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Myeloid Leukemia in Down Syndrome

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Abstract

Although adults with Down syndrome (DS) show a decreased incidence of cancer as compared to individuals without DS, children with DS are at an increased risk of leukemia. Nearly half of these childhood leukemias are classified as acute megakaryoblastic leukemia (AMKL), a relatively rare subtype of acute myeloid leukemia (AML). Here, we summarize the clinical features of myeloid leukemia in DS, review recent research on the mechanisms of leukemogenesis, including the roles of *GATA1* mutations and trisomy 21, and discuss treatment strategies. Given that trisomy 21 is a relatively common event in hematologic malignancies, greater knowledge of how the genes on chromosome 21 contribute to DS-AMKL will increase our understanding of a broader class of patients with leukemia.

Keywords

AMKL; leukemia; transient myeloproliferative disorder; trisomy 21; GATA-1; MPL

INTRODUCTION

Individuals with Down Syndrome (DS) display various developmental abnormalities, including craniofacial dysmorphism, cardiovascular defects and learning disabilities. Paradoxically, individuals with DS have a decreased frequency of solid tumors (epidemiological studies in Denmark, Finland, and Australia indicated an incidence ratio respectively of 0.50, 0.57, and 0.44^{1–3}), but a higher incidence of leukemia (10–20 fold).⁴ Even more strikingly, young children (<4 years) with DS have a 500-fold increased incidence of acute megakaryoblastic leukemia (AMKL, also known as ML-DS).⁵ The natural history of leukemia in children with DS suggests that trisomy 21 directly contributes to the malignant transformation of hematopoietic cells. In addition, somatic mutations of the *GATA1* gene have been detected in nearly all DS AMKL cases and are notably absent in non-DS AMKL.⁶ In this review, we will highlight the clinical manifestations, outcomes and new observations related to signaling pathways aberrantly controlled by trisomy 21 or *GATA1* mutations during DS-AMKL leukemogenesis.

CLINICAL FEATURES

There is a well-recognized preceding transient myeloproliferative disorder (TMD), aka transient leukemia (TL), occurring in the neonatal period in 10% of infants with DS.^{7–9} TMD is a clonal pre-leukemia characterized by an accumulation of immature megakaryoblasts in the fetal liver and peripheral blood.⁵ The incidence of TMD may be underestimated as not all cases come to medical attention. The median age of presentation of TMD, based on pooled data from > 200 neonates, is 3–7 days.^{10–12} The clinical presentation

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of neonates with TMD ranges from a healthy appearance to bruising, respiratory distress, fulminant hepatic failure, *hydrops fetalis* or even death in 15–20% of cases that have been diagnosed. Overall, though, the majority of cases resolve spontaneously with normal blood counts at a mean of 84 days.¹³ After a latency period of 1–4 years, a subset of these children (20–30%), develop acute megakaryoblastic leukemia.¹⁴ In a series of 112 patients with AMKL, the median age of DS patients was 1.8 years vs. approximately 8 years in non-DS cases.^{15–16} Patients with AMKL develop anemia, thrombocytopenia, myelofibrosis, organomegaly, extensive skeletal lesions,^{17–18} and leukocytosis although white blood counts are lower than in non-DS.^{19–20} CNS involvement is unusual.¹⁶

Diagnosis

Histological examination of the bone marrow in AMKL shows replacement with megakaryoblasts and reticulin deposition. Megakaryoblasts are identified by a positive platelet peroxidase reaction,²¹ and by immunophenotyping for glycoprotein IIb/IIIa or the von Willebrand factor protein.²² These blasts are non-reactive for myeloperoxidase and express stem/progenitor markers CD33, CD34, CD117, erythroid markers CD36 and glycophorin A, the lymphoid antigen CD7 and the megakaryocytic markers CD41 and CD42b.^{23–25} Of note, cytogenetic differences between DS and non-DS AMKL include the absence of the translocation t(1;22), and instead, the presence of trisomies involving chromosomes 8 and 1,⁷ as well as monosomy 7.^{26–27} Since Down syndrome is the most common cytogenetic abnormalities seen at birth (1/700), improved non-invasive prenatal diagnosis is an area of active research. Strategies are emerging based on screening differentially methylated regions (DMRs) of fetal DNA for chromosome 21 dosage assessment.²⁸ Moreover, murine models of DS have helped identify differentially expressed genes in DS-fetal livers, some of which may represent potential chromosome 21 specific biomarkers.²⁹

Prognosis

Prospective, multi-institutional studies in the US, Germany and Japan have examined the natural history of TMD in 264 infants.^{10–12} Early death occurred in up to 20% of infants and was significantly correlated with higher white blood cell count at diagnosis, increased bilirubin and liver enzymes, and a failure to normalize the blood count. Later development of leukemia occurred in 19% of infants at a mean of 20 months and was significantly correlated with karyotypic abnormalities in addition to trisomy 21, including trisomy 11, del 16q, der(14;21), t(5;13), and tetrasomy 21.¹⁰ In DS-AML age at diagnosis had independent prognostic significance, primarily a result of poor remission induction in older patients.³⁰ Cytogenetic abnormalities such as monosomy 7 confer an adverse prognosis in non-DS and DS-AMKL in some studies.²⁶

MECHANISMS

Pathogenesis

From trisomy 21 to TMD towards AMKL: an incremental process of leukemogenesis—If trisomy 21 is considered the first genetic event in DS-AMKL leukemogenesis, the second hit is a mutation of the X-linked gene *GATA1*, encoding a blood-specific transcription factor essential for development of the erythroid and megakaryocytic lineages. *GATA1* mutations are present in nearly all TMD patient samples as early as 21 weeks gestation.^{31–34} Using the variable length of nucleotide insertions and deletions as a marker of individual TMD clones, sequential samples collected from the same patient during TMD, remission, and AMKL showed identical *GATA1* mutations that disappeared during remission.³³ This confirms the clonal nature of AMKL and its evolution from TMD.

TMD is a critical model to understand the natural history of AMKL, 20% of TMD cases evolve into AMKL either overtly, or following an apparent remission. AMKL and TMD blasts express erythroid markers such as gamma globulin and delta aminolevulinate synthase as well as *GATA-1* and *GATA-2* suggesting origin from the megakaryocyte-erythroid progenitor cells.³⁵ Myeloid and erythroid dysplasia are common as well as the presence of karyotypic abnormalities in metaphases from CFU-GM and BFU-E mimicking those seen in megakaryoblasts.³⁶

Fetal liver origin of leukemia initiating cell—*GATA1* mutations most likely occur in utero, based on neonatal blood spot testing, and may precede disease development.^{37–38} Mice expressing a *GATA-1* mutant ortholog of the one seen in human DS specimens display sustained proliferation of a yolk sac/early fetal liver megakaryocyte progenitor implicating this as the target cell for leukemic transformation in DS-AMKL and TMD.^{39–40} Moreover, *GATA1* mutations were detected in 2 of 9 liver samples from terminated fetuses with DS (as early as 21 to 23 weeks of gestation) supporting the fetal liver origin of TMD.³¹

Role of trisomy 21—Second trimester DS fetal livers (FLs) show increased megakaryocyte-erythroid progenitor frequency and increased clonogenicity.⁴¹ Enhanced erythroid and megakaryocytic differentiation was seen in NOD/SCID mice transplanted with DS FL mononuclear cells.⁴² Those observations were obtained from 13 to 23 week trisomic FL, preceding the acquisition of any *GATA1* mutation.

Through a high-resolution map of DS was generated using a panel of 30 individuals with rare segmental trisomies 21, Korbelt *et al.* identified a critical region of 8.35 Mb (35–43.35) that is likely contributing to the risk increase for both TMD and AMKL. This region includes previously known oncogenes, such as *RUNX1*, *ERG* and *ETS2*.⁴³ Using mouse ES cells (ESC) bearing an extra copy of human chromosome 21 (Hsa21), disturbances in early hematopoietic differentiation were observed and related to increased expression of *GATA-2*, *Tie-2* and *c-kit*. An siRNA silencing study implicated increased level of *RUNX1* in abnormal *Tie-2* and *c-kit* expression. Using a panel of ESCs partially trisomic mapped with tiling arrays, two non-overlapping regions of Hsa21 were correlated to abnormal hematopoiesis.⁴⁴ The distal region contains *RUNX1*, *DYRK1A*,⁴⁵ *ETS2* and *ERG* while the pericentromeric region frequently harbors chromosome rearrangements and increased disomic homozygosity of DNA markers in DS-TMD and DS-AMKL.⁴⁶

Both *ERG* and *ETS2* bind the hematopoietic enhancer of *SCL/TAL1*, a key regulator of hematopoietic stem cell and megakaryocytic development.⁴⁷ Overexpression of *ETS2* and *ERG* increase the megakaryocytic differentiation of *GATA-1*s progenitors, and immortalize *Gata1*s fetal liver progenitors in replating assays.⁴⁸ Coexpression of *ERG* and *GATA-1*s *in vivo* results in leukemia with an immature megakaryocytic phenotype.⁴⁹

In parallel, several mouse models of DS have been developed to identify dosage-sensitive genes that contribute to specific hematopoietic phenotypes (see Figure 1). The Ts65Dn mouse, trisomic for 104 orthologous genes of human chromosome 21, develops a macrocytic anemia and a myeloproliferative disorder (MPD) associated with thrombocytosis.⁵⁰ Interestingly, unlike trisomy of *Runx1* in the Ts65Dn mouse model of DS, reduction to functional disomy of *Erg* using a loss-of-function allele, corrects the pathologic and hematologic features of myeloproliferation.⁵¹ Other segmental trisomy mouse models include the Tc1 mouse model (212 genes)⁵² and the Ts1Cje mouse (81 genes).⁵³ Notably, none of these mouse strains develops TMD or AMKL alone or in cooperation with *GATA-1*s expression, suggesting undiscovered cooperating mutations.

There are also 5 micro-RNAs encoded on chromosome 21, of which miR-125b2 is overexpressed in TMD and AMKL. In both fetal liver and human CD34+ cells, overexpression of *miR-125b-2* led to hyperproliferation and enhanced self-renewal of megakaryocytic progenitors attributed to repression of *DICER1* and the tumor suppressor *ST18*.⁵⁴

Role of GATA-1—The first insight into the mechanism of DS-AMKL was the discovery of acquired mutations in the *GATA1* gene. These mutations were restricted to the leukemic clones and were not found in normal remission samples.⁶ The mutation is not detectable in non-DS leukemia or other subtypes of DS leukemia,⁵⁵ emphasizing the specific cooperation of *GATA1* mutation with trisomy 21 in megakaryocytic leukemia. DS and non-DS-AMKL samples exhibit distinct gene expression profiles and a specific signature for DS-AMKL was identified with relatively increased expression of GATA-1 transcripts (as GATA-1s) and failure to down-regulate proliferation-promoting genes that are normally repressed by GATA-1.^{56–57} In almost all DS-AMKL and TMD samples, mutations in *GATA1* are detectable in exon 2 producing a premature stop codon within the N terminal activation domain.^{55,58} These mutations prevent the generation of full-length GATA-1, but preserve the translation of GATA-1s, a truncated form of GATA-1 lacking the N terminal activation domain. Distinct regions in the GATA-1 N terminus are required for terminal megakaryocyte differentiation and controlling growth of immature precursors.^{59–60} Analysis of the mutational spectrum at *GATA1* in DS TMD and AMKL blasts shows predominance of insertions/deletions, duplications (74%) and base substitutions (26%).⁶¹ A recent study concluded that the different classes of *GATA1* mutations result in variable translation efficiency of GATA-1s, and further, that the level of GATA-1s protein correlates with risk of progression to leukemia.⁶² However, a subsequent study showed that the *GATA1* mutational spectrum did not differ between TMD or AMKL, and that the type of *GATA1* mutation was unable to predict evolution from TMD to AMKL.⁶³

Mice with lineage-specific mutations of the *GATA1* promoter show impaired maturation and dysregulated proliferation of megakaryocytes.⁶⁴ Expression profiles of GATA-1s and full-length GATA-1 expressing murine fetal megakaryocytes have been contrasted and showed that GATA-1s fails to repress a number of transcription factor genes (including *Gata2*, *Ikaros*, *Myb* and *Myc*) that have “pro-proliferative” effect on hematopoietic cell growth.^{39, 60} Of note, in 2006, a family was discovered with a germline *GATA1* mutation in which affected males generated only the GATA-1s isoform and exhibited anemia and trilineage dysplasia, but failed to develop leukemia.⁶⁵ This observation established that trisomy 21 is necessary for leukemogenesis in the presence of mutated *GATA1*.

Cooperating mutations—Mutations in the p53 tumor suppressor gene have been demonstrated in a proportion of patients after transformation from TMD to AMKL suggesting a role in disease evolution. To date, only a single case of a p53 mutation in TMD has been reported.^{66–67} Several activating mutations of the *JAK3* gene have been identified in TMD, DS AMKL and non-DS AMKL patients as well as in DS-AMKL cell lines (CMK and CMY). These mutations result in constitutive JAK signaling^{13,68–69} and confer responsiveness to treatment with JAK3 inhibitors in vitro.⁷⁰ Both JAK3 A572V and the recently identified JAK3 P132A^{68,71} mutants appear to be oncogenic in a murine models. However, recent data show that the purported activating *JAK3* mutations are present in DNA samples from normal blood donors, at a frequency similar to that observed in patients with AML, suggesting that they may represent SNPs.⁷¹ Further study in this field is required to clarify the leukemogenic role of *JAK3* mutations in DS-AMKL. In addition, activating mutations affecting *FLT3*, *JAK2*, and *MPL* genes were also identified within DS-AMKL.^{72–73} A summary of the stepwise acquisition of mutations is shown in Figure 2A.

Aberrant signaling Pathways in DS-AMKL—Fetal liver hepatic stromal cells support hematopoietic stem cell (HSC) expansion by secreting insulin-like growth factor 2 (IGF-2).⁷⁴ Constitutive activation of IGF signaling was demonstrated in DS-AMKL and TMD blast cells, as well as in DS-AMKL mouse model.⁷⁵ Klusmann et al. showed that mutated GATA-1 fails to restrict IGF-mediated activation of the E2F transcription network. This aberrant response converges with overactive IGF signaling to promote enhanced proliferation and increased survival of DS fetal liver progenitors, and revealing a fetal stage-specific regulatory network (Figure 2B).

More than 20 genes involved in oxidative metabolism are localized to chromosome 21, including superoxide dismutase (*SOD*)⁷⁶ and Cystathionine Beta Synthase (*CBS*). *CBS* overexpression in DS directs homocysteine to cystathionine synthesis and away from methionine remethylation, creating a folate trap and thymidylate imbalance. Perturbed folate metabolism in turn results in the accumulation of uracil and its misincorporation into DNA. This altered metabolism, when paired to oxidative stress caused by increased *SOD1* activity seen in DS, has been implicated in a model linking chromosome 21 genes (*CBS* and *SOD1*) to the generation of mutations in the *GATA1* gene.⁶¹ Additionally AMKL blasts, unlike TMD cells, have demonstrable telomerase activity, implicating telomerase with the malignant character of a leukemic proliferation.⁷⁷

THERAPY

Treatment Options

One of the first clinical trials for this malignancy studied 12 children with DS-AML. These patients (POG8498) showed heightened sensitivity to high dose cytarabine and anthracycline based therapy with a significantly superior event-free survival compared to non-DS AML (3 yr EFS 100% in DS-AML vs 33% in non-DS AML).⁷⁸ In subsequent trials, intensive induction showed unacceptable toxicity and increased mortality in DS-AML as did autologous and allogeneic transplant.¹⁵ AMKL has been treated on protocols involving either conventional (100–300mg/m²)²⁶ or high dose cytosine arabinoside (3g/m²) with reported 3 yr OS>80%. However significant toxicity has been reported with the high dose Ara-C.^{16, 19, 30, 79} Low dose subcutaneous Ara-C induced remission in almost all cases of AMKL and complicated TMD^{80–81} with comparable 5 year EFS and OS to standard chemotherapy.⁸²

There was a significant improvement in clinical trials survival outcomes in DS between 1993 and 1998 mainly due to reduction in treatment related mortality. This resulted from reduced anthracycline and cytarabine dosing and longer intervals of recovery between therapy.⁸³ Due to the limitations of toxic deaths, infections, and cardiac toxicity in treating DS-AMKL, new, less-intensive protocols have been conducted in the United States, Japan and Europe.^{16, 84} In a single prospective study treatment of TMD with low dose cytarabine (0.5–1.5 mg/kg) improved 5 year EFS from 28% to 52% in children with risk factors for early death. Treatment of TMD did not alter risk of developing subsequent AMKL.¹¹ The ML-DS prevention trial (EudraCT no. 2006-002962-20) is currently ongoing to assess if the progression from TL to ML-DS may be blocked by eradication of the GATA-1s clone using low-dose cytarabine treatment and monitoring for minimal residual disease (MRD).

Chemosensitivity in DS-AMKL

The enhanced sensitivity of DS myeloblasts to Ara-C is due to greater extent of Ara-C incorporation into DNA, and increased relative numbers of double strand DNA strand breaks,⁸⁵ attributed to dosage effect of genes localized to chromosome 21 including *CBS*. In vitro, DS myeloblasts generate higher concentrations of Ara-CTP, the active cytarabine

metabolite. This is thought to be due to increased CBS expression and an elevated ratio of deoxycytidine kinase (*Cdk*) to cytidine deaminase (*CDA*). *CDA* metabolizes Ara-C to the inactive metabolites uridine arabinoside (ara-U) and its levels are lower in DS-myeloblasts than non-DS myeloblasts. GATA-1 binding sites in the *CDA* promoter suggest the potential role of GATA-1 in regulating *CDA* transcription.⁸⁶

Blast cells from DS patients are also significantly more sensitive to daunorubicin, melphalan, mitoxantrone, 4-hydroperoxy-cyclophosphamide, vincristine, etoposide, bleomycin, and pirarubicin than those from non-DS patients in MTT assays.⁸⁷ Low levels of bone marrow stromal-cell antigen 2 (*BST2*) in DS megakaryoblasts may lead to decreased interaction of leukemia cells with bone marrow stroma, a mechanism of protection from chemotherapy-induced apoptosis. This may be explained by decreased stimulation of *BST2* promoter activity by GATA-1s compared with the full-length protein.⁵⁶ DS-AMKL and good prognosis non-DS AMKL blasts demonstrate high expression of CD36, the thrombospondin receptor. CD36 plays a role in fatty acid transport and may exacerbate drug-triggered apoptosis by intracellular lipid accumulation in AMKL.⁸⁸ *RUNX1* expression is lower in DS megakaryoblasts compared with non-DS megakaryoblasts.⁵⁷ This suggests that *RUNX1* may play a role in chemotherapy resistance and contribute to the poor outcomes in non DS-AMKL. Inhibition of *RUNX1* may further chemosensitize leukemia cells by inhibition of the PI3 kinase survival pathway.⁸⁹

CONCLUSIONS

It is clear that myeloid/megakaryocytic leukemia in DS is the result of a series of genetic events therefore representing a useful model to understand the role of the chromosome 21 in leukemia in general. A trisomic background results in oxidative stress and altered folate metabolism predisposing to the acquisition of *GATA1* mutations, which then allow for the development of TMD. The discovery that mutated GATA-1 is unable to suppress E2F transcription in fetal liver cells may explain the cellular origin of TMD. Research to identify dosage-sensitive genes (or regulators) on chromosome 21 that contribute to megakaryocyte proliferation, implicate the ETS proteins *ERG* and *ETS2*. Recently, overactive IGF signaling and overexpression of miR-125b-2, which allow for dis-inhibition of tumor suppressor genes, have also been highlighted. Subsequent clonal selection and evolution to AMKL requires additional insults, including putative cooperating mutations in *JAK3*, *FLT3*, *MPL* or *TP53*. The multi-step progression to AMKL provides insight into the steps by which normal HSC/progenitors are transformed into leukemic cells. Moreover this is an excellent disease model to understand cell type-specific signaling pathways and their intersection with oncogenes during malignant transformation.

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Abbreviations

DS	Down syndrome
AMKL	acute megakaryoblastic leukemia
AML	acute myeloid leukemia
ML-DS	myeloid leukemia Down syndrome

DMR	differentially methylated region
TMD	transient myeloproliferative disorder
TL	transient leukemia
CFU-GM	colony forming unit, granulocyte macrophage
BFU-E	burst forming unit erythroid
NOD/SCID	non-obese diabetic/severe combined immunodeficient
FL	fetal liver
ESC	ES cells
Hsa21	human chromosome 21
siRNA	small interfering RNA
MPD	myeloproliferative disorder
SNP	single nucleotide polymorphism
HSC	hematopoietic stem cell
IGF-2	insulin-like growth factor 2
SOD	superoxide dismutase
CBS	cystathionine beta synthase
EFS	event free survival
OS	overall survival
Ara-C	cytarabine
MRD	minimal residual disease
ara-U	arabinoside
CDA	cytidine deaminase
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
BST2	bone marrow stromal-cell antigen 2

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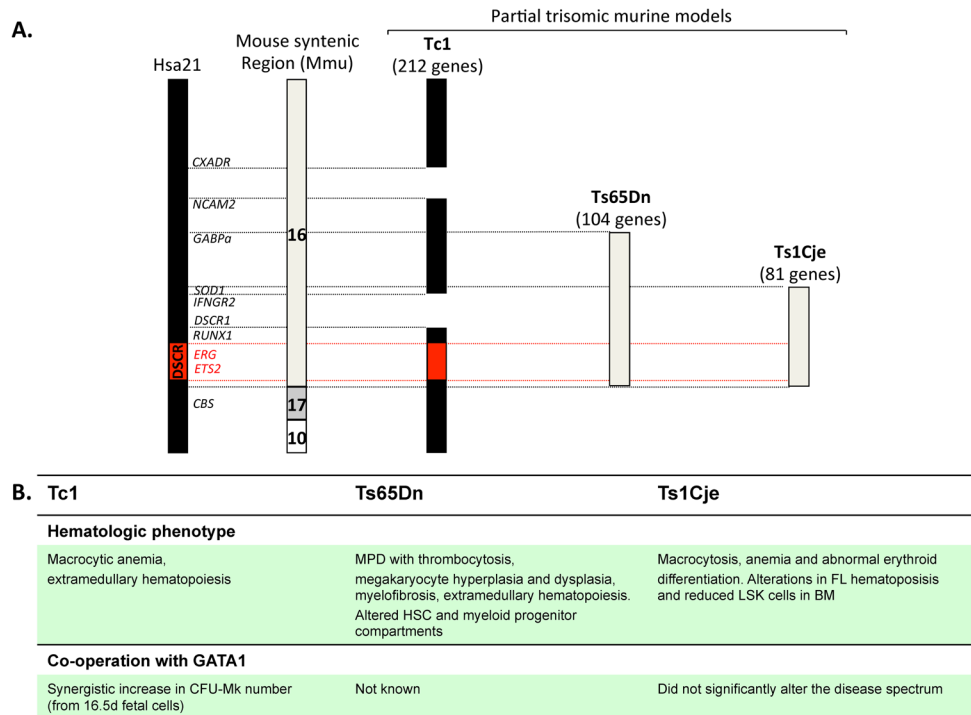


Figure 1. Diagram of Hsa21 and the regions of trisomy in the various mouse models of DS
 A) Human chromosome 21 and specific genes in the DS critical region (DSCR) that may contribute to the development of leukemia are shown. The syntenic murine Mmu16 with varying degrees of trisomic representation in the different mouse models is depicted on the right. B) Summary of the hematopoietic phenotype of the murine models and the effect of coexpression of GATA-1s.

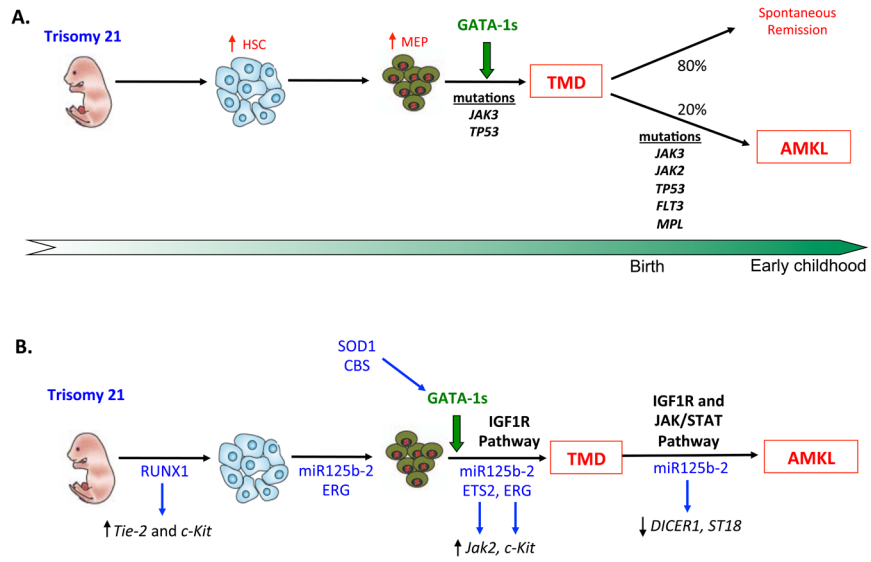


Figure 2. Multi-step model of leukemogenesis in Down syndrome

A) Sequential acquisition of known genetic abnormalities and their role in the evolution of DS-AMKL. B) Aberrant signaling pathways implicated in the pathogenesis of DS-AMKL. The chromosome 21 specific genes that appear to have a functional impact in these pathways are highlighted in blue.