It Takes Two

Special ID Grand Rounds
April 6, 2017

Dilek Ince, MD
Laura Whitmore, PhD

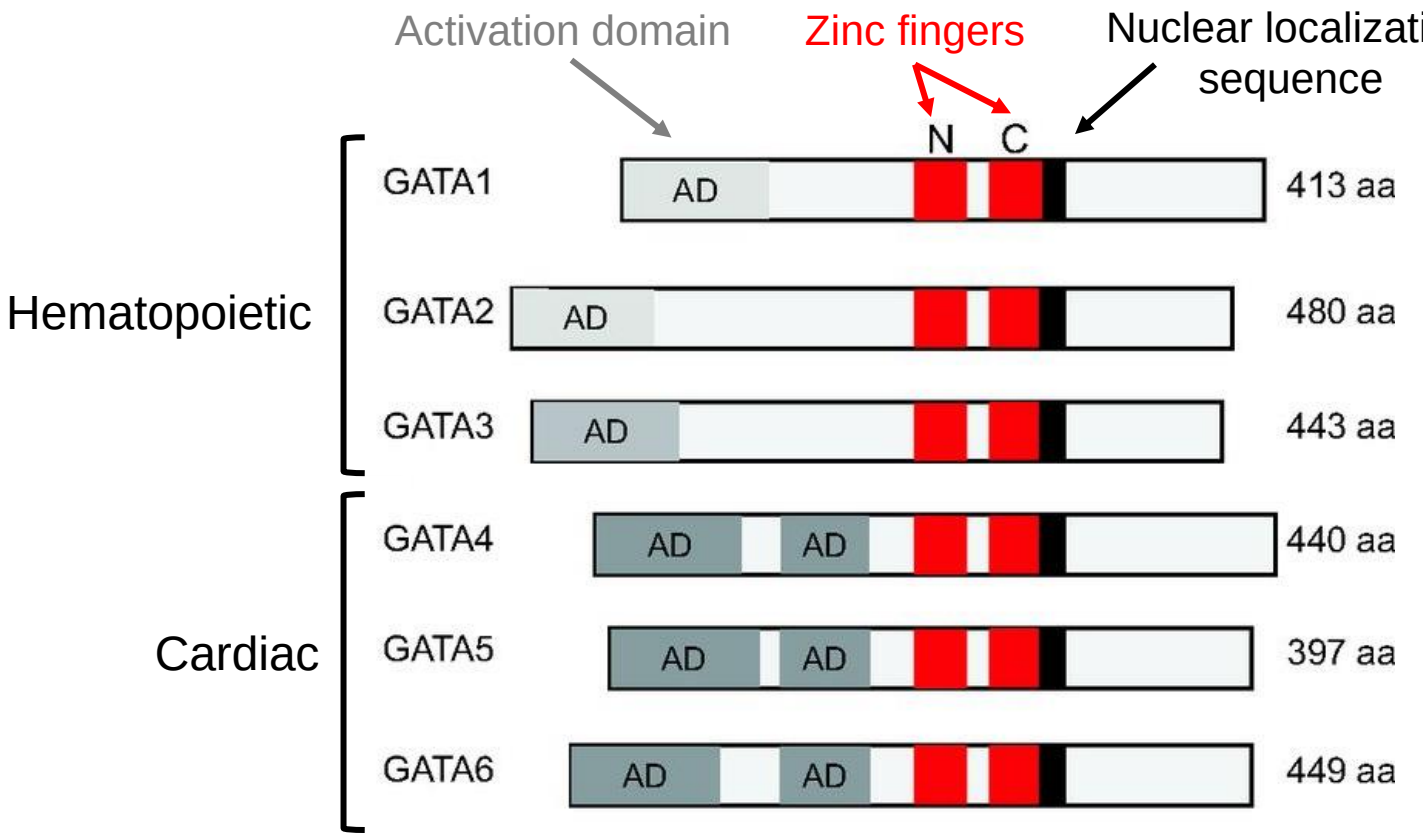
GATA2

1. What is the role of GATA2?

2. Why do *Gata2* mutations make patients prone to certain infections and hematologic malignancies?

GATA transcription factors

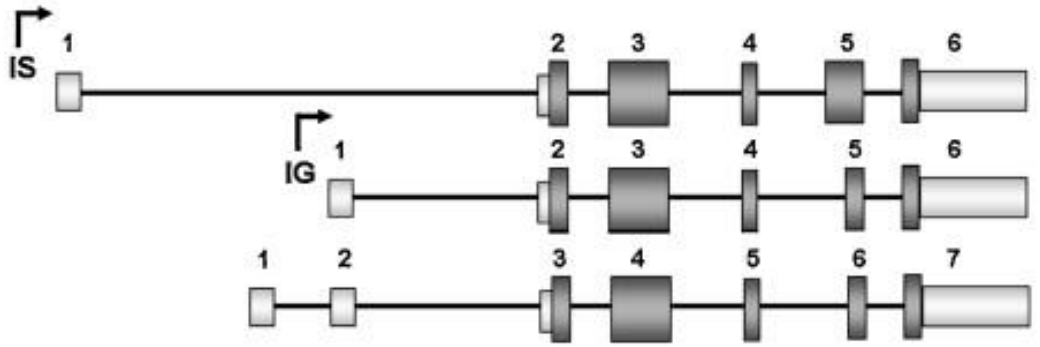
- 6 human GATA transcription factors
- Bind to DNA consensus sequence (A/T)GATA(A/G)
- Mediated through two Cys4 zinc fingers (Cys-X_2-Cys-X_{17}-Cys-X_2-Cys)



Adapted from Chlon & Crispino Development 2012;139:3905-3916

GATA2 structure and expression

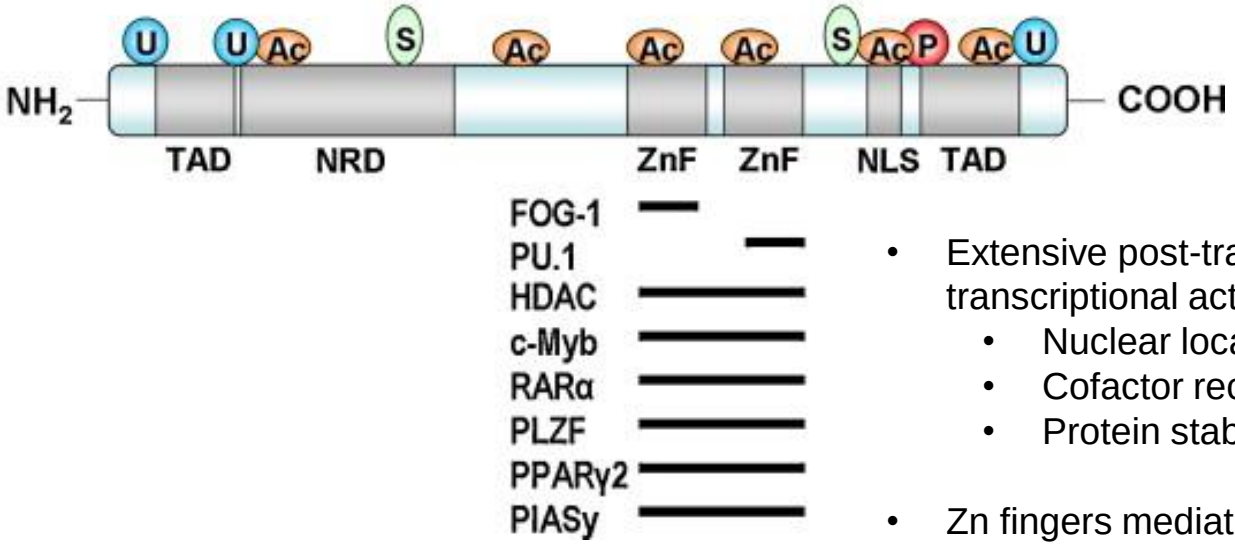
***GATA2* genomic structure**



Multiple transcripts:

- Hematopoietic cells & CNS
- Also in endothelial cells, placenta, fetal liver, and fetal heart
- Unknown (recently reported in NCBI database)

GATA2 protein structure



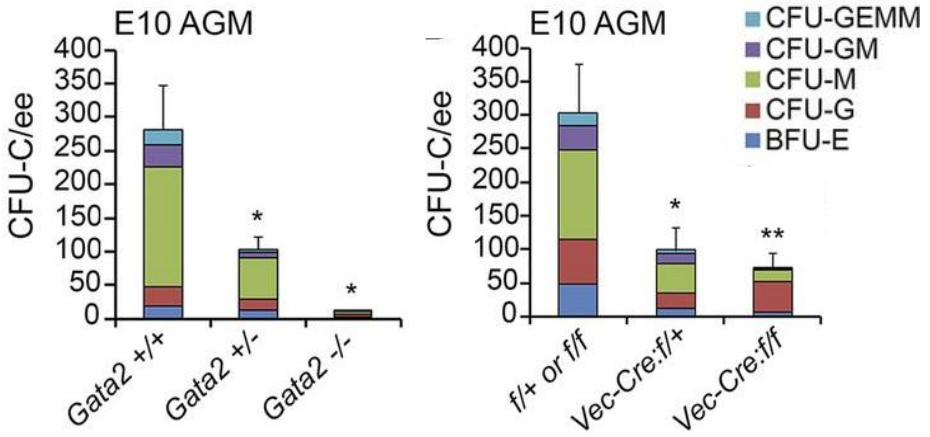
- Extensive post-translational modification alters transcriptional activity
  - Nuclear localization
  - Cofactor recruitment
  - Protein stability
- Zn fingers mediate binding between specific DNA sequences and cofactor complexes

Vicente et al. Critical Reviews in Oncology/Hematology, 2012; 82(1):1–17

GATA2 is required for HSC generation and survival

- GATA2 is pivotal in the endothelial to hematopoietic transition that produces the first adult HSCs
- Homozygous knockout of *Gata2* is lethal due to the failure of hematopoiesis

AGM – aorta-gonad-mesonephros



CFU-GEMM – Granulocyte, erythrocyte, macrophage, megakaryocyte
CFU-GM – Granulocyte, macrophage
CFU-G – Granulocyte
CFU-M – Macrophage
BFU-E – Burst forming unit-erythroid

De Pater et al. J Exp Med 2013;210:2843-2850

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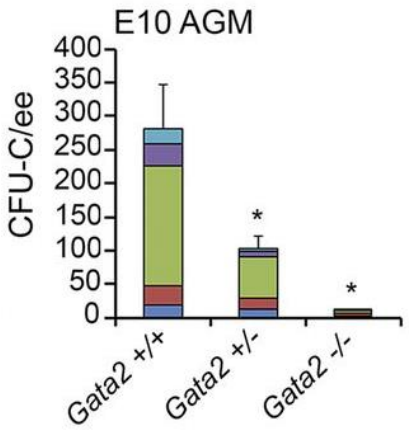
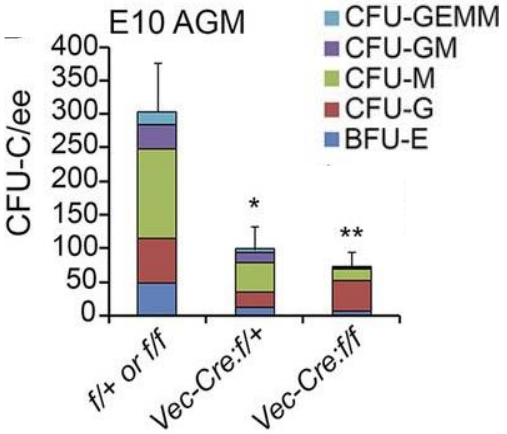
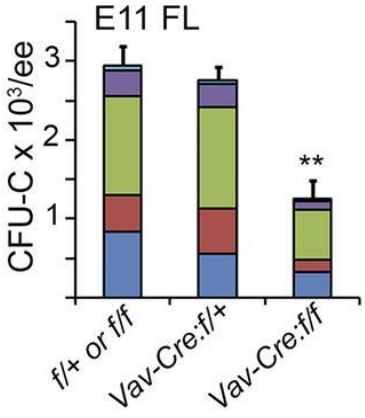
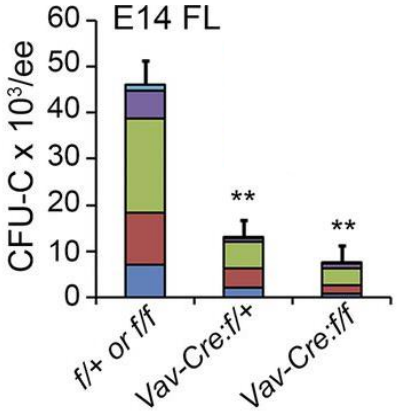
AGM – aorta-gonad-mesonephros
FL – fetal liver





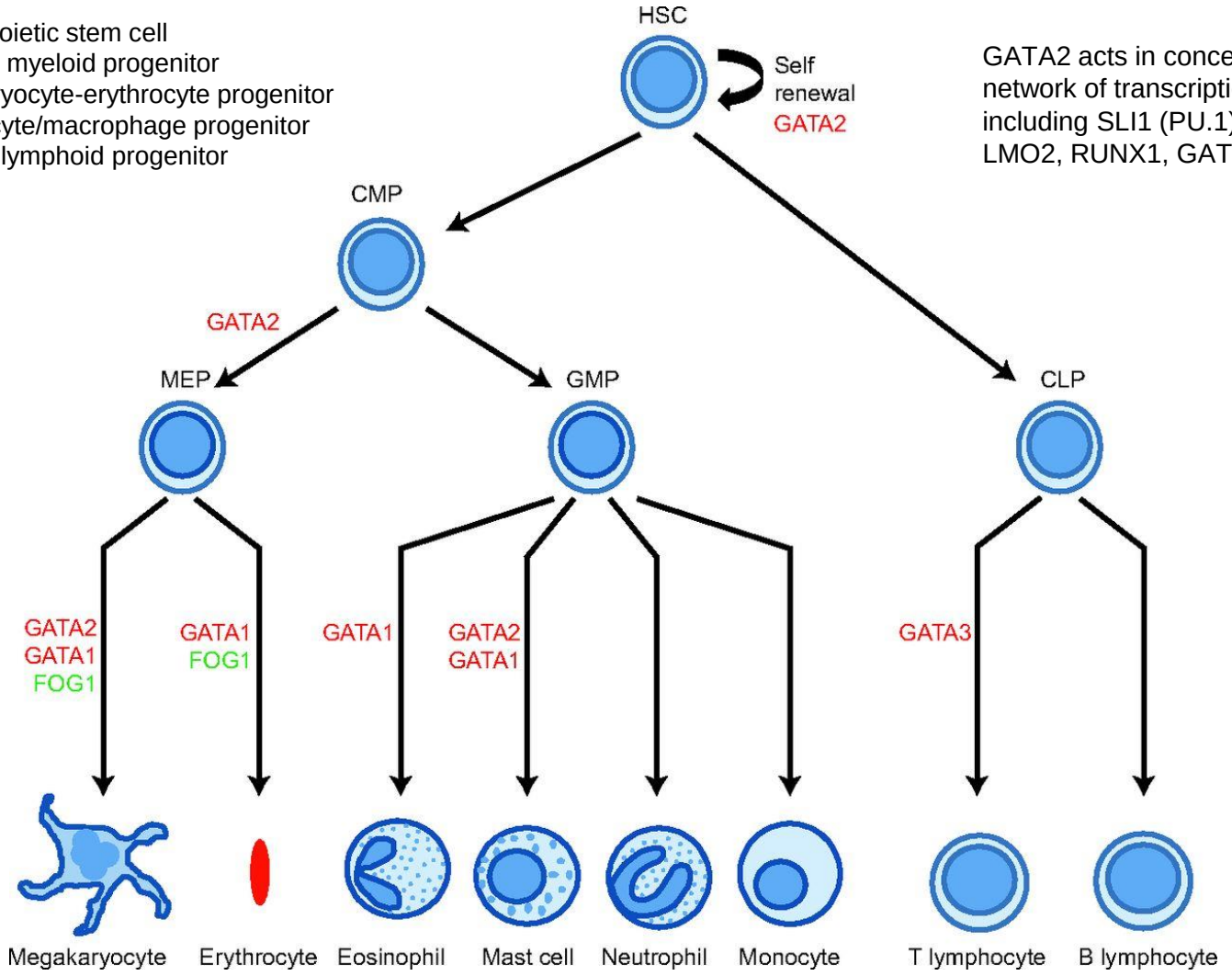




De Pater et al. J Exp Med 2013;210:2843-2850

GATA2 is required for the development of specific cell lineages

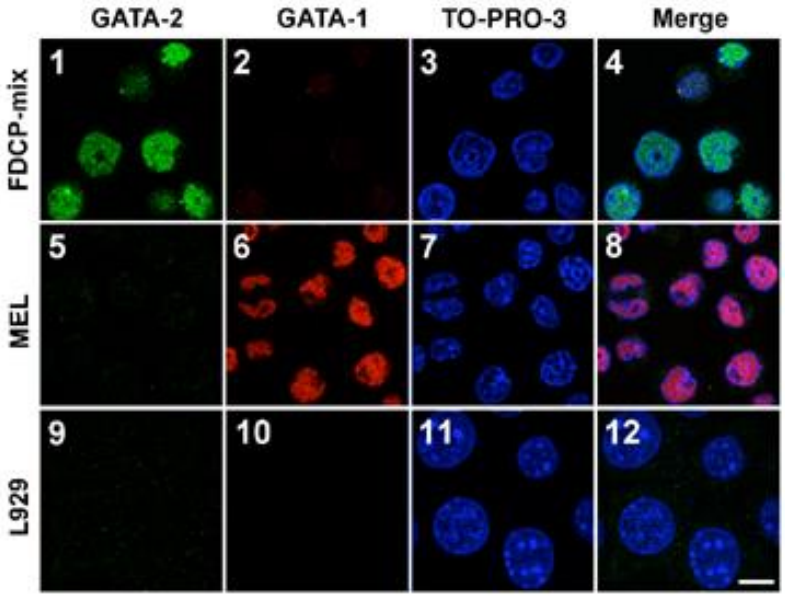
HSC - hematopoietic stem cell
CMP - common myeloid progenitor
MEP - megakaryocyte-erythrocyte progenitor
GMP - granulocyte/macrophage progenitor
CLP - common lymphoid progenitor



GATA2 acts in concert with a large network of transcription factors, including SLI1 (PU.1), FLI1, SCL, LMO2, RUNX1, GATA1, and CEBPα

Chlon & Crispino Development 2012;139:3905-3916

GATA2 is replaced by GATA1 during erythropoiesis



Anguita et al. EMBO J. 2004;23:2841-2852

FDCP (factor-dependent cell Paterson) **mix cells** – murine multipotent hematopoietic progenitors

MEL (mouse erythroleukemia cells) – transformed erythroid cells

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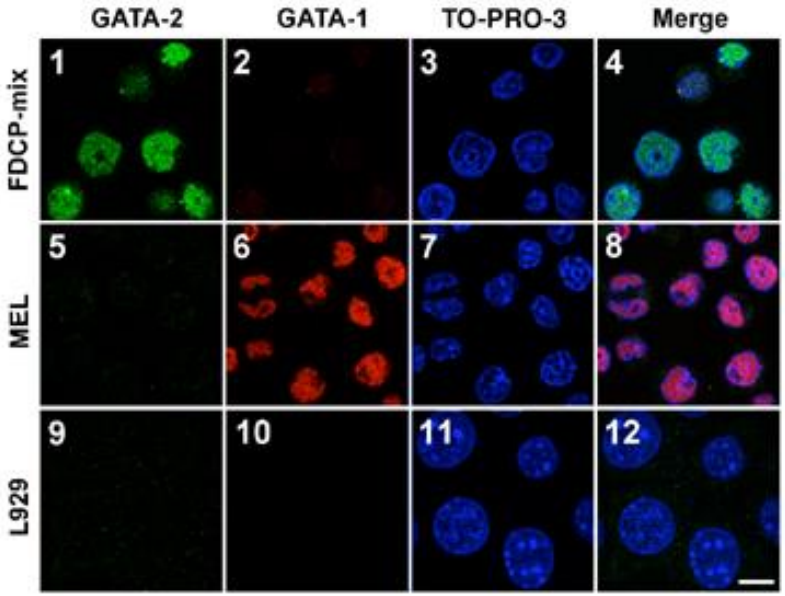

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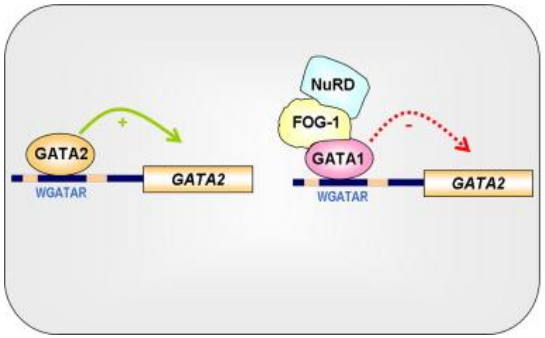
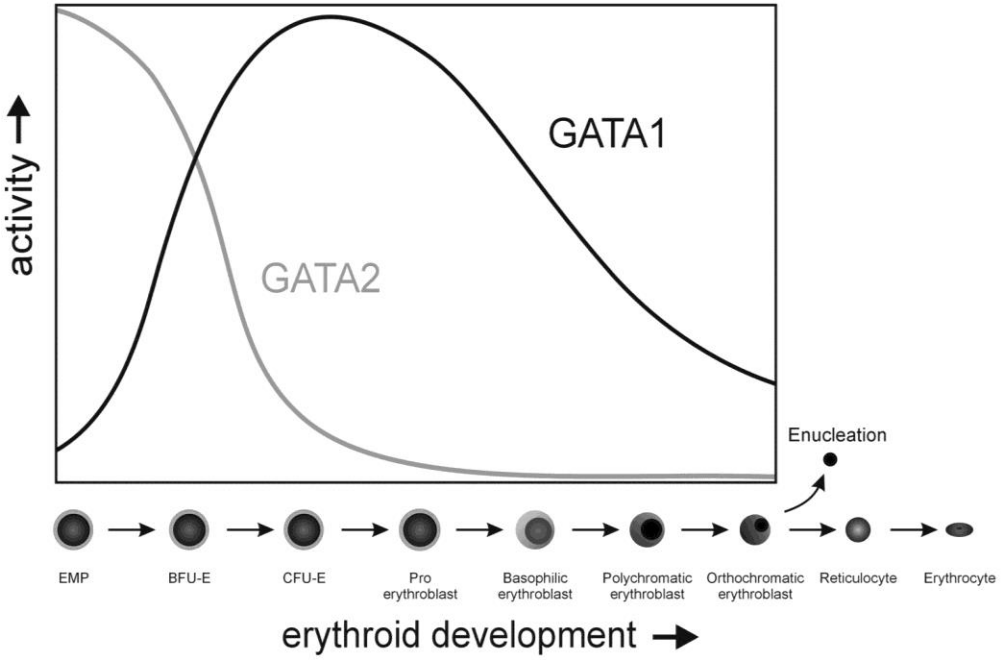


"GATA switch"



Ferreira et al. Mol. Cell. Biol. 2005;25:1215-1227
Vicente et al. Crit Rev in Oncol Hematol, 2012; 82(1):1–17

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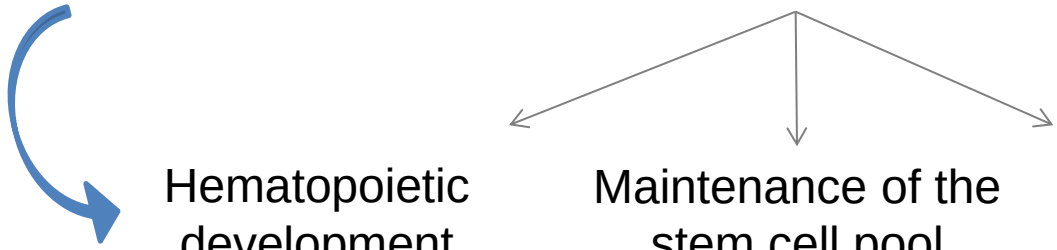
GATA2



Hematopoietic development

Maintenance of the stem cell pool

Development of specific cell lineages

1. What is the role of GATA2?

GATA2

2. Why do *Gata2* mutations make patients prone to certain infections and hematologic malignancies?

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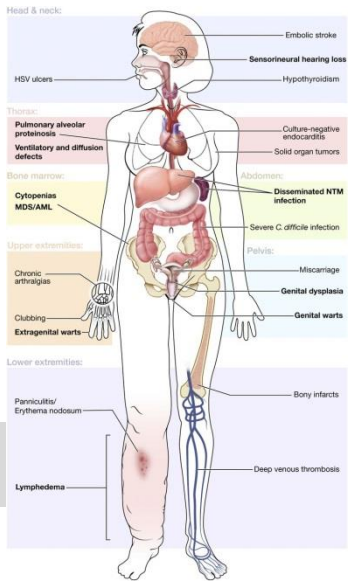
Maintenance of the stem cell pool

Development of specific cell lineages

Mycobacterial and viral infections, MDS/AML, lymphedema



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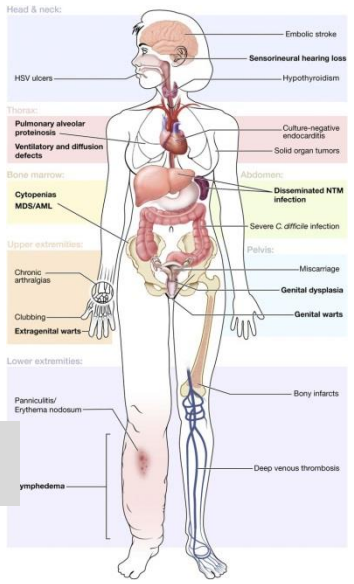
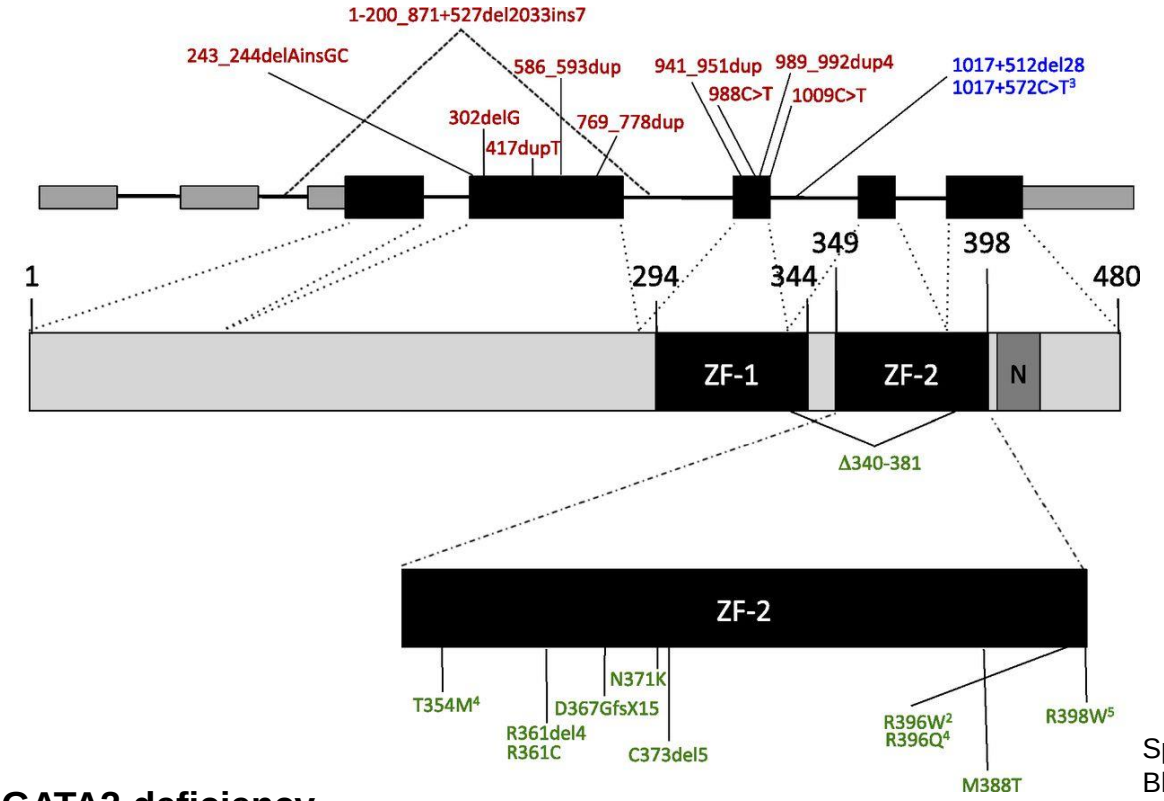
HAPLOINSUFFICIENCY

It Takes Two…Alleles!



Mycobacterial and viral infections, MDS/AML, lymphedema

Gata2 mutations



Spinner et al.
Blood 2014; 123:809-821

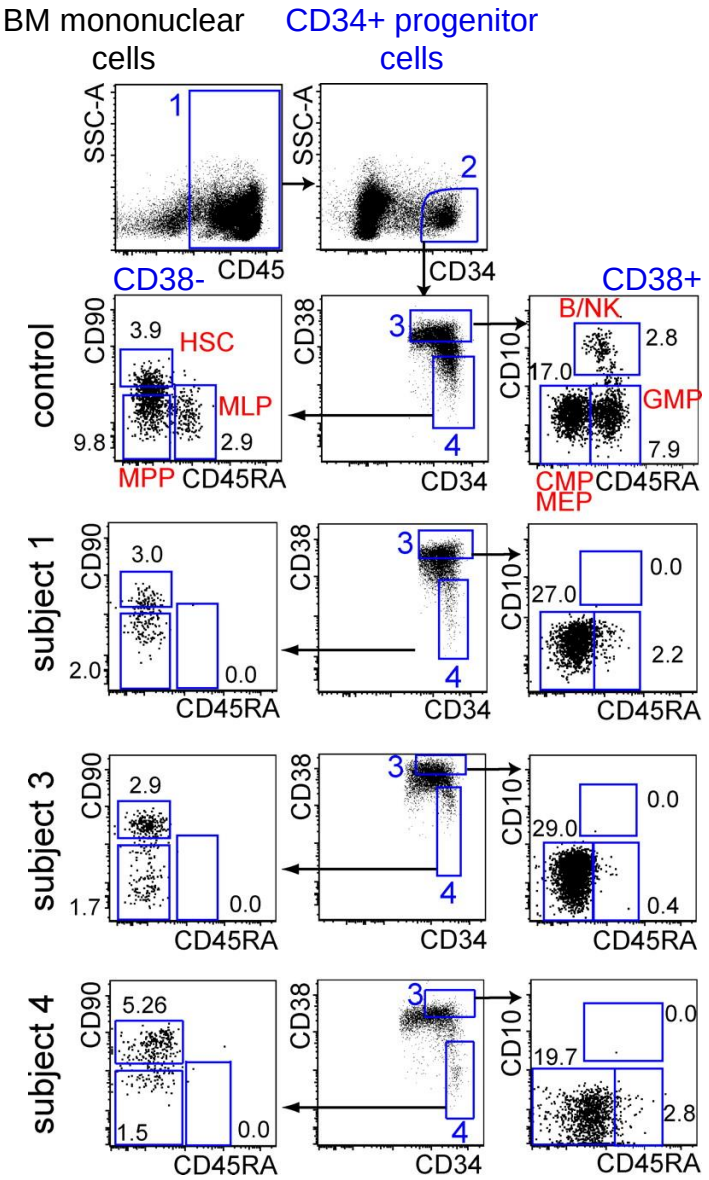
Mechanisms of GATA2 deficiency

Type of mutation (location)	Reported mechanisms	Probable effect
Regulatory (non-coding regions)	Mutation in intron 5 enhancer	Haploinsufficiency due to reduced transcription
***Insertion/deletion; nonsense (across coding region)**	Nonsense-mediated decay; premature stop codon; disrupted splice site	Haploinsufficiency due to loss of expression or severe truncation of protein
SNP; missense (conc. in Zn fingers)	Non or hypo-functional protein; dominant negative functional protein	Haploinsufficiency due to expression of GATA2 protein with reduced function

*Tended to have earlier age of clinical presentation (18 vs 26 yrs)

Adapted from Collin et al. BJH Rev 2015;169:173-187

Depletion of specific populations of BM CD34+ cells



GATA2 haploinsufficiency results in BM depletion of:

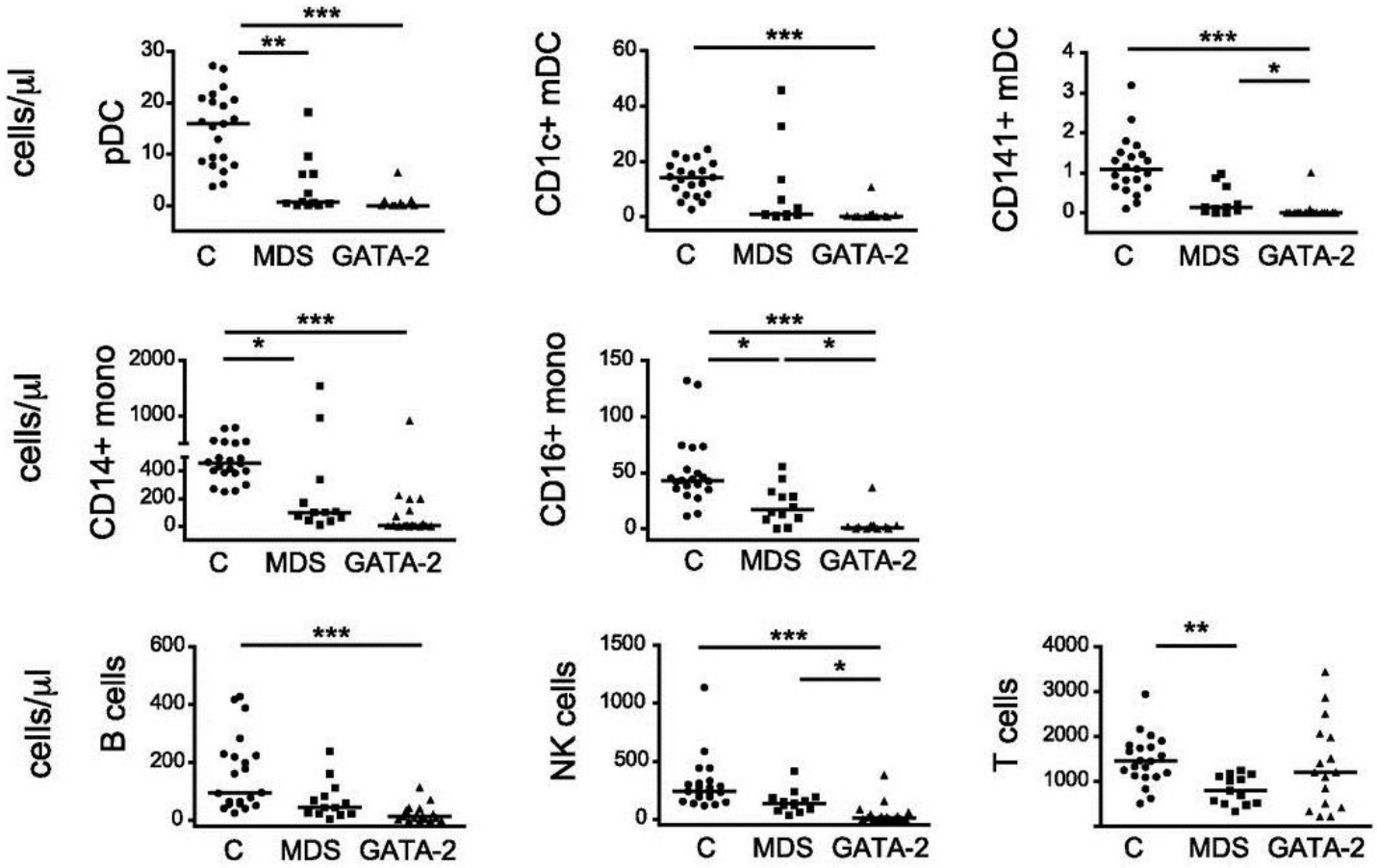
MLP: Multilymphoid progenitors
($CD38^{-}CD90^{lo}CD45RA^{+}$)

↳ B/NK precursors
($CD38^{+}CD10^{+}$)

GMP: Granulocyte macrophage progenitors
($CD38^{+}CD10^{-}CD45RA^{+}$)

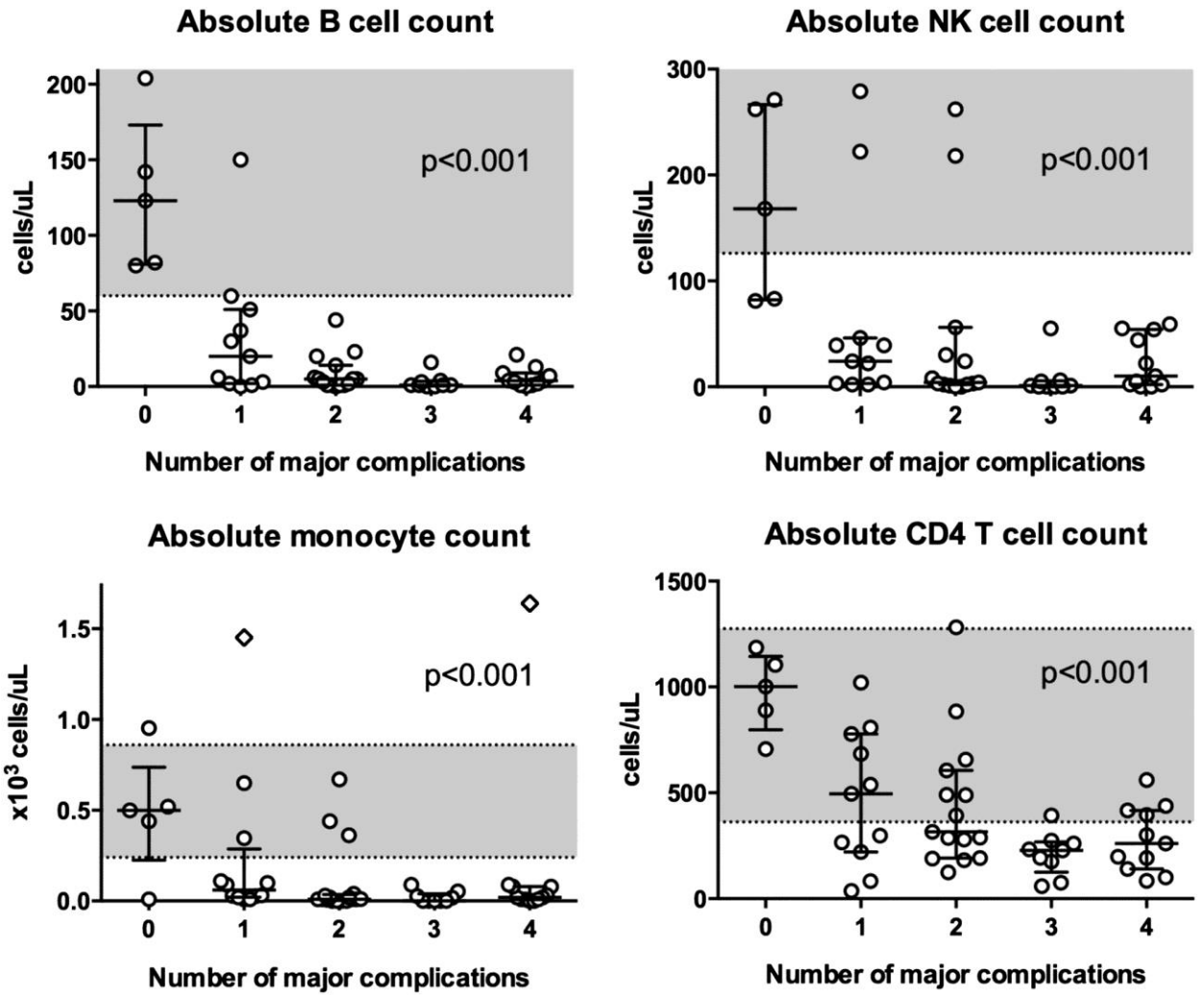
Venetia Bigley et al. J Exp Med 2011;208:227-234

Depletion of certain peripheral cell populations



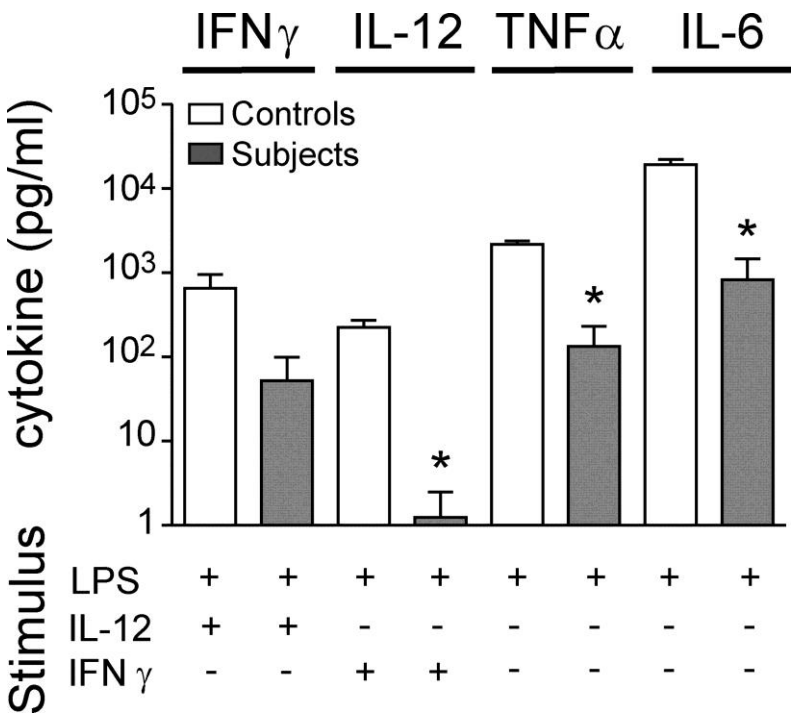
Dickinson et al. Blood 2014;123:863-874

Blood cell counts correlate with disease severity



Michael A. Spinner et al. Blood 2014;123:809-821

Dysfunction of the IL-12/IFN-γ axis



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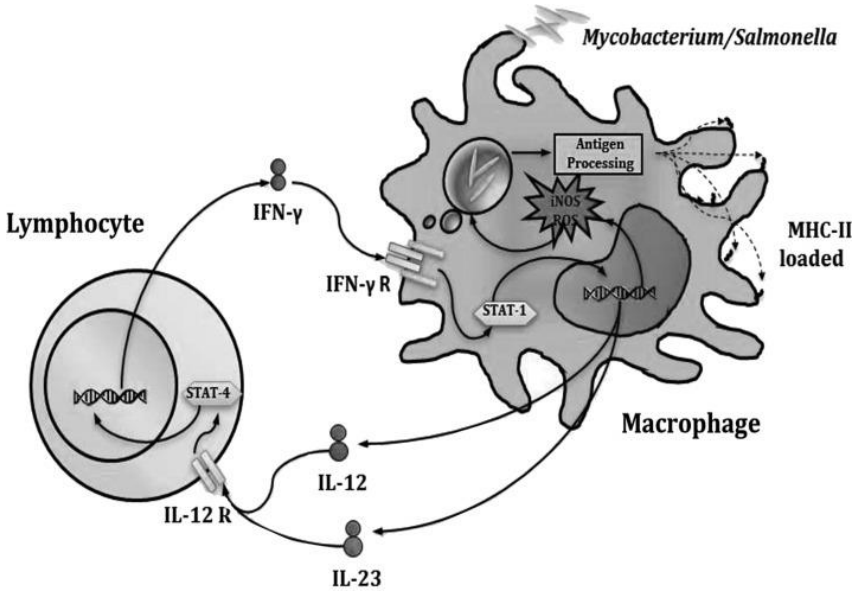
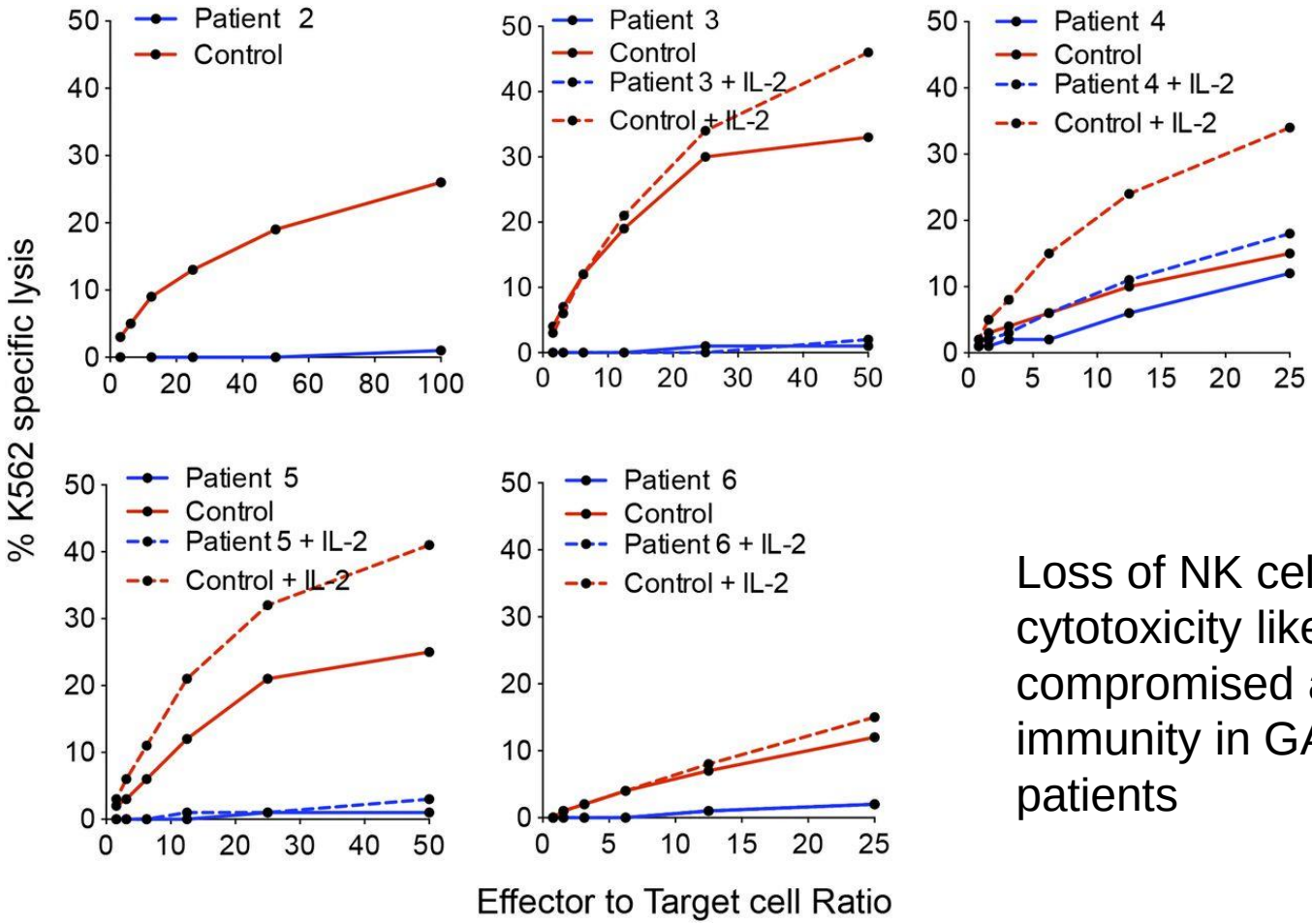


Ramirez-Alejo & Santo-Argumedo. 2014. 34(5):307-317.

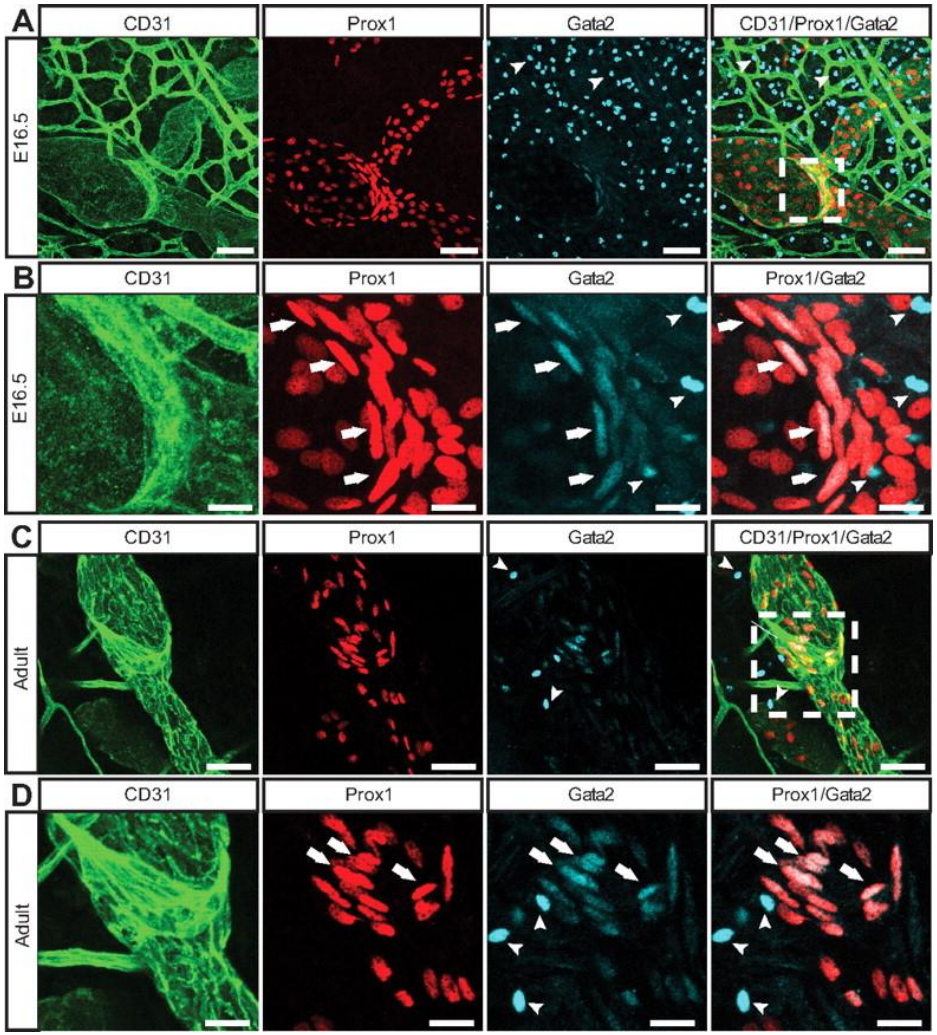
GATA2-deficient patients have defective NK cell cytotoxicity



Loss of NK cell cytotoxicity likely leads to compromised antiviral immunity in GATA2 patients

Emily M. Mace et al. Blood 2013;121:2669-2677

GATA2 localizes to lymphatic valves, suggesting a key role in lymphatic vascular development

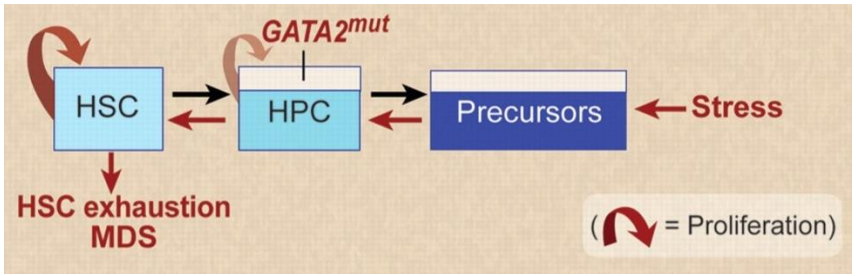


Jan Kazenwadel et al. Blood 2012;119:1283-1291

Does GATA2 haploinsufficiency lead to altered development and, hence, early onset lymphedema?

GATA2 and MDS/AML

- 30-50% of patients at presentation
- 30-year median onset
- 90% lifetime risk

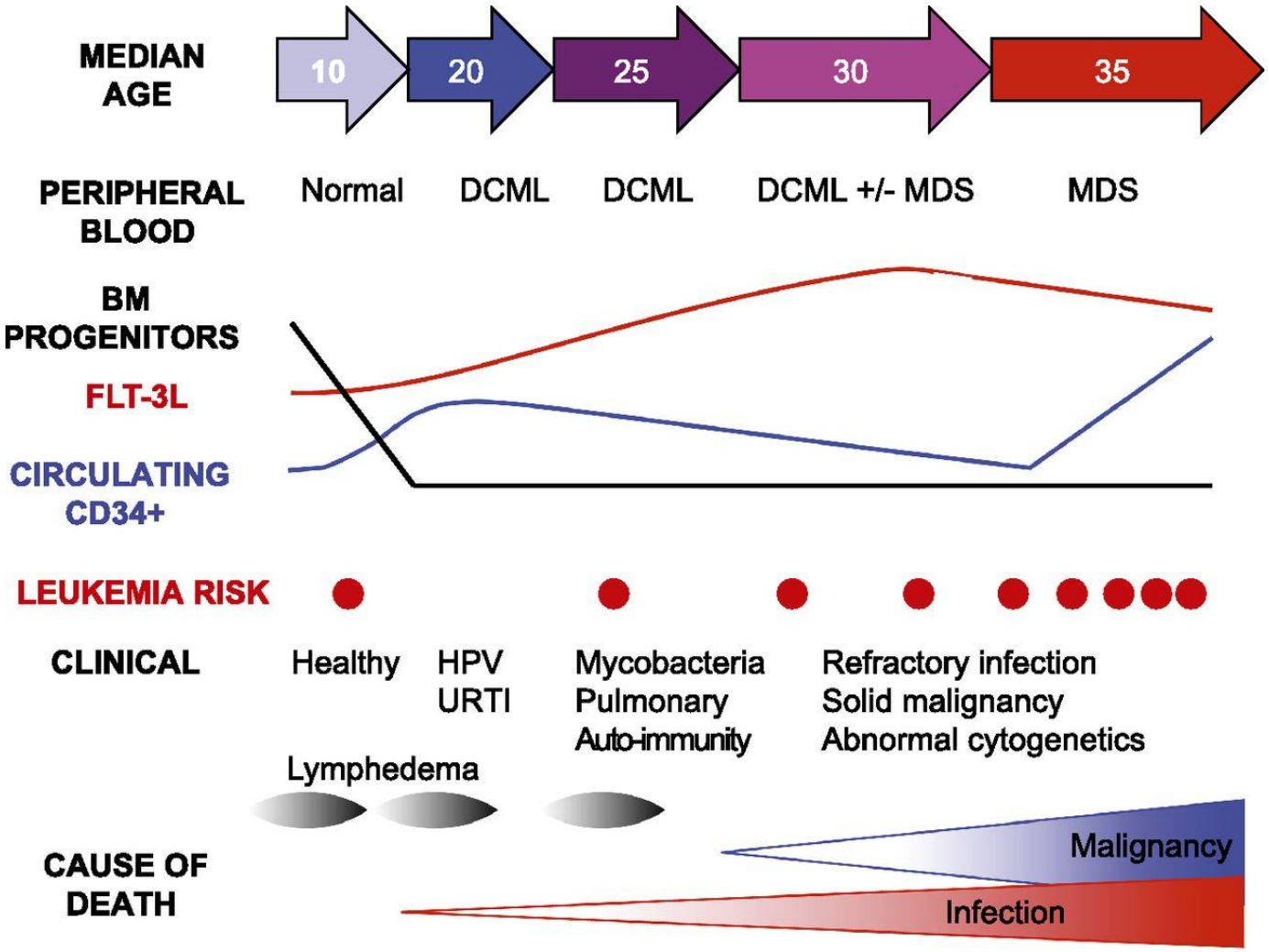


Migliaccio, & Bieker Blood 2011;118:2647-2649

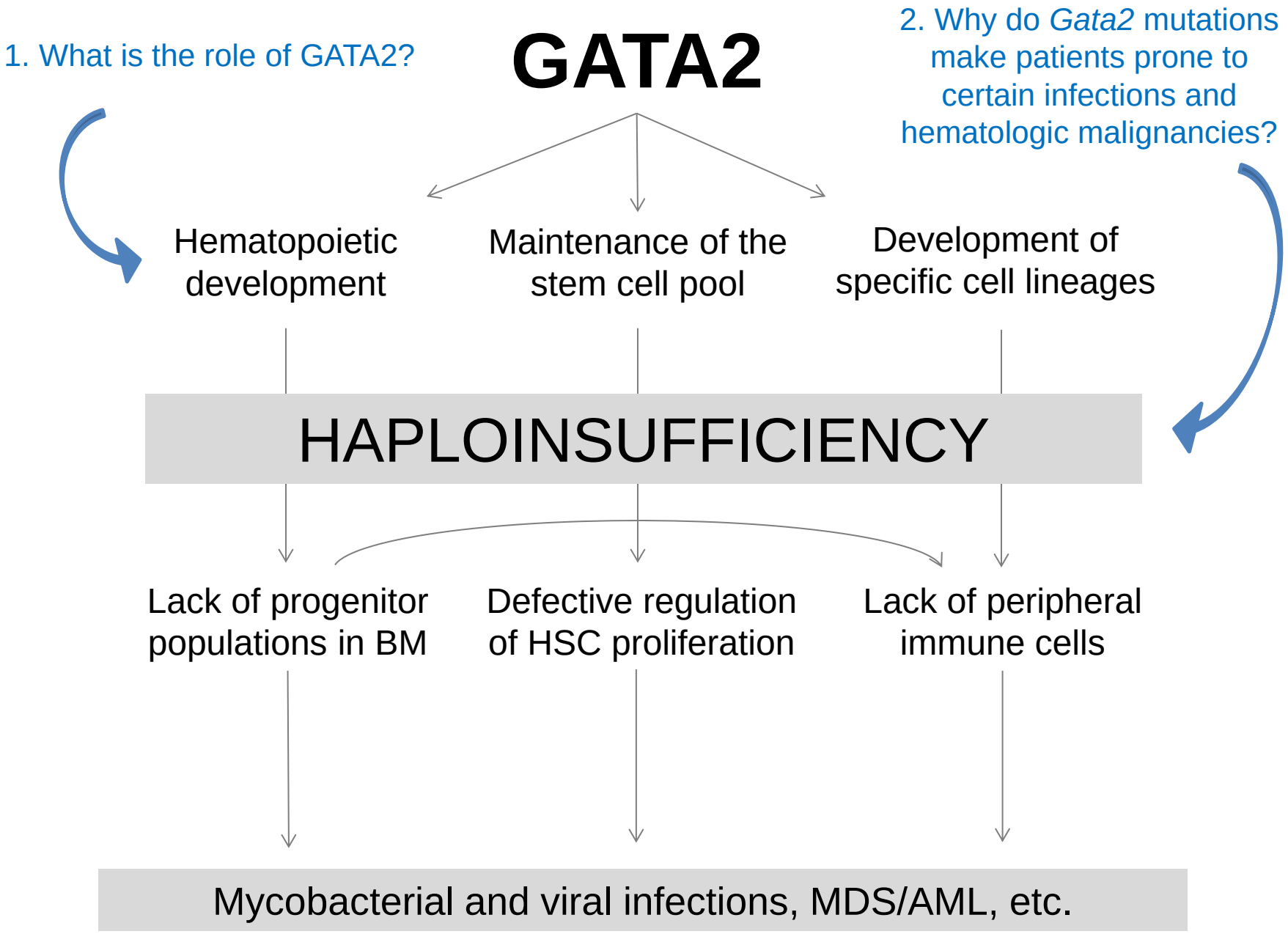
GATA2 configuration	Associated with	Outcome
Germline heterozygous mutation	***ASXL1***	High risk MDS/AML
	monosomy 7	
	trisomy 8	
	trisomy 21	
	der(1;7), +1q −7q	
	EZH2	
	HECW2	
	GATA1	

Collin et al. 2015. BJH Rev 169:173-187

Evolution of cellular deficiency with GATA2 mutation



Rachel E. Dickinson et al. Blood 2014;123:863-874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Lack of progenitor populations in BM
Defective regulation of HSC proliferation
Lack of peripheral immune cells
Mycobacterial and viral infections, MDS/AML, etc.

Questions