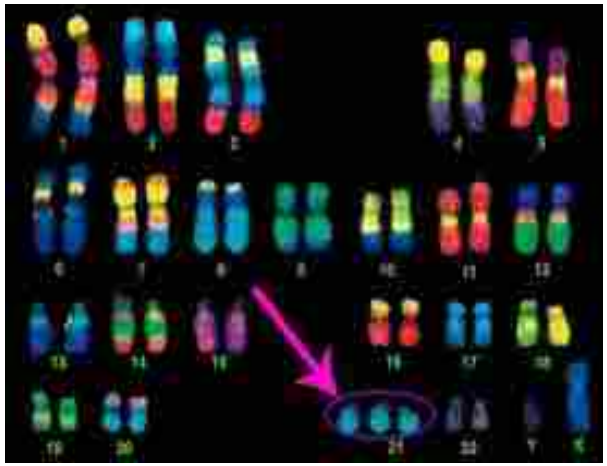
Too much and too little: how does an extra chromosome cause leukaemia in children with Down syndrome?

Sir Thomas and Lady Edith Dixon Lecture, April 2021



Professor Irene Roberts

Professor of Paediatric Haematology, University of Oxford

Weatherall Institute of Molecular Medicine

MRC Medical Research Council

University of Oxford

Department of Paediatrics

Increased susceptibility to leukaemia in Down syndrome (DS)

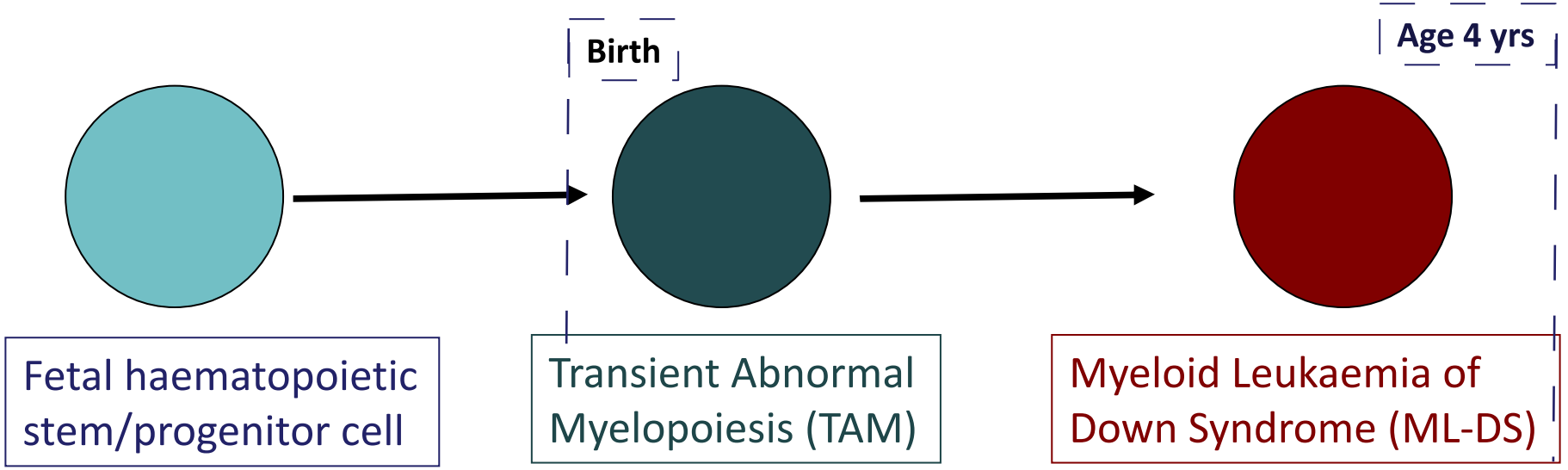
Type of malignancy	Standardised incidence ratio
Acute myeloid leukaemia age 0-4 yrs age 5-29 yrs	 153.9 10.3
Acute lymphoblastic leukaemia age 1-4 yrs age 5-29 yrs	 27.0 12.4
Solid tumours	0.45

* Both myeloid and lymphoid leukaemias are more common
* Young children are especially susceptible
* Solid tumours are less frequent

Hasle et al, Genet Med 2016, Hasle et al, Lancet, 2000, Browne et al, 2018

Myeloid Leukaemia of Down Syndrome (ML-DS)

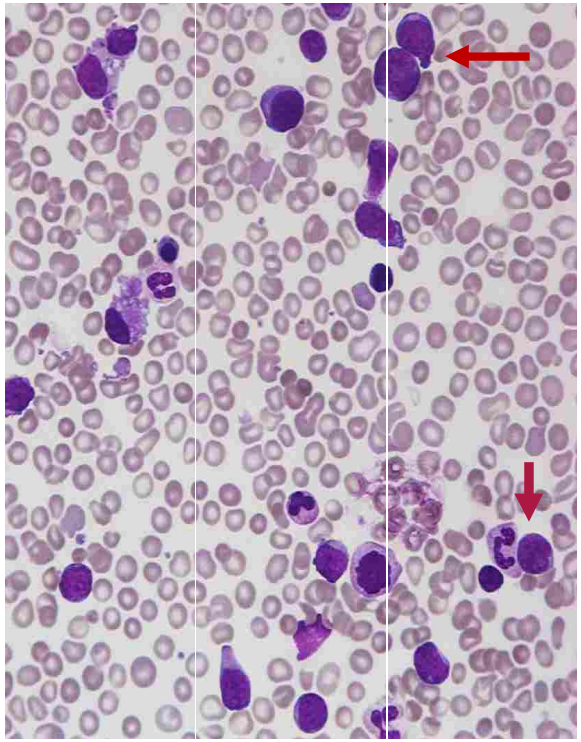
- ML-DS originates in fetal life and presents before the age of 4 yrs
- Preceded by a neonatal leukaemia unique to DS known as Transient Abnormal Myelopoiesis (TAM)



Zipursky et al, 1987

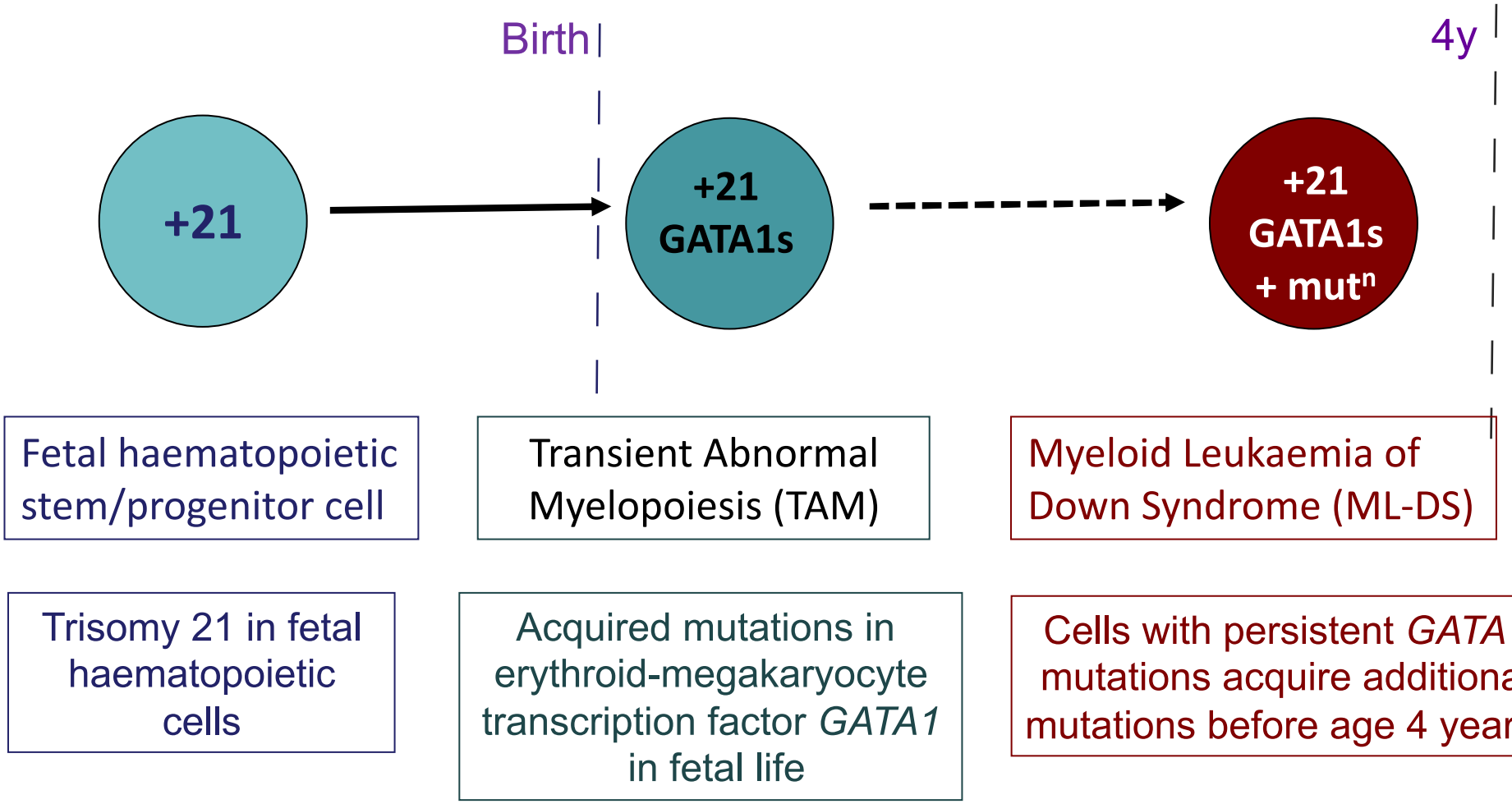
Transient Abnormal Myelopoiesis (TAM)

- Clonal haematological disorder characterised by increased circulating blast cells in a baby with Down syndrome
- Unique to babies with trisomy 21
- Usually presents in the first few days of life; sometimes in fetal life, never after 3 months of age



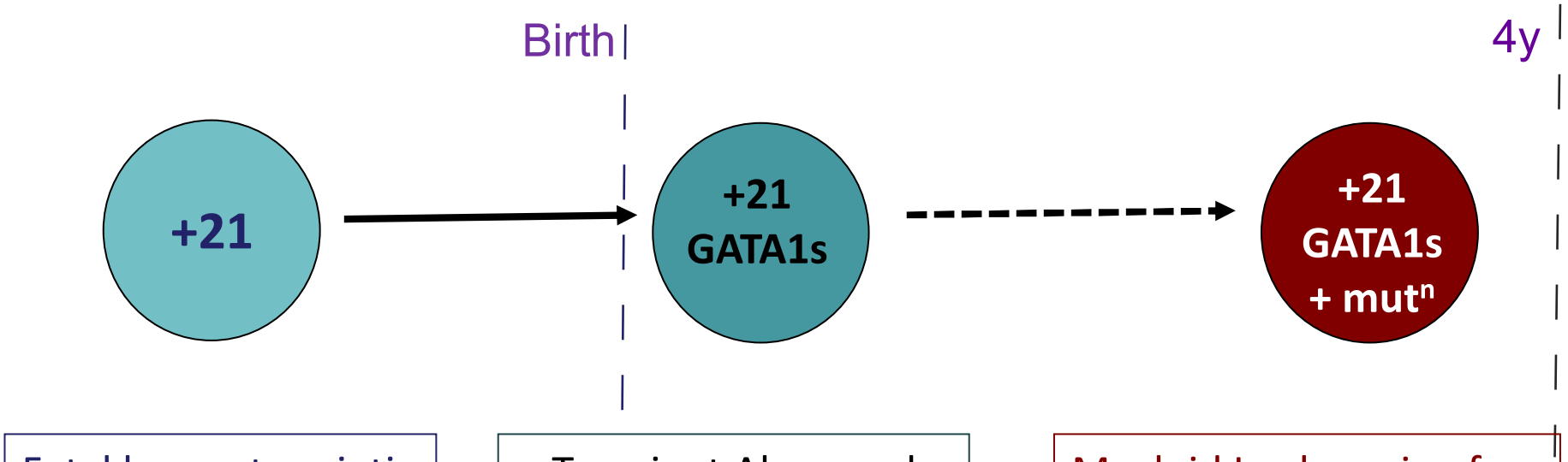
TAM is a fetal disorder strongly linked to T21

Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome



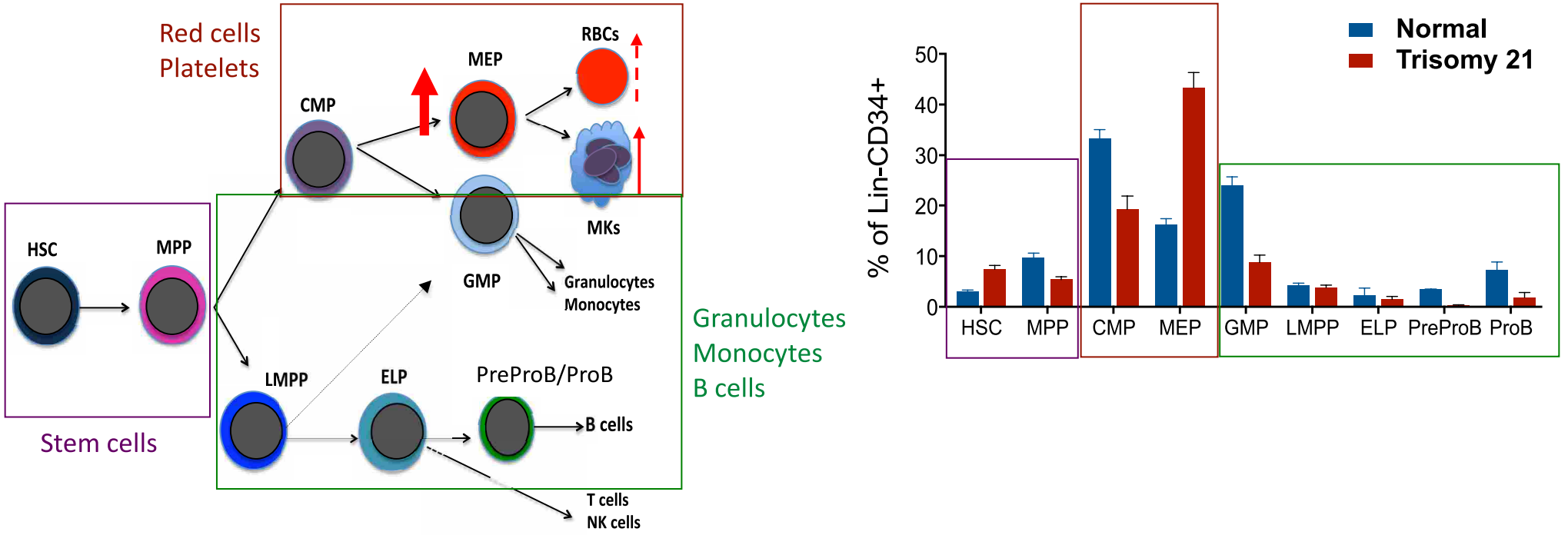
Wechsler et al, 2002; Mundschau et al, 2003; Rainis et al, 2003; Ahmed et al, 2004; Alford et al, 2011; Labuhn et al, 2019

Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome



How does trisomy 21 affect fetal and neonatal blood cells?

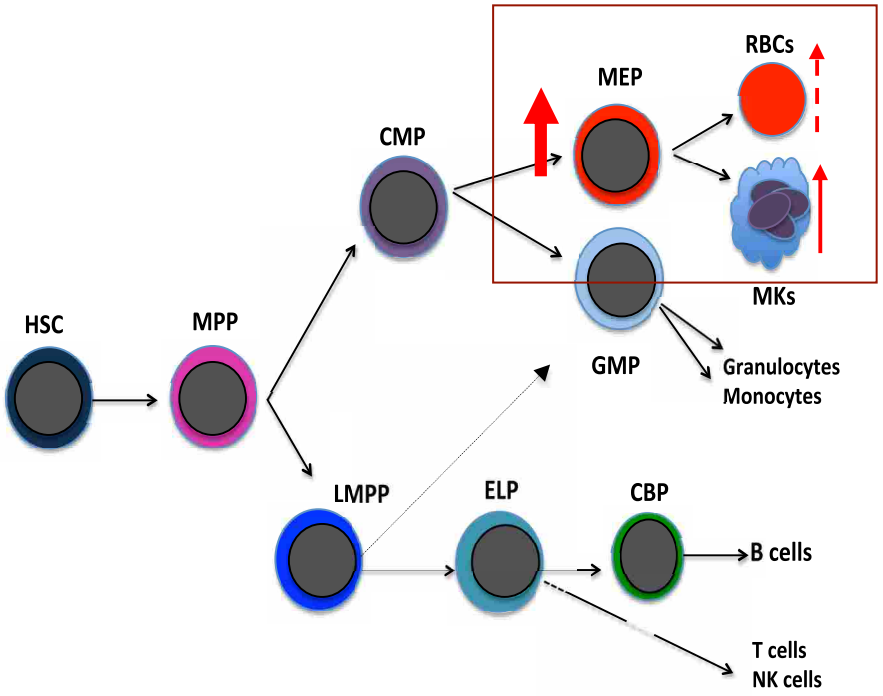
Trisomy 21 perturbs fetal haematopoiesis in the absence of acquired *GATA1* mutations

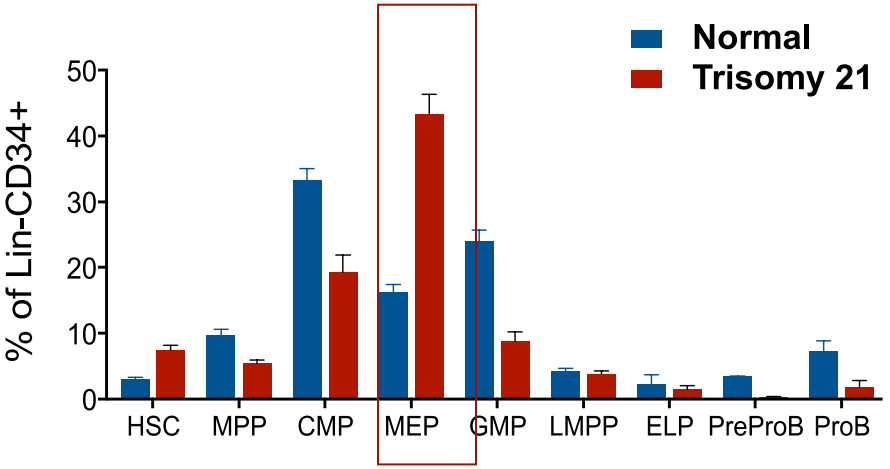


Trisomy 21 causes multiple changes in fetal haematopoietic stem cells and blood progenitors of all lineages

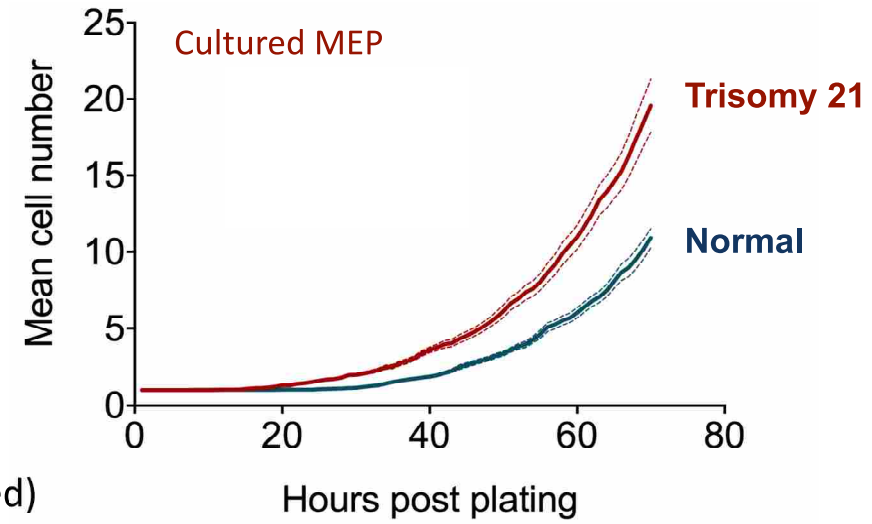
Tunstall, O *et al.* 2008; Roy, A. *et al.* 2012; Natalina Elliott (unpublished)

Trisomy 21 perturbs fetal haematopoiesis in the absence of acquired *GATA1* mutations





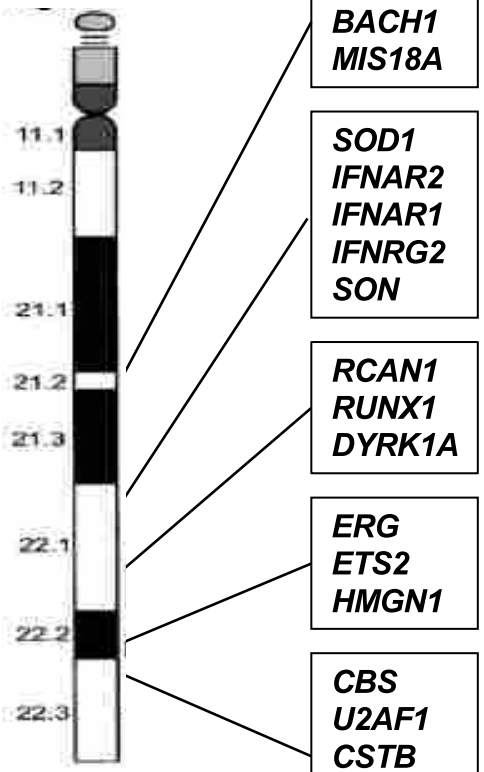
Fetal Megakaryocyte-Erythroid Progenitor (MEP) cells are increased in frequency and highly proliferative in trisomy 21



Tunstall, O *et al.* 2008; Roy, A. *et al.* 2012; Natalina Elliott (unpublished)

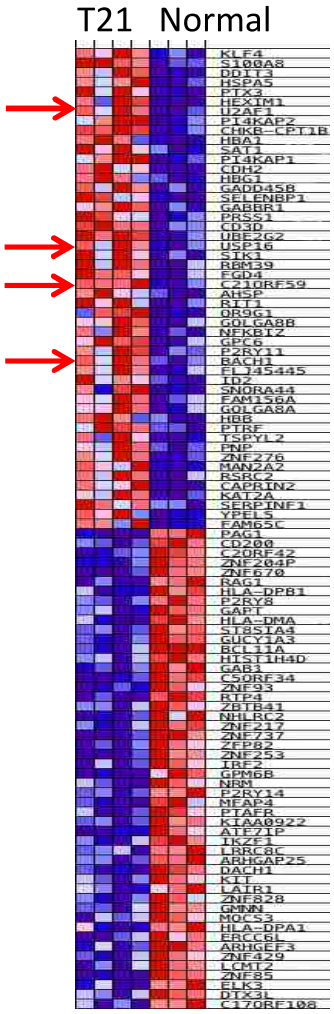
Trisomy 21 causes genome-wide perturbation of gene expression in fetal haematopoietic stem and progenitor cells

Chromosome 21



~240 protein coding genes

Gene expression profiling of fetal CD34+ cells

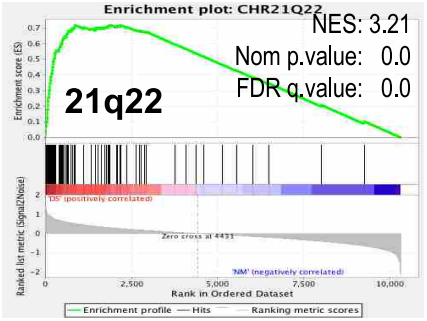


Liu et al, 2015

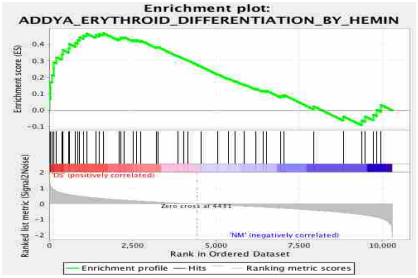
833 differentially expressed genes

34 on Hsa21

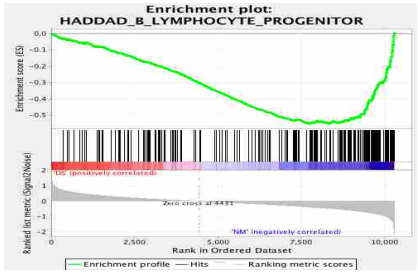
799 not on Hsa21



Enrichment of 21q22 gene set

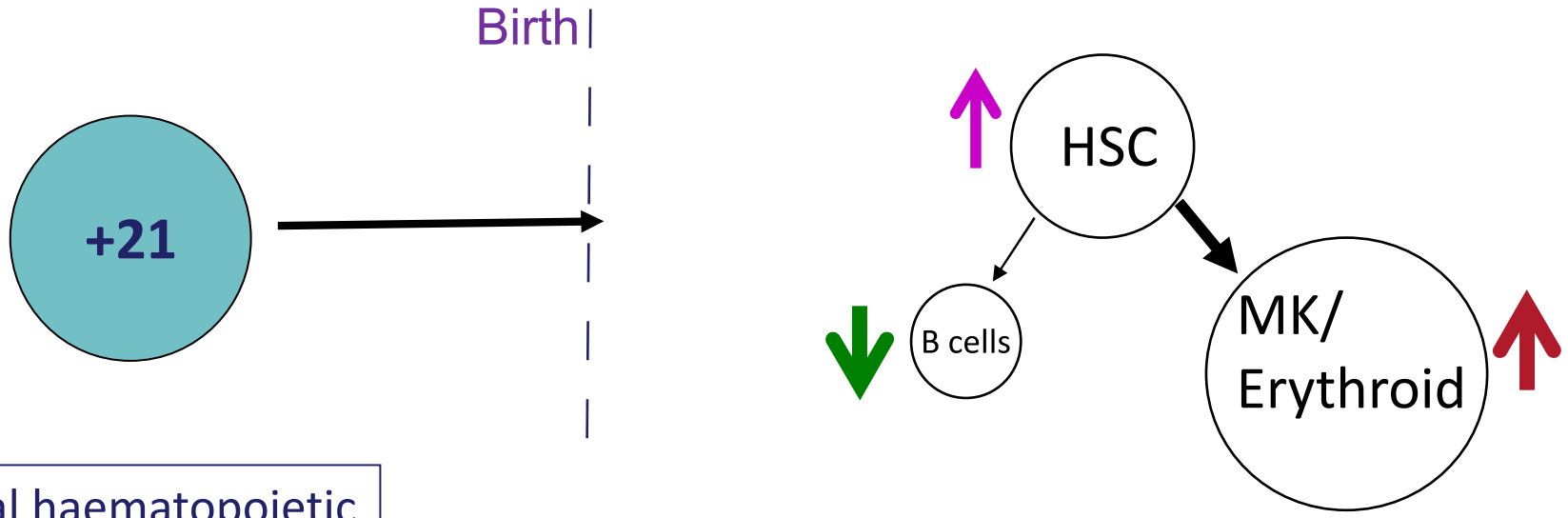


Increased expression of erythroid genes



Reduced expression of B-lymphoid genes

Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome



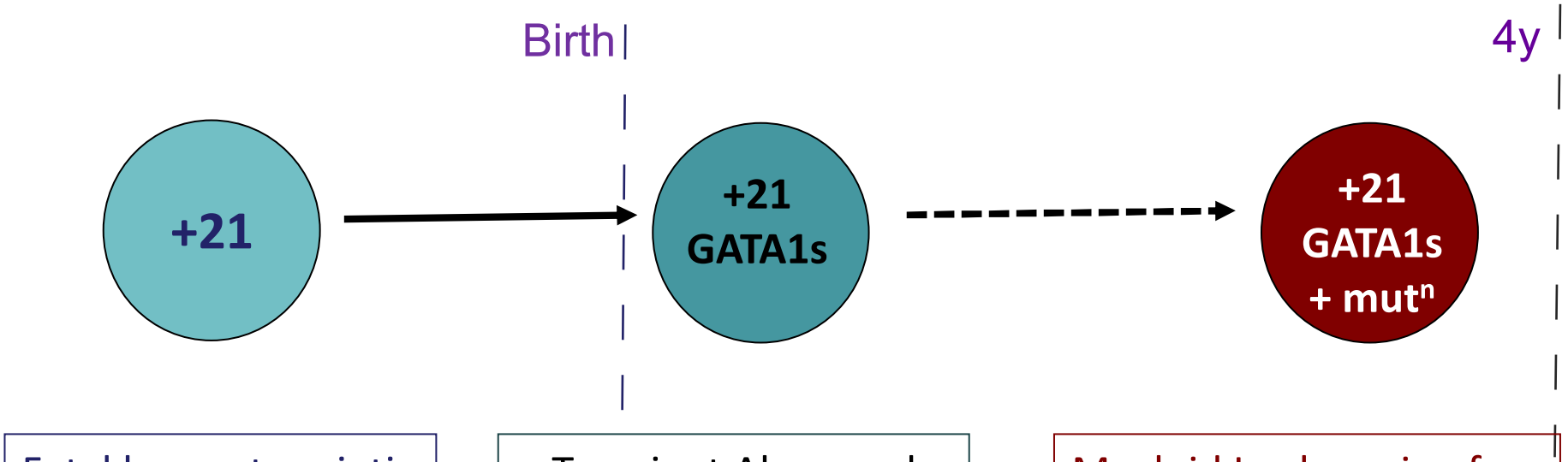
Fetal haematopoietic stem/progenitor cell

How does trisomy 21 affect fetal and neonatal blood cells?

Trisomy 21 causes an expanded proliferating HSC/myeloid progenitor pool with MK/ erythroid bias due to complex genome-wide changes in gene expression, most likely controlled by epigenetic mechanisms which allow survival of trisomic cells

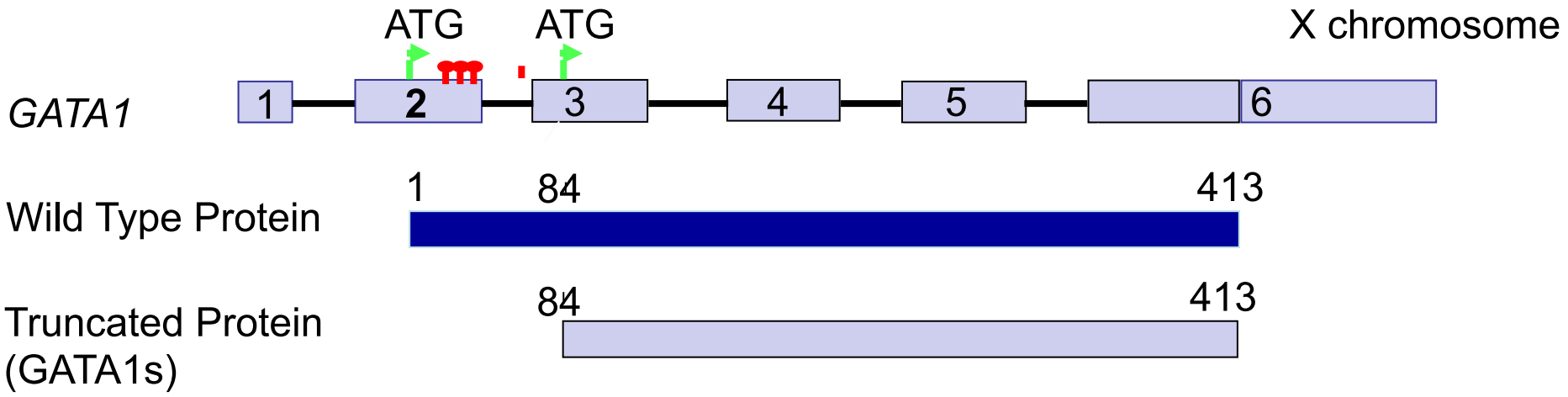
Muskens et al, 2021

Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome



Acquired N-terminal mutations in the *GATA1* gene uniquely transform fetal cells with trisomy 21 (Down syndrome)

- TAM is caused by acquired mutations in the N terminal of the *GATA1* gene
- *GATA1* is a transcription factor that controls red cell & platelet/megakaryocyte development
- Mutations cluster in exon 2 and result in the translation of a short protein, Gata1s
- N terminal *GATA1* mutations are not leukaemogenic in the absence of trisomy 21
- *GATA1* mutations are already present at birth

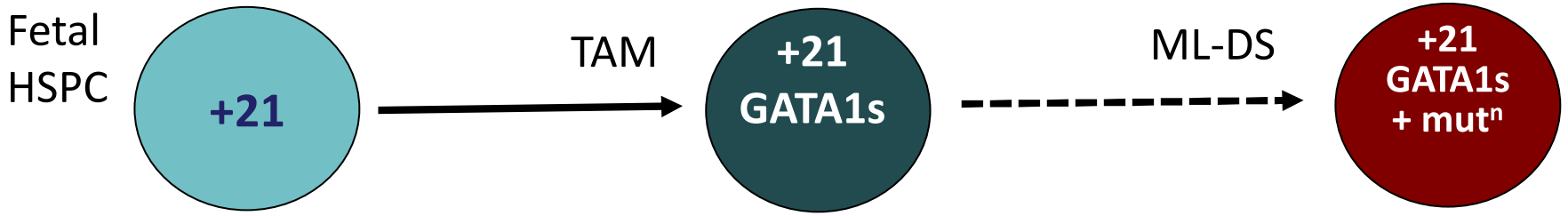


Wechsler et al, 2002; Mundschau et al, 2003; Rainis et al, 2003; Ahmed et al, 2004; Hollanda et al, 2005; Alford et al, 2011

The Oxford Down Syndrome Neonatal Study: Aims

- To determine the frequency of acquired *GATA1* mutations in neonates with Down syndrome (DS)
- To identify the clinical, haematological and molecular features of DS neonates with and without *GATA1* mutations
- To determine the natural history of *GATA1* mutations in DS and the true risk of subsequent leukaemia

Roberts et al, Blood 2013



Oxford Down Syndrome Neonatal Study- design

- Prospective, multicentre study to precisely describe the haematological abnormalities in neonates with Down syndrome
- FBC, film and *GATA1* mutation analysis (PCR, NGS) in first week of life
- Serial samples and follow up until age 4 years
- Definition of TAM: blood blasts >10% and a *GATA1* mutation in a DS neonate

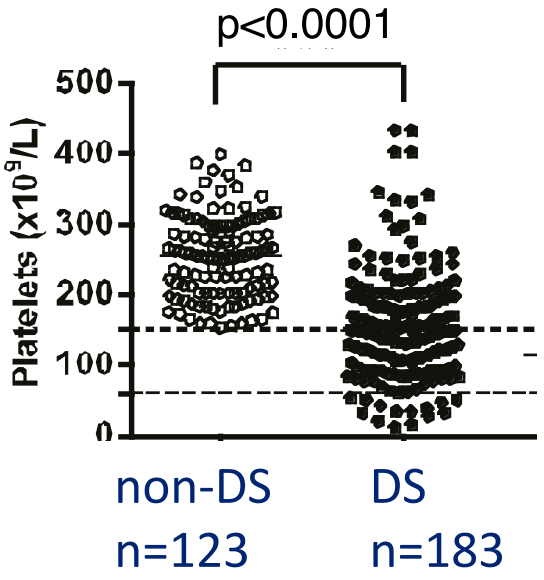





Helen Richmond, Neha Bhatnagar, Laure Nizery, Joanna Bonnici, Natalina Elliott, Paresh Vyas
Kelly Perkins, Marlen Metzner, Alison Kennedy, Gemma Buck

Abnormal platelet production in DS neonates and no *GATA1* mutation

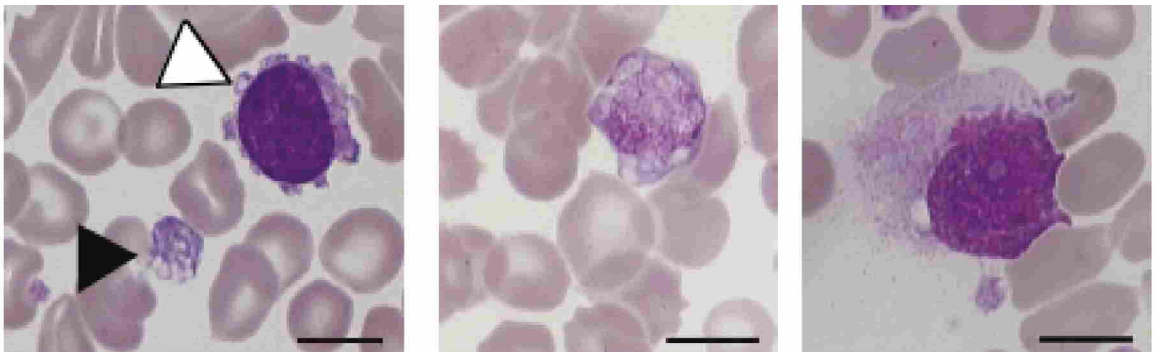
Roberts et al, 2013



Giant platelet and megakaryoblast

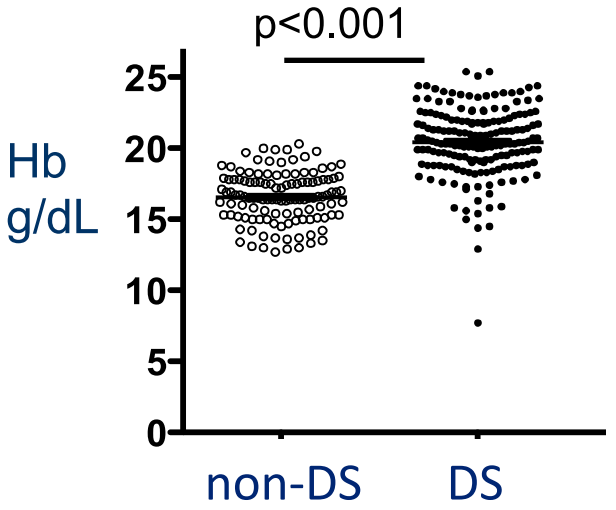
Megakaryocyte fragment

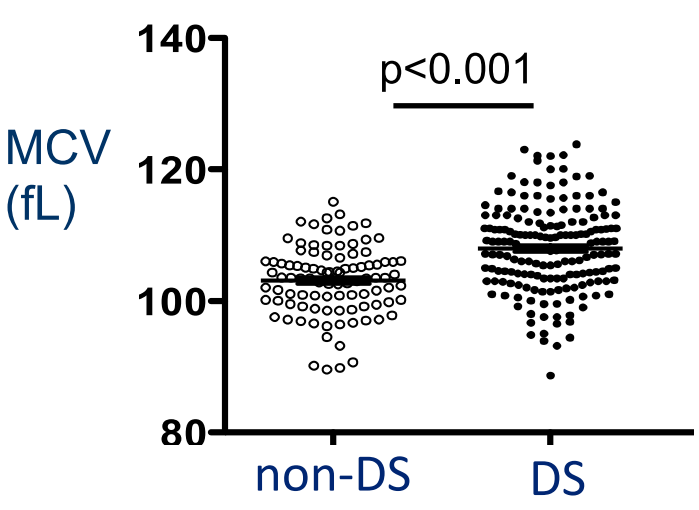
Circulating megakaryocyte

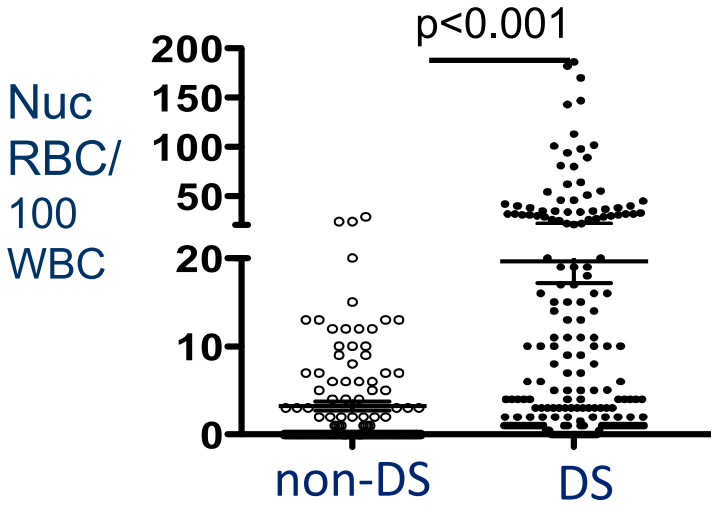


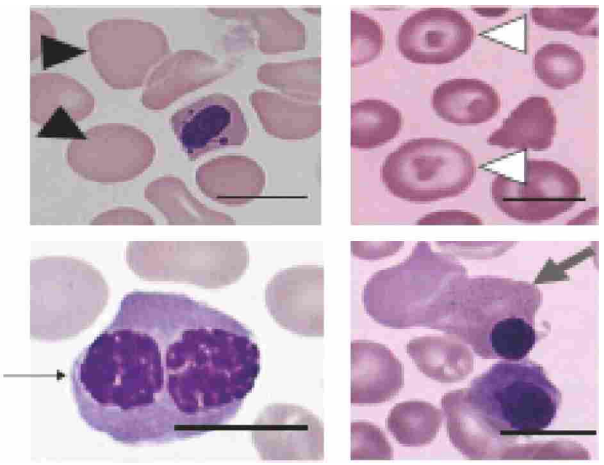
- Thrombocytopenia is common in neonates with Down syndrome
- Trisomy 21 perturbs neonatal megakaryopoiesis and platelet production in the absence of *GATA1* mutations

Abnormal erythropoiesis in DS neonates and no *GATA1* mutations







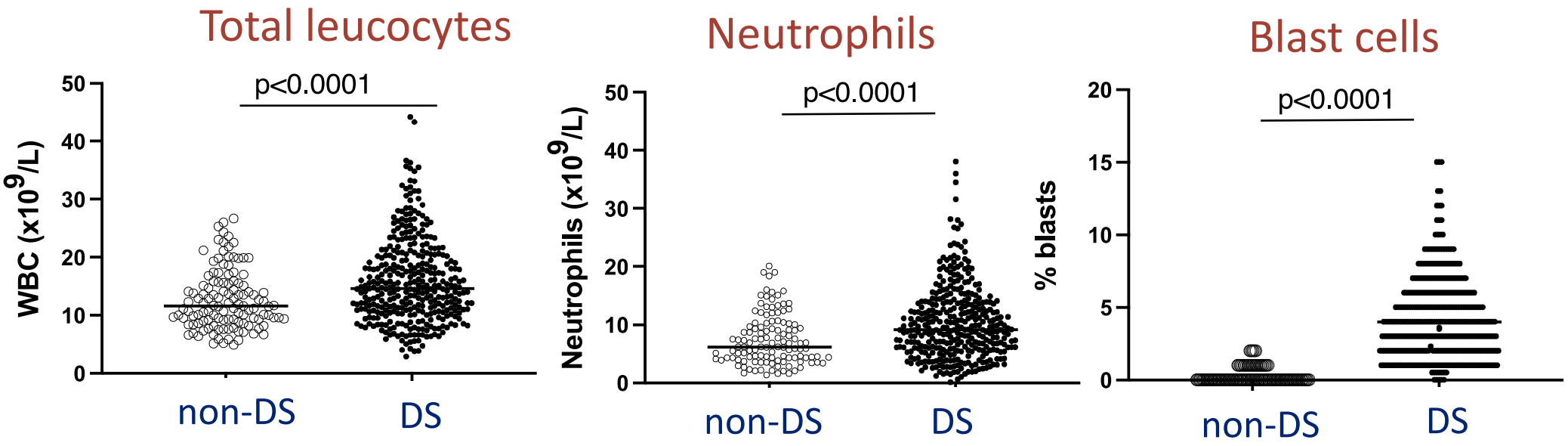


Non-DS: n=123
DS: n=183

Increased red blood cell production and dyserythropoiesis are seen in almost all neonates with Down syndrome suggesting that trisomy 21 itself alters erythropoiesis

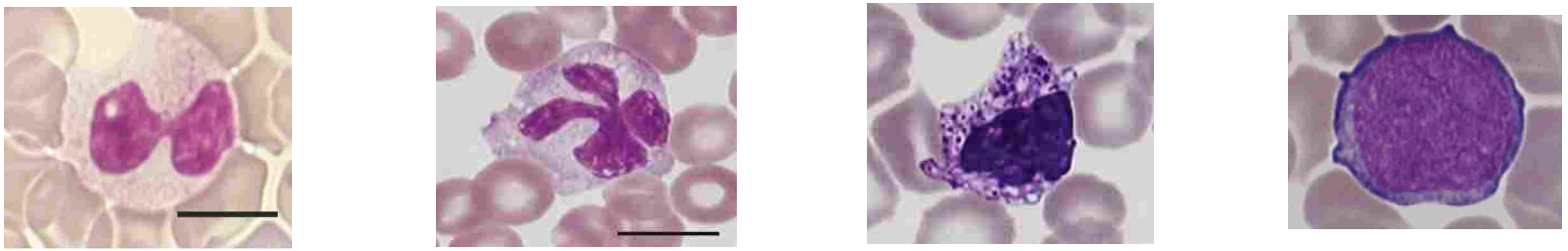
Roberts et al, 2013

Abnormal leucocytes in neonates with DS and no *GATA1* mutation



Non-DS: n=123

DS: n=183

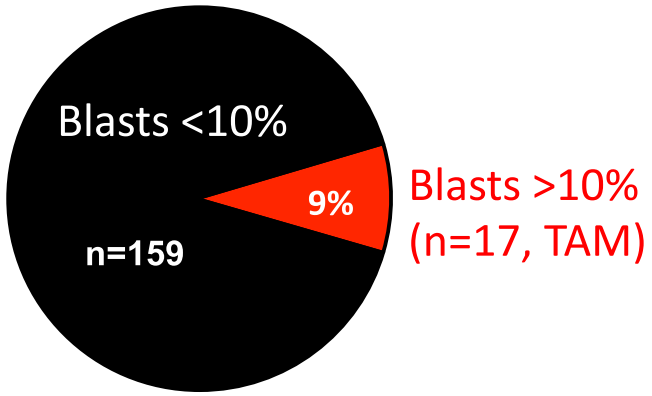


Trisomy 21 causes trilineage perturbation of neonatal haematopoiesis

Roberts et al, 2013

Detection of mutant *GATA1* clones in blood cells from neonates with Down syndrome: sensitive tests reveal a high frequency

Direct sequencing (n=186)

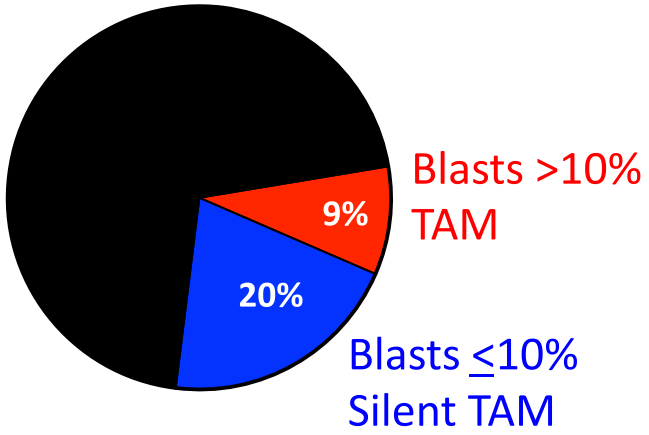


GATA1 mutation (Sanger)

No *GATA1* mutation

GATA1 mutation (NGS)

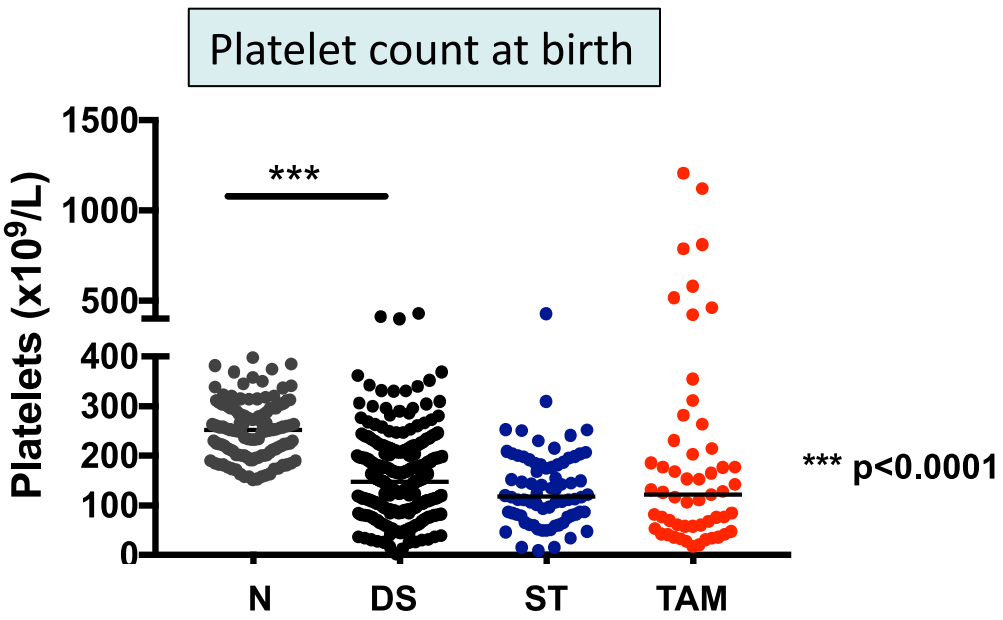
Next Generation Sequencing (NGS)

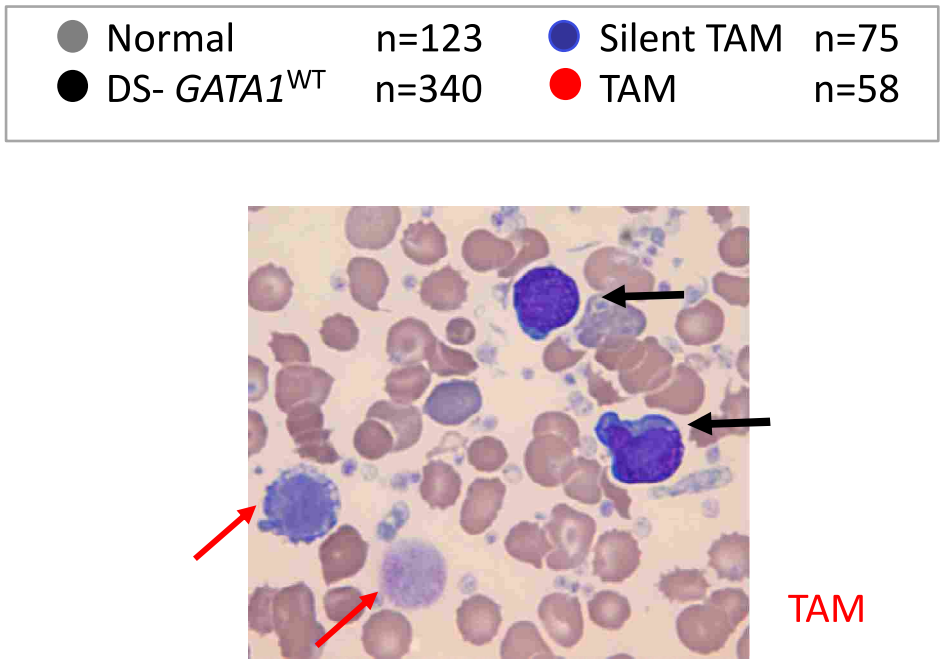


- Using direct sequencing *GATA1* mutations were detected in 9% of neonates with DS, all had blasts >10% and were designated as TAM
- Using NGS, *GATA1* mutations were detected in 29% of DS neonates; most (20% overall) had blasts ≤10% and no clinical signs of TAM- we designated these as Silent TAM

Oxford DS Study, Roberts et al, 2013

Abnormal platelet production in DS neonates: impact of *GATA1s* mutations



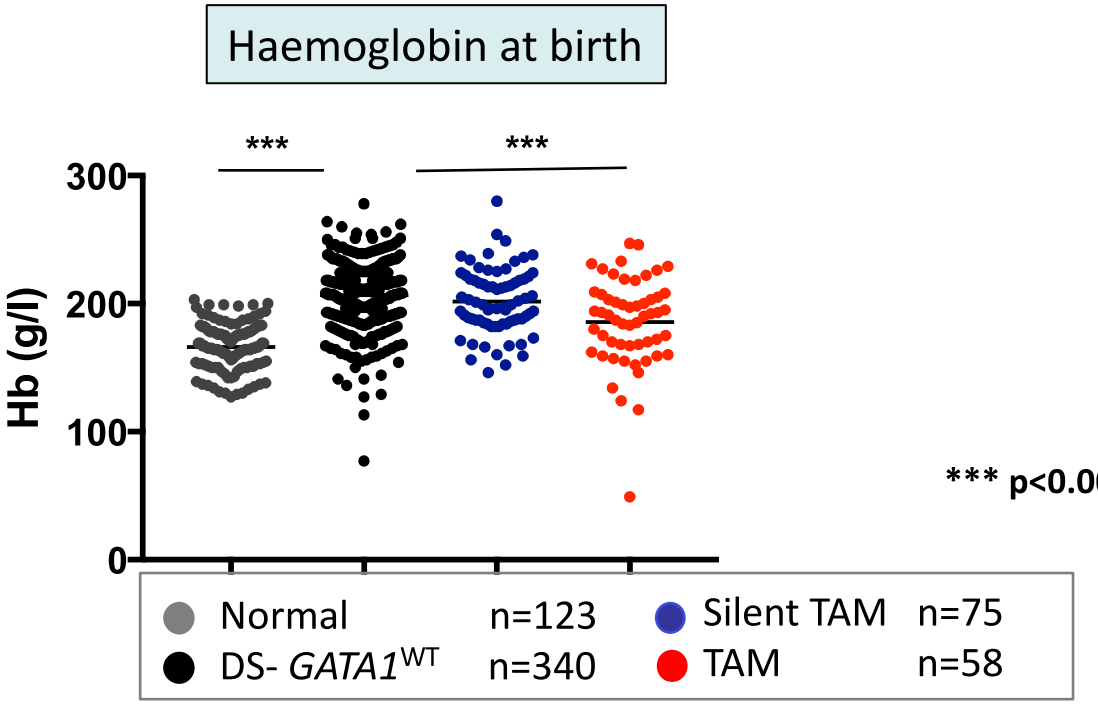


- Trisomy 21 perturbs neonatal platelet production in the absence of *GATA1* mutations
- The platelet count is not further reduced in TAM or Silent TAM but giant platelets and MK fragments are frequent suggesting Gata1s increases T21-associated dysmegakaryopoiesis

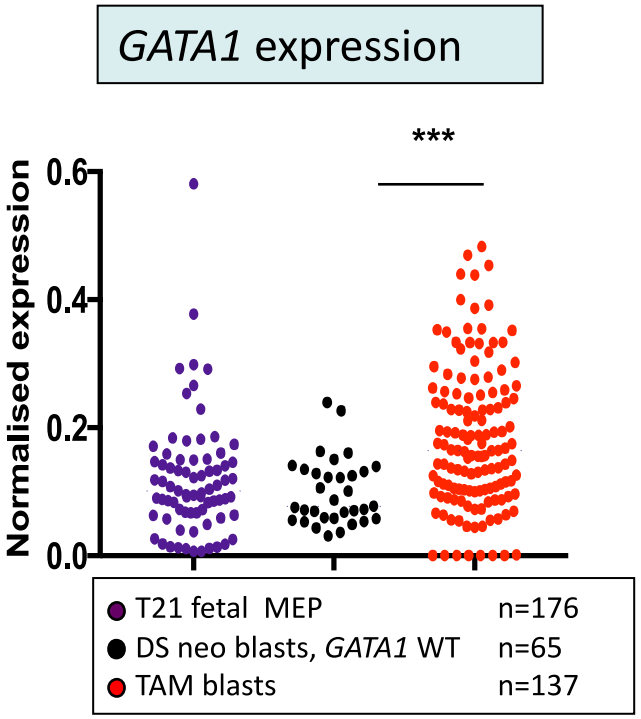
Oxford DS Study

Neha Bhatnagar, Laure Nizery, Amelie Chaussade, Natalina Elliott

Increased erythropoiesis in DS neonates: impact of *GATA1* mutations



*** p<0.0001



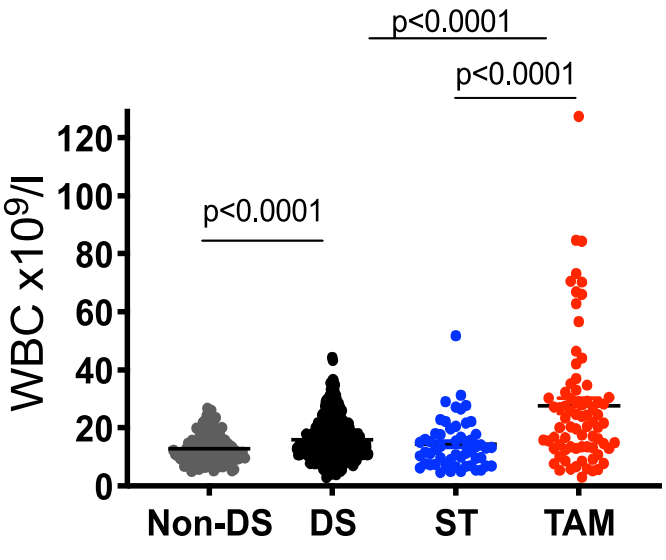
- Trisomy 21 increases neonatal erythropoiesis in the absence of *GATA1* mutations
- Hb is slightly lower in TAM (not silent TAM) but very few babies are anaemic perhaps because *GATA1* expression is sufficient to maintain red blood cell production

Oxford DS Study

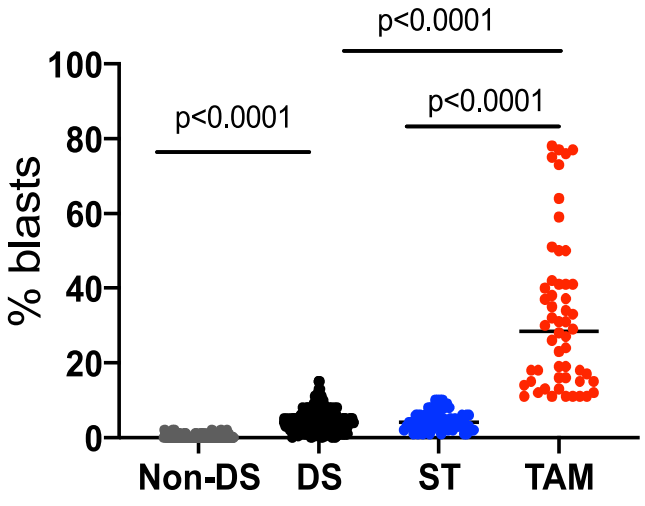
Neha Bhatnagar, Laure Nizery, Amelie Chaussade, Natalina Elliott

Leucocyte production in DS neonates: impact of *GATA1* mutations

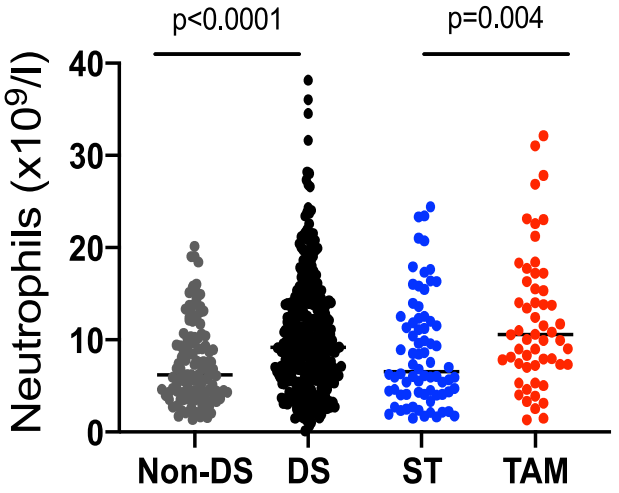
WBC count



Blasts



Neutrophils

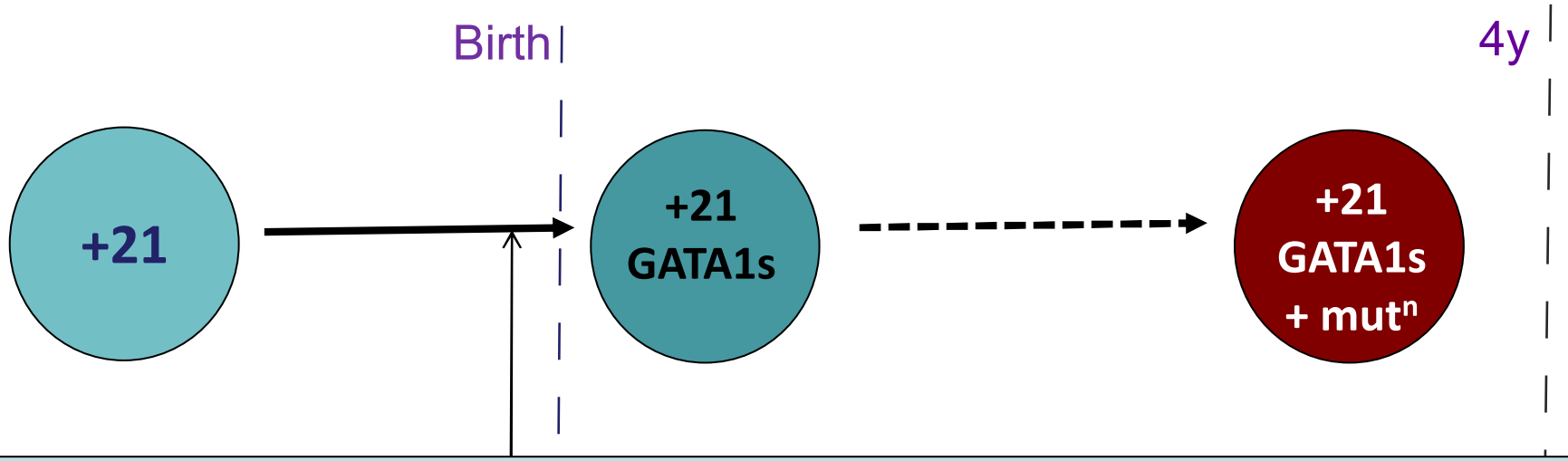


- T21 increases leucocytes, blast cells and neutrophils in the absence of *GATA1* mutations
- Leucocyte and blast cell counts are increased in DS neonates with TAM but not Silent TAM

Oxford DS Study

Neha Bhatnagar, Laure Nizery, Amelie Chaussade, Natalina Elliott

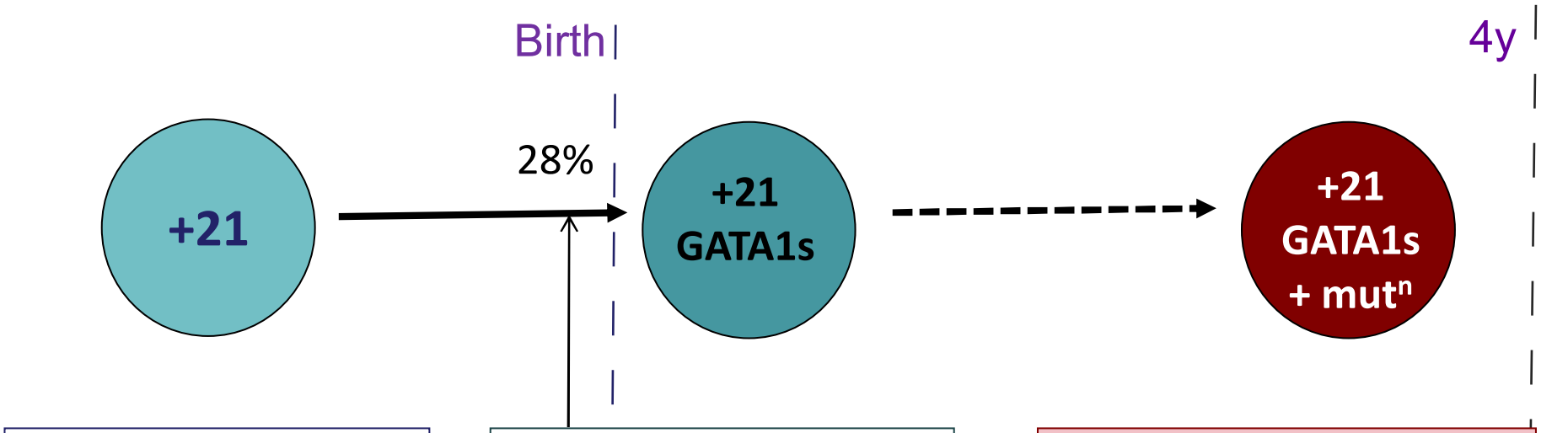
Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome



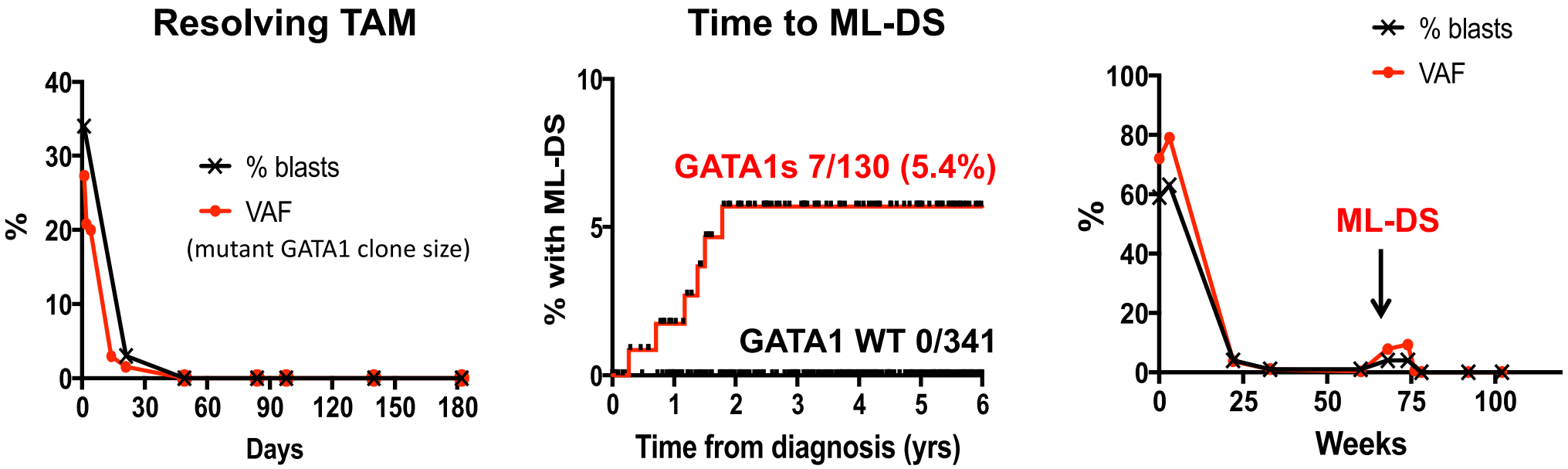
Trisomy 21 and *GATA1* mutations in DS neonates

- Trisomy 21 (T21) causes tri-lineage perturbation of fetal and neonatal haematopoiesis with expansion of a proliferative megakaryocyte-erythroid progenitor (MEP) pool
- *GATA1* mutations occur at high frequency (28%) of DS neonates suggesting that T21-induced expansion of MEPs causes a potent selection advantage for mutant cells
- GATA1s further perturbs haematopoiesis but platelet and red cell production are usually preserved perhaps due to high levels of *GATA1* expression in trisomic cells

Molecular pathogenesis of TAM and ML-DS: 3 steps to leukaemia in children with Down Syndrome

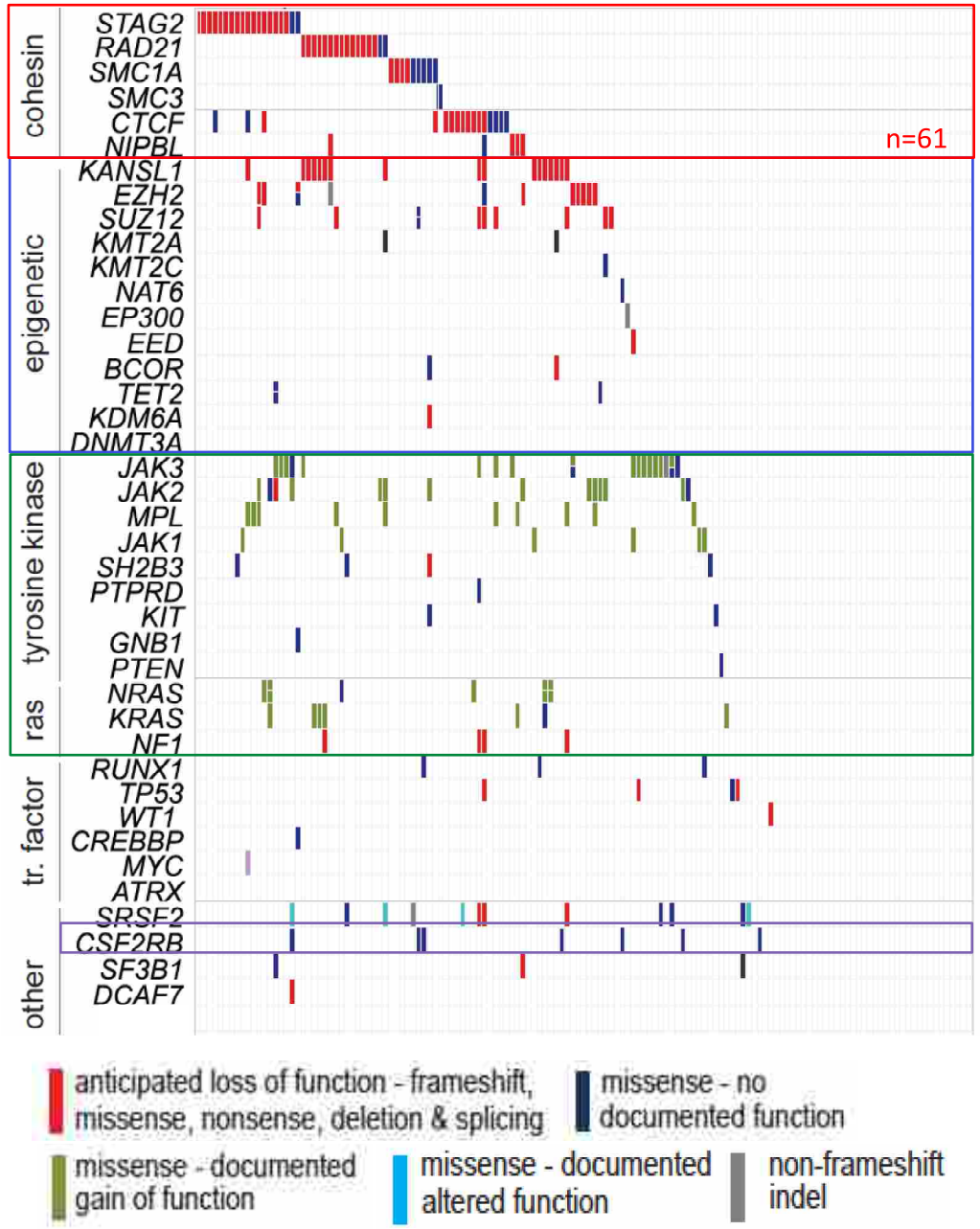


Natural history of DS neonates with acquired *GATA1* mutations in the Oxford DS Study- follow up to age >4 years



- In >90% of DS neonates, *GATA1* mutations and blasts disappear over the first 2-3 months of life
- 0/341 DS neonates without a *GATA1* mutation at birth developed ML-DS or acquired a *GATA1* mutation postnatally
- 7/133 (5.3%) DS neonates with a *GATA1* mutation developed ML-DS at a median age of 16 months

Progression to ML-DS (n=141)



Cohesin and *CTCF* mutations are the most frequent driver mutations acquired after the neonatal period in patients with TAM who develop ML-DS

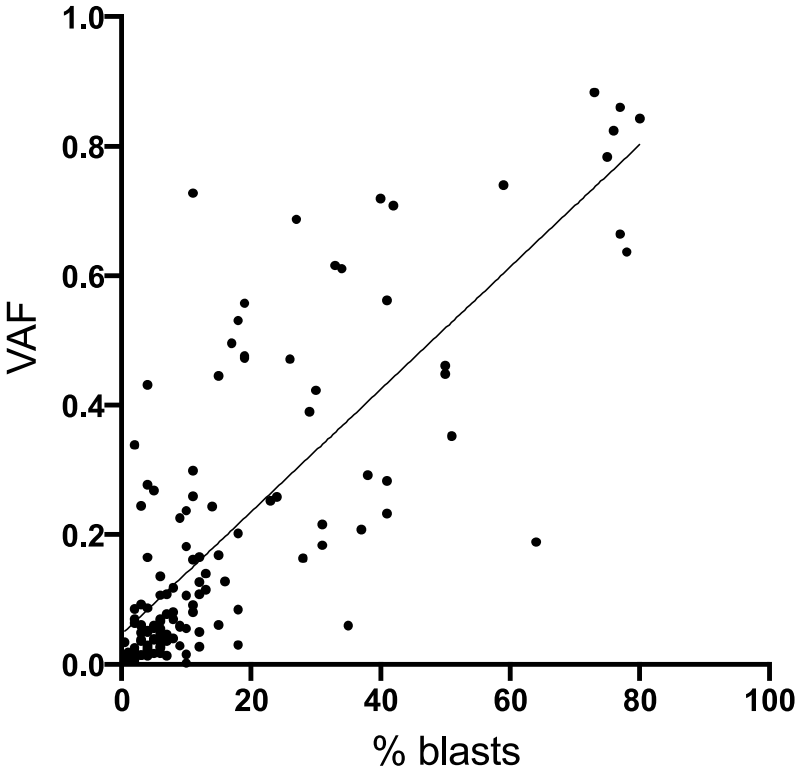
Labuhn, Perkins et al, Cancer Cell, 2019

Yoshida et al, Nat Genet 2013; Nikolaev et al, Blood 2013

What factors predict for leukaemic transformation of TAM?

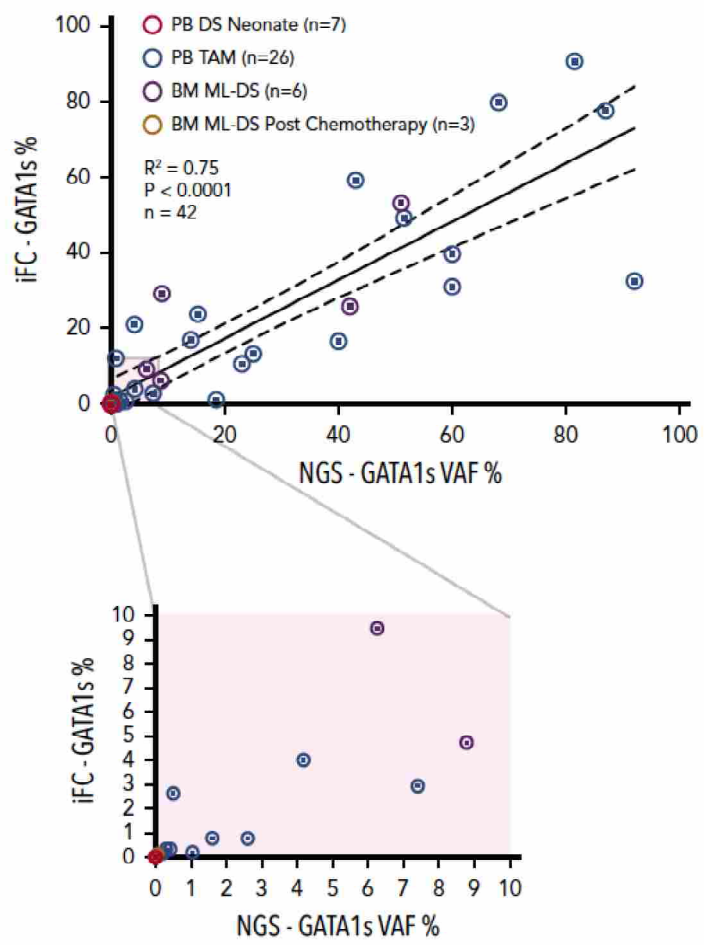
- Size of mutant *GATA1* clone?
- Clinical features?
- Type of *GATA1* mutation?
- Number of *GATA1* mutations?
- Percentage of peripheral blood blasts?

DS neonates with high blast % have larger GATA1s clones (VAF)



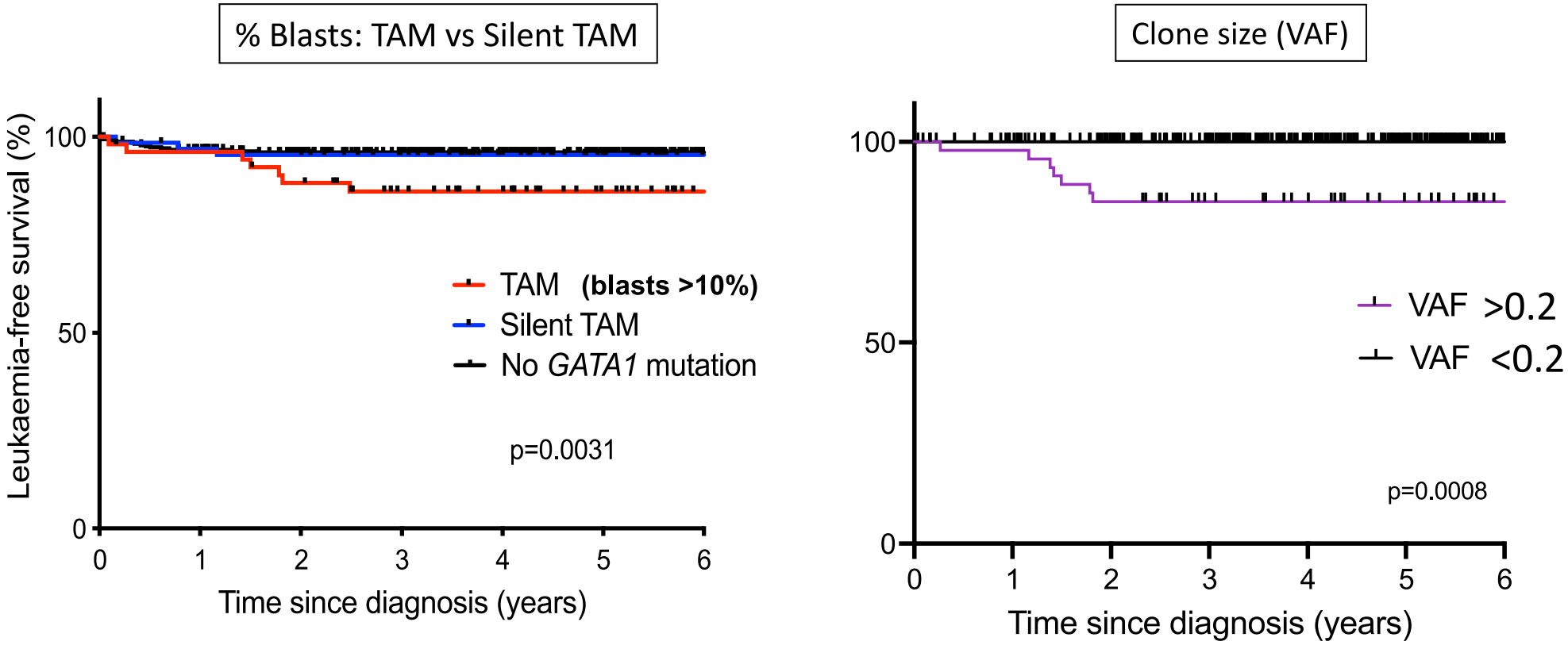
r=0.0794
p<0.0001

Oxford DS Study 2021 (unpublished)



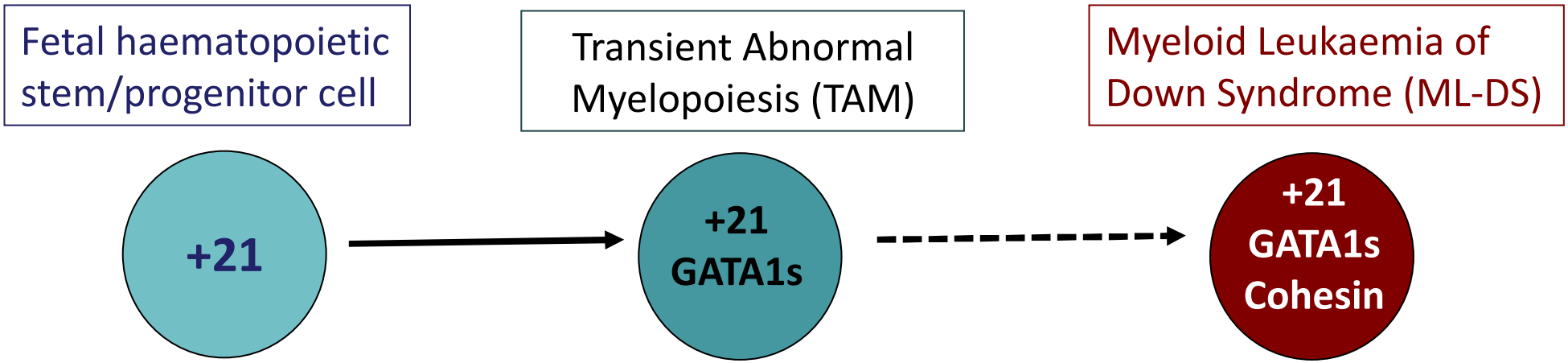
David Cruz Hernandez et al,
Blood 2020

Only the size of the mutant GATA1s clone (and % blasts) predict for transformation to ML-DS



Oxford DS Study 2021 (unpublished)

Summary



- Acquired N-terminal truncating mutations in *GATA1* occur at very high frequency in newborns with Down syndrome (~28%).
- These mutations are acquired only in fetal cells and usually occur (and/or expand) after 20 weeks gestation.
- Mutant *GATA1* clones are usually small, clinically silent, resolve spontaneously and confer an extremely low risk of ML-DS
- DS neonates with larger mutant *GATA1* clones have a higher chance of acquiring additional mutations (eg in cohesin) and developing ML-DS

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Bloodwise
Beating blood cancer since 1960

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