on MKs, independent of *GATA1* (37). *ERG* and *FLI-1* both lead to immortalisation of hematopoietic progenitor cells in *GATA1* mutant mice, probably through JAK/STAT pathway activation (37).

Confirmatory studies implicate *ERG* in dysregulated megakaryopoiesis in *GATA1* mutant mice models (37), in immortalisation of hematopoietic progenitors (36,37) and as contributor to leukemogenesis in adult bone marrow cells (55). Perhaps the most striking observation is that *ERG* can cooperate with GATA1s to create a TMD-like defect *in vivo* and potentially lead to myeloid leukemia (38). *ERG* alone led to increased MKPs in fetal liver (38). Earlier models, with large triplicated regions of orthologues found on human chromosome 21 (Hsa21), failed to demonstrate myeloproliferation despite triplicated copies of *ERG* (56). Modulation of the Ts65Dn mouse, which converted the mice from *ERG* trisomy to *ERG* disomy [Ts65Dn(Erg$^{+/+/mld2}$)], led to a complete reversal of the myeloproliferative phenotype observed in Ts65Dn mice (39). Studies using human cell lines have demonstrated that *ERG* (an ETS transcription factor) is found in hematopoietic stem cells (HSCs), megakaryocytic cell lines and primary leukemia cells in DS (41).

ERG can alter HSC and megakaryocytic development by switching differentiation from erythroid to megakaryocytic phenotypes, through activation of *gp1*b and *gpIIb* promoters *in vivo*; and, together with *ETS2*, by binding the hematopoietic enhancer *SCL/TALI in vivo* (41).

In addition, *ERG*-expanded adult bone marrow T-cells with an added *NOTCH1* gene mutation, developed ALL (55). However, *ERG*-expressing pro-B cells in culture but could not induce B-cell leukemia (55). ERG expression is a poor prognosticator in adult T-ALL and cytogenetically-normal AML, although its role in childhood leukemias is uncertain (55). A crucial role in maintenance of leukemia in adult HSCs is shown by shRNA knockdown of *ERG*, which resulted in reduced cell growth in erythroid, myeloid, T and B cells (55).

RUNX1 and *DYRK1A* genes lie in the DS Critical Region (DSCR), a region at 21q22 that is thought to be responsible for DS phenotypic features.

RUNX1 is an aetiologic factor in AML (8) although there is conflicting evidence regarding *RUNX1* in ML-DS (*Table 1*). Human germline *RUNX1* mutation leads to familial platelet disorder and AML (44). Results from Tc1, Ts65Dn and Ts1Cje mouse studies would indicate that *RUNX1* does not cause abnormal hematopoiesis (57), myeloproliferation (43) or MKP expansion (56). However, there may be a role for RUNX-1 in potentiation of

Table 1 Potential candidates in ML-DS leukemogenesis model

Target	Location	Description	Supporting evidence
ERG	21q22.3	V-ets avian erythroblastosis virus E26 oncogene homolog	Required for normal megakaryopoiesis. Synergistic with *GATA1* mutation and leads to immortalisation of fetal megakaryocyte progenitors (MKPs) *in vivo* (36). Overexpression leads to enhanced megakaryopoiesis in wild-type, Gata1-knockdown, and Gata1s knockin fetal liver progenitors, likely signalling through JAK-STAT pathway (37). *ERG* can act with GATA1s to create TMD-like defect (*ERG*/GATA1s mouse) (38). Ts65Dn (Erg+/+/mld2) mice (i.e., converting trisomy *ERG* to disomy) show complete reversal of myeloproliferative Ts65Dn phenotype (39). Note, not overexpressed in iPS derived DS progenitors (fetal definitive hematopoiesis stage) (24). Overexpressed in non-DS-AMKL (40)
ETS2	21q22.2	V-ets avian erythroblastosis virus E26 oncogene homolog 2	Transcripts elevated in ML-DS and non-DS-AMKL; has role in differentiation switch from erythroid to megakaryocyte (MK) line (41). Overexpression leads to enhanced megakaryopoiesis in wild-type, Gata1-knockdown, and Gata1s knockin fetal liver progenitors (37). Note, not overexpressed in iPS derived DS progenitors (fetal definitive hematopoiesis stage) (24)
RUNX1	21q22.3	Runt-related transcription factor 1	Joint role with *GATA1* in MK differentiation (42); increased expression in AMKL samples (41). Evidence in ML-DS is conflicting. *RUNX1* trisomy not required in Ts65Dn myeloproliferative model (43) and not overexpressed in iPS derived DS progenitors (fetal definitive hematopoiesis stage) (24). *RUNX1* may prolong survival of MKPs (43). Human germline *RUNX1* mutation leads to familial platelet disorder and AML (44)
GABPA	21q21.3	GA binding protein transcription factor	Required for normal megakaryopoiesis. Expressed in MK lineage [reviewed in (4)]. iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45)
BACH1	21q22.11	BTB and CNC homology 1, basic leucine zipper transcription factor 1	Repressor of normal megakaryopoiesis. iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45). Significantly increased expression in ML-DS (DS-AMKL) compared to non-DS-AMKL (46)
DYRK1A	21q22.13	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45). *DYRK1A* overexpressed in both murine and human samples at each step of leukemogenesis (trisomy 21, *GATA1* mutation and subsequent megakaryocytic leukemia). Additional factors likely involved in dysregulation of megakaryopoiesis (40). Leads to increased megakaryopoiesis in mouse modelling (Ts1Rhr/GATA1s) and cell lines (40). Third hit, *MPL*, leads to megakaryocytic leukemia. *DYRK1A* inhibits calcineurin/NFAT pathway, which results in inhibition of VEGF-mediated angiogenesis (40)
SON	21q22.11	SON DNA binding protein	Homology with MYC family. iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45). Increased expression in ML-DS (DS-AMKL) cells after *GATA1* activation, compared to non-DS-AMKL (46)
MYCN	2p24.3	V-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog	Consistently higher expression in DS-TMD as compared to ML-DS and healthy non-DS controls (47). *ERG*/GATA1s-derived MKPs show increased significantly levels of Mycn (38)

Table 1 *(continued)*

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Target	Location	Description	Supporting evidence
SLC37A1	21q22.3	Solute carrier family 37 (glucose-6-phosphate transporter), member 1	Consistently higher expression in DS-TMD as compared to ML-DS (47)
PRAME	22q11.22	Preferentially expressed antigen in melanoma	ML-DS (DS-AMKL) samples had significantly higher *PRAME* antigen levels and significantly higher CDKN2C levels than DS-TMD samples (47). CDKN2C is the effector of *GATA1* mediated cell cycle arrest. *PRAME* is a potential immunotherapeutic target. *PRAME* expressed in non-DS-AML and in paediatric B-ALL
MPL	1p34	*MPL* proto-oncogene, thrombopoietin receptor	Ts1Rhr/*GATA1* mice with *MPL* as third hit developed megakaryocytic leukemia (40). *ERG*/GATA1s-derived MKPs show significantly increased expression of mpl (38)
MYC	8q24.21	V-myc avian myelocytomatosis viral oncogene homolog	*GATA1* mediates the inhibition of this transcription factor (28). GATA1s fails to repress *MYC* in ML-DS (DS-AMKL) (46). Activation of WNT, JAK-STAT and MAPK-P13K pathways all result in *MYC* overexpression (34)
FLI1	11q24.1-q24.3	Fli-1 proto-oncogene, ETS transcription factor	Required for normal megakaryopoiesis. *FLI1* can lead to immortalisation of mutant *GATA1* hematopoietic progenitors, but not wild-type, likely signalling through JAK-STAT pathway (37)
JAK3	19p13.1	Janus kinase 3	*JAK3* mutation present in ML-DS samples [reviewed in (4)]
FLT3	13q12	Fms-related tyrosine kinase 3	*FLT3* mutation present in ML-DS, suggests activation of MAPK-PI3K pathway in ML-DS (34)
JAK2	9p24	Janus kinase 2	*JAK2* mutation found in DS-ALL (48) and reported in one study of ML-DS samples (33)
IKZF1	7p12.2	IKAROS family zinc finger 1 (Ikaros)	*GATA1* mediates the inhibition of this transcription factor (28)
GATA2	3q21.3	GATA binding protein 2	MK transcription factor. *GATA1* mediates the inhibition of this transcription factor (28). GATA1s fails to repress *GATA2* in ML-DS (DS-AMKL) and *GATA2* is upregulated after *GATA1* activation of ML-DS cells (46). *ERG*/GATA1s-derived MKPs show increased expression of *GATA2* (38)
EZH2	7q35-q36	Enhancer of zeste 2 polycomb repressive complex 2 subunit	Possible role in early stage of ML-DS development (33). Encodes catalytic subunit of *PRC2*, which has role in H3K27 dimethyl and trimethylation. Mutations in 33% of ML-DS (DS-AMKL) and 16% of non-DS-AMKL (33)
MYB	6q22-q23	V-myb avian myeloblastosis viral oncogene homolog	iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45). *GATA1* mediates the inhibition of this transcription factor (28)
BRWD1	21q22.2	Bromodomain and WD repeat domain containing 1	iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45)
HLCS	21q22.13	Holocarboxylase synthetase	iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased expression compared to euploid controls (45). Physically interacts with chromatin-modifying proteins eg DNMT1 (49)
HMGN1	21q22.2	High mobility group nucleosome binding domain 1	iPS-derived DS progenitors (primitive yolk-sac stage) demonstrate significantly increased gene expression compared to euploid controls (45,49). Epigenetic changes related to *HMGN1* expression may lead to B-cell proliferation and leukemogenesis

Table 1 *(continued)*

Table 1 *(continued)*

Target	Location	Description	Supporting evidence
KIT	4q12	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	GATA1s fails to repress *KIT* in ML-DS (DS-AMKL) (46). *KIT* expression is increased after *GATA1* activation of ML-DS cells (46). Kit-overexpression demonstrated in TMD samples, but not AMKL samples (50). Kit inhibited by imatinib. *KIT* encodes c-kit
CTCF	16q21-q22.3	CCCTC-binding factor (zinc finger protein)	Possible role in early stage of ML-DS development (33). Interaction with cohesin and *CTCF* proteins may result in inhibited long-range regulation of gene expression (33). *CTCF* mutations present in TMD (2%), ML-DS (20%) and non-DS-AMKL (21%) (33)
PSTPIP2	18q12	Proline-serine-threonine phosphatase interacting protein 2	*PSTPIP2* is a direct *GATA1* target gene in megakaryopoiesis. *GATA1* deficiency *in vitro* led to dysregulation of PSTPIP2 and abnormal MK terminal differentiation (51). This phenotype may involve Src family kinases and *ERK*
Mir-125b-2	21q21.1	MicroRNA 125b-2	Overexpressed in DS-TMD and ML-DS. Leads to phenotype of increased MKP proliferation, self-renewal and myeloid differentiation arrest (52). Synergistic with GATA1s. Inhibition of mir-125b-2 reverses phenotype
CHAF-1B		Chromatin assembly factor 1, subunit B (protein)	Chromatin-assembling factor, encoded by *CHAF-1B* on 21q22.13. Requires further study in ML-DS (40)
Cohesin		Multiprotein complex	53% of ML-DS samples had a mutation that led to loss of cohesin function (33)
KANSL1		KAT8 regulatory NSL complex subunit 1 (protein)	Required for acetylation of H4K16 and acetylation of TP53, which leads to transcriptional activation. Encoded by *KANSL1* on 17q21.31. Signalling through *KANSL1* pathway may involve interaction with MLL (33)
Pathway			
MAPK-PI3K		Includes RAS (Rat sarcoma viral oncogene homolog)-MAPK (mitogen activated kinase-like protein); PI3K (phosphatidylinositol 3-kinase)-AKT (v-AKT murine thymoma viral oncologene homolog 1) pathway	Pathway activation may be involved in DS-mediated leukemogenesis (34,50,53). PI3KC2A mutation present in TMD (34). RAS pathway mutations in 16% of ML-DS and NRAS recurrently mutated in ML-DS (33). MicroRNA-486-5p is a candidate erythroid onco-miR in ML-DS and is an activator of AKT (53). MiR-486-5p is regulated by *GATA1* and GATA1s
WNT		WNT (wingless-related integration site) pathway	Pathway activation may be involved in DS-mediated leukemogenesis (34). APC mutation in ML-DS may lead to WNT pathway activation (34)
Interferon (IFNAR1, IFNAR2)	21q22.11	IFNAR pathway. Interferon alpha, beta and omega receptors 1 and 2, are both on Hsa 21	Proposed in (12). Fetal liver-derived MKPs have low Type 1 interferon expression. Restoration of IFN-response may lead to resolution of fetal-liver derived MKPs responsible for TMD phenotype (54). IFN-α could be explored as treatment for postnatal life-threatening TMD
JAK-STAT		Janus kinase/signal transducer and activator of transcription pathway	Immortalisation of hematopoietic progenitors in *GATA1* mutant mice involved activation of JAK-STAT pathway (37). *JAK1* mutation in ML-DS leads to activation of JAK-STAT pathway (34)
TP53		Tumor protein p53 pathway	ML-DS sample with *PARK2/PAKRG* mutation, resulting in TP53 pathway activation (34). Encoded by TP53 on 17p13.1

megakaryopoiesis, as survival of the MKPs was longer in trisomic RUNX-1 mice (43). *GATA1* interacts with *RUNX1* to facilitate normal megakaryopoiesis in cell lines (42). *RUNX1* accounted for some of the increased gene expression observed in DS-hyperproliferation *in vitro*, including a significant increase in c-Kit and Tie-2 expression (58). The mechanism by which altered GATA1s/RUNX1 interaction may lead to ML-DS is unclear (42).

DYRK1A is overexpressed in DS-TMD and ML-DS (40). DYRK1A expression leads to increased megakaryopoiesis and inhibition of the calcineurin/NFAT (nuclear factor of activated T-cells) pathway (40). Dysregulation of the calcineurin/NFAT pathway is thought to account for increased leukemic incidence and reduced solid tumour incidence in DS (40).

MicroRNAs encoded by chromosome 21 may also play a role in leukemogenesis (8). miR-125b-2 is overexpressed in ML-DS (DS-AMKL) and DS-TMD, is found to increase proliferation and self-renewal of MKPs; and causes a myeloid differentiation arrest (52). miR-125b-2 and GATA1s synergistically increased proliferation of relevant hematopoietic precursors, and inhibition of miR-125b-2 caused impaired growth in DS-AMKL/DS-TMD (52). MicroRNA-486-5p, on chromosome 8, has recently been shown to be regulated by both GATA1 and GATA1s; and to act as an erythroid onco-miR in ML-DS (53).

Epigenetic targets on chromosome 21 include *BRWD1*, *HLCS*, and *HMGN1* (45,49) (*Table 1*).

Other candidate drivers not encoded by chromosome 21 include *JAK3* (4), *MYCN* (47), *MYC* (34), *PRAME* (47) and additional epigenetic modifiers such as *EZH2* (*Table 1*). GATA1-target genes identified through non-DS models may also reveal candidates, such as *PSTPIP2* (51).

Additional models to study ML-DS

Development of a DS TMD/ML-DS model remains a challenge. One group was successful in creating AMKL mouse xenografts, however a mouse model of DS-TMD evolving to ML-DS has not been described (59). Promising models to date include one that mimics the function of GATA1s (28) and a model of *ERG*/GATA1s mice that developed a TMD-like expression profile and MKP proliferation defect (38). The *ERG*/GATA1s mice were reported to develop myeloid leukemia at 3 months (38). A recent xenograft incorporating patient-derived TMD samples demonstrated emergence of genomic features of ML-DS after serial transplantation. These genomic changes were present in small subclones in the original TMD samples (60).

Well studied murine models of DS include Tc1, Ts65Dn, Ts1Cje (43,56,57) and GATA1 knock-in mice (28). Ts1Rhr mice have also been used to study DS phenotypes, including ML-DS (40) and DS-ALL (61). These models differ in the number of relevant triplicated genes present; and this in part depends on which mouse chromosomes (and thus orthologues) are present (62). The Ts65Dn and Ts1Cje mouse models use mouse chromosome 16 (Mmu16), on which the majority of Hsa21 genes are located (56). Mouse chromosome 10 and 17 contain the remainder of Hsa21 orthologues (57).The most widely used model, Ts65Dn model, is trisomic for the distal end of chromosome 16q, and this is fused to a genetically-poor region of 17p (43). Tc1 is trisomic for 269 genes orthologues on Hsa21 whilst Ts65Dn includes 104 gene orthologues, 94 of which are from the DSCR (Hsa21q22) (43,57). The Ts1Cje mouse has 97 orthologues and Ts1Rhr has 31 orthologues, analogous to part of the DSCR (56,61). Tc1/GATA1$^{\Delta e2}$ double mutant mice did not develop TMD or leukemia, despite this model offering the largest number of Hsa21 orthologues to study hematopoiesis (57). Only the Ts65Dn model developed a myeloproliferative phenotype, albeit progressive and occurring at 15 months of age (43). Therefore, although useful, this model does not replicate human ML-DS.

The role of GATA1s in myeloproliferation has been studied in mouse models. The GATA1 knock-in model (GATA1$^{\Delta N}$) confirmed a megakaryocytic proliferative phenotype in yolk sac and fetal liver hematopoiesis, that was sensitive to the effects of GATA1s (28). The GATA1$^{\Delta e2}$ model closely replicates the GATA1s truncated protein that is produced by N-terminal *GATA1* gene disruption. MK proliferation occurred, without an accompanying differentiation block, possibly through loss of inhibition of growth regulatory genes (28). N-terminal disruption of *GATA1* can be compensated for by signalling through the C-terminal transactivation domain (C-TAD) of *GATA1*, and C-TAD has a role in embryonic hematopoiesis and megakaryopoietic proliferation (63) .

Due to limitations of using mouse models, including the lack of a TMD phenotype with spontaneous resolution, other hematopoietic systems have been studied. Recent work has focused on *in vitro* differentiation of isogenic human pluripotent cells. *In vitro* studies of fetal DS (24,45) report varying gene expression results, according to the stage of fetal hematopoiesis. Characteristic MKP proliferation was detected in fetal DS hematopoiesis (24), specifically definitive fetal hematopoiesis only (23). In addition, Roy *et al*.

demonstrated impaired B-lymphoid differentiation in DS fetal liver tissue (23). *BACH1*, *GABPA*, *SON*, *DYRK1A* were significantly up-regulated in DS progenitor cells in early fetal hematopoiesis, compared to euploid human cell lines (45). No alteration of *ETS*, *ERG*, *RUNX-1* or *IGF* signalling genes were found in definitive fetal hematopoiesis in human DS samples (24). *In vitro* studies are limited by the small magnitude of gene expression changes that are detected, therefore stage-specific changes would ideally be confirmed by additional disease models.

Can children with DS who will progress to ML-DS be identified before frank leukemia develops?

The most likely candidate markers for DS patients at risk of progressing to ML-DS are the GATA1s protein itself or the various mutations which cause this protein to be prematurely truncated. The GATA1s level in peripheral blood or bone marrow at the diagnosis of TMD and its pattern of change over time, which predicts the subsequent risk of ML-DS, is unknown. *GATA1* mutations are restricted to blast cells, and therefore clear after resolution of TMD or ML-DS (15,27,64,65). Therefore, the true prevalence of *GATA1* mutations in DS, and the timing of re-emergence of the *GATA1* mutant clone remain unanswered questions. There is debate regarding whether the type of *GATA1* mutation predicts progression to ML-DS. The largest study to date of TMD (134 samples) and ML-DS (103 samples) did not show any correlation between type of *GATA1* mutation and progression to ML-DS (32). However, another study found a surprising association between low GATA1s protein levels, associated with particular types of *GATA1* mutations, and a significantly higher risk of ML-DS (66).

To definitively answer these questions, a prospective, longitudinal study of all DS patients is needed. With current next-generation sequencing techniques we may be able to further elucidate drivers and repressors of leukemogenesis, using matched TMD and ML-DS samples. To our knowledge, there are no published prospective longitudinal data that have evaluated the pattern of *GATA1* gene mutations and resultant gene expression over time. In our own research study, the PreP21 (Predicting and Preventing Leukemia in Children with DS) study (Clinical trials reference number: ACTRN12613000861752), we aim to study longitudinal changes over time in patients with TMD. Prospective sampling will allow detection of subclinical TMD. *GATA1* mutations and additional drivers for leukemogenesis may be assessable in a longitudinal manner, in a similar manner to MRD monitoring in ALL therapy. This may then become a reliable biomarker for risk of progression to ML-DS. We hope to identify factors that promote regression of TMD in DS patients, or subsequent progression to ML-DS. In addition, we may be able to identify molecular targets for therapeutic targeting to prevent DS-mediated leukemogenesis.

Can progression from TMD to ML-DS be prevented?

Three prospective studies (POG, BFM, COG) found an incidence of 19-23% for DS patients with TMD developing ML-DS at a later stage (9). This occurred at a median time of 1.2-1.5 years from TMD (9). COG trial A2971 prospectively enrolled DS-TMD and ML-DS patients, and found that 43% of patients with ML-DS had prior TMD (19). The trial was run before PCR studies on *GATA1* mutations revealed its importance, thus no *GATA1* analysis was performed.

Therapeutic intervention for patients with TMD has been undertaken in small studies. Low dose cytarabine was used for babies who were symptomatic from severe TMD, with dose ranges varying from 1.2-1.5 mg/kg/dose twice daily for 7 days (subcutaneous or intravenous) (12) or 0.5-1.5 mg/kg for 3-12 days (11). Higher doses (3.33 mg/kg/day for 5 days) used in another trial led to Grade 3-4 toxicity in 96% of patients, without a discernible survival improvement (13).

Klusmann *et al*. studied 146 newborns with TMD and found that cytarabine therapy improved outcomes for those with risk factors for early death (11). Cytarabine was administered to those DS patients with TMD and clinical compromise due to high white cell count, thrombocytopenia or liver dysfunction. Patients with TMD who were treated with cytarabine developed ML-DS at the same frequency as those with TMD who did not received cytarabine, although numbers of treated patients were small (n=28). Patients in this study who went on to develop ML-DS with a history of TMD (n=29) had a significantly higher EFS than those without a history of TMD who developed ML-DS during the same period of study (n=142) (EFS 91% compared to 70%, P=0.039) (11).

Taken together, prior evidence suggests that treatment of TMD does not prevent progression to ML-DS, based on three prospective studies [reviewed in (9)]. Previous studies were not always able to test for *GATA1* mutations; and if *GATA1* was analysed, there were limitations in sensitivity of *GATA1* mutation detection techniques and

adequate samples. There is one current study (EudraCT no. 2006-002962-20) which aims to assess feasibility of low-dose cytarabine therapy to prevent progression from TMD to ML-DS by eradication of GATA1s and use of MRD monitoring (62). Therefore further study is required, in particular incorporating routine, sensitive *GATA1* mutation testing.

Novel therapies in ML-DS

Therapeutic targets for further study in ML-DS include c-Kit inhibition using imatinib (50), miR-125b-2 inhibition (52) and interferon therapy (54). Low dose interferon may restore inhibition of MKP proliferation (54) and may therefore be an effective treatment strategy for residual MKPs in TMD (6).

Recent pre-clinical work has elucidated other potential drug therapies, such as wee1 kinase inhibitor MK-1775 in ML-DS (67) and Aurora–A Kinase (AURKA) inhibitors [such as dimethyl fasudil and MLN8237(59)] in AMKL. MK-1775 enhanced cytarabine-induced cytotoxicity *in vitro* in ML-DS cell lines and in *ex vivo* primary patient samples (67). *AURKA* inhibitors lead to polyploidisation, mature cell-surface marker expression and apoptosis of malignant (non-DS) AMKL cells (59). The role for treatment of ML-DS with AURKA inhibitors has not yet been established.

Understanding of *GATA1* biology in ML-DS can be applied to non-DS AML. For example, elegant pre-clinical studies demonstrated that high *GATA1* expression correlated with increased Bcl-xl protein levels (68). Knock-down of GATA1 in megakaryocytic cell lines partly reduced Bcl-xl expression, resulting in increased apoptosis and increased chemosensitivity. There is a Bcl-2 inhibitor GX15-070 (obatoclax) currently in early phase clinical trials in leukemia (68). Sodium valproate, in the same study, downregulated GATA1 expression and led to enhanced cytarabine-induced apoptosis *in vitro*. Therefore Bcl-2 inhibitors and sodium valproate, a histone deacetylase inhibitor, are potential therapeutic agents in non-DS AML with high GATA1 expression (68).

ALL in DS: "DS-ALL"

This type of leukemia, derived from lymphoid precursors, is more common in the general (non-DS) population than AML. The most common types of ALL we see in paediatric practice are B-lymphoblastic leukemia (B-ALL), affecting B-lymphocytes; T-lymphoblastic leukemia (T-ALL, T-lymphocytes) and mixed phenotype acute leukemia (MPAL) (69).

We will discuss DS-ALL in the context of new translational targets that may be used for future treatment strategies. The treatment landscape in DS-ALL is very different to ML-DS.

Treatment and outcome for DS-ALL

In the non-DS population, the overall event-free survival (EFS) for ALL is 85% or greater (70). In contrast, overall survival (OS) for children with DS-ALL is closer to 70% compared to 89% for non-DS ALL (P<0.0001) (71). Increased chemosensitivity documented in ML-DS cells has not been observed in DS-ALL cells (8).

Genetic features that may have an adverse impact on overall survival from DS-ALL include JAK2 mutations (that are found in 20% of DS-ALL) (48) and aberrant expression of the type 1 cytokine receptor CRLF2 in 60% of children with DS-ALL (72). CRLF2-positive DS-ALL is likely to be classified as high-risk ALL if present in combination with IKAROS (IKZF1) gene deletion (71). Recently, *RAS* driver mutations *(KRAS*, *NRAS)* were identified in 1/3 cases of DS-ALL; occurring virtually exclusively of, and at a similar frequency to, JAK2 mutations (73). Potential good prognostic features include *ETV6/RUNX1* fusion and high hyperdiploidy (71). Other factors that influence OS include increased rate of treatment-related toxicity and increased risk of infectious deaths compared to non-DS ALL (74,75). Treatment-related toxicity, for example due to methotrexate, may be explained by altered metabolic profiles in non-leukemic cells, caused by constitutional trisomy 21 [reviewed in (76)].

Potential reasons for the increased rate of infectious deaths in children with DS-ALL, which can occur during less intensive maintenance therapy, are firstly an immunodeficient state characterised by partial B-cell deficiency with resulting dysgammaglobulinemia and secondly, dysregulation of T-cell function due to inhibition of the calcineurin/NFAT pathway [reviewed in (74)]. Epigenetic studies indicate this may be due to reduced expression of proteins involved in cell signalling pathways, such as cytokine-cytokine receptor interaction pathways (61).

Two recent studies reported that children with DS-ALL still have a high risk of relapse, even after hematopoietic stem cell transplantation (HSCT) (71). Therefore the challenge is identifying those children with high-risk DS-ALL who require treatment intensification to prevent

subsequent relapse; and correctly identifying those children with DS-ALL who have favourable molecular features that will permit treatment de-intensification and still achieve lasting remission (74,77). MRD response could be used to discriminate DS-ALL patients with a low-risk of relapse and to intensify treatment for DS-ALL with a poor MRD response (75). Successful use of MRD-risk directed therapy was described for a large cohort of children with *BCR-ABL1-like* ALL, which included some children with DS (78). The heterogeneity of DS-ALL, with respect to biological features and treatment response, remains a clinical challenge.

Drivers of leukemogenesis in DS-ALL

The relevance of recent identification of altered JAK-STAT and RAS signalling in DS-ALL has therapeutic potential. JAK-STAT inhibitors are already in clinical use for adults with myelofibrosis (77) and systematic trials in paediatric DS-ALL are awaited (74). JAK-STAT inhibitors, such as the dual JAK1/JAK2 inhibitor ruxolitinib, may in future be used as adjunct therapy for children with CRLF2 positive-ALL to induce remission (74) and provide a bridge to HSCT for high-risk paediatric DS-ALL. A recently developed KRAS inhibitor, deltarasin, may be of benefit to patients with DS-ALL and *RAS*-mutations (73); and this would need to be studied in early phase trials.

One potential translational research question is whether there are any pre-leukemic initiating events that occur in DS-ALL, similar to ML-DS. Of interest, is the known *in utero* transforming event of *ETV6/RUNX1 (TEL-AML1)* fusion that can be detected postnatally and leads to increased leukemogenic potential of the transformed B-cells (6,79). *RUNX1*, as previously discussed, is located on chromosome 21 and has a role in megakaryopoiesis. A recent study implicated mir-125-b2 as a potential independent driver in *ETV6/RUNX1* non-DS ALL (80). However, in DS-ALL, there is a decreased prevalence of both favourable (e.g., E*TV6/RUNX1*) and unfavourable chromosomal aberrations (e.g., *BCR-ABL*) (74,81), suggesting that there may be a different driver of leukemogenesis in DS-ALL.

DS and leukemia: epigenetics and future directions

Epigenetics may also play a broad role in DS-ALL and ML-DS. Two recent studies used Ts1Rhr mouse models to analyse epigenetic changes, the first in ML-DS (82) and the second study in DS-ALL (61).

Malinge *et al*. found that trisomy 21 led to global hypomethylation; and that DS-TMD samples featured new, focal gains of DNA methylation. Hypomethylation of the DSCR in particular may lead to increased expression of trisomic genes that predispose to DS-mediated myeloid leukemogenesis (82). In contrast, the transcriptome and epigenome of DS-TMD samples compared to ML-DS samples were very similar (82).

In DS-ALL, a transcriptional profile was defined based on analysis of B-lymphocytes (61). The analyses revealed highly enriched clustering in pathways related to polycomb repressor 2 (*PRC2*) targets and sites of trimethylated Lys 27 of histone 3 (H3K27me3). H3K27me3 is the repressive epigenetic mark added by PRC2. DS-ALL demonstrates global reduction in H3K27me3, which in turn leads to an increased gene expression pattern that drives B-cell development. By using a histone demethylase inhibitor, GSK-J4, H3K27me3 expression was increased in Ts1Rhr B cells, and led to reversal of the B-cell leukemogenic phenotype (61). The study found a potential candidate *HMGN1* in Ts1Rhr mice that resulted in global suppression of H3K27me3. Sole *HMGN1* overexpression in shRNA modified- Ts1Rhr mouse models led to phenotypic changes seen in the normal Ts1Rhr mice, therefore providing further proof that *HMGN1* is largely responsible for the B-cell leukemogenic changes. The authors provide a proof of principle that by reversing global suppression of H3K27me3, which is likely due to *HMGN1*, B-cell leukemogenesis may be blocked (61).

An elegant study of monozygotic twins, discordant for trisomy 21, also describes profound genome-wide changes associated with trisomy 21. The additional chromosome 21 is thought to promote changes in the transcriptome of trisomic cells, affecting protein-coding genes and lncRNAs (49). These transcriptional changes were organised in well-defined chromosomal domains, termed "gene expression dysregulation domains" (GEDDs). GEDDs were replicated in additional models, including induced pluripotent stem cells derived from fibroblasts from the discordant twins; and the Ts65Dn mouse model (49). GEDDs may be the result of Hsa21-based genes that modify the chromatin environment of the nuclear compartments in trisomic cells. Candidate genes on chromosome 21 that may contribute to epigenetic changes include holocarboxylase synthetase (*HLCS)*, and proteins HMGN1, DYRK1α, RUNX1, BRWD1 (49). This hypothesis requires further study, including whether the transcriptional changes are due

to chromosome 21-based candidates; or as a general result of any human trisomy. These findings could then be applied to both DS-ALL and ML-DS.

Lastly, targeting pre-leukemic cells and eradicating leukemic stem cells that account for relapse will be a major challenge. Potentially, further study could assess whether an early HSC precursor is mutated, prior to lymphoid/myeloid pathway commitment (58). This is plausible, due to HSC expansion induced by trisomy 21 alone. Genetic alterations in a pluripotent HSC could explain the increased incidence of both myeloid and lymphoid malignancies in children with DS; and also explain rare cases of non-contemporaneous AML and ALL occurring in the same individual (74).

Conclusions

Therefore, the ultimate aim is to identify novel therapeutic targets that may improve outcome for all children with DS, pre-leukemia and leukemia. Insights into TMD/ML-DS may help us understand how early fetal hematopoietic development promotes leukemogenesis in disparate patient populations and may help us understand the aetiology of DS-ALL. Prospective *GATA1* analysis may provide a platform for identification and intervention, to prevent ML-DS and improve quality of life in children with DS. Robust DS-TMD/ML-DS xenograft models will help to define the overall role of GATA1s and to understand the elusive third and subsequent "hits". Xenograft models may also promote further understanding of chemosensitivity and, inversely, resistance of DS-leukemia cells. Translational models of ML-DS and DS-ALL will permit dynamic analysis of Hsa21 genes and provide a platform for development of targeted agents for high-risk leukemia. Moreover, this knowledge may collectively provide insights that may be applied to non-DS leukemia and possibly all embryonal cancer.

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Footnote

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