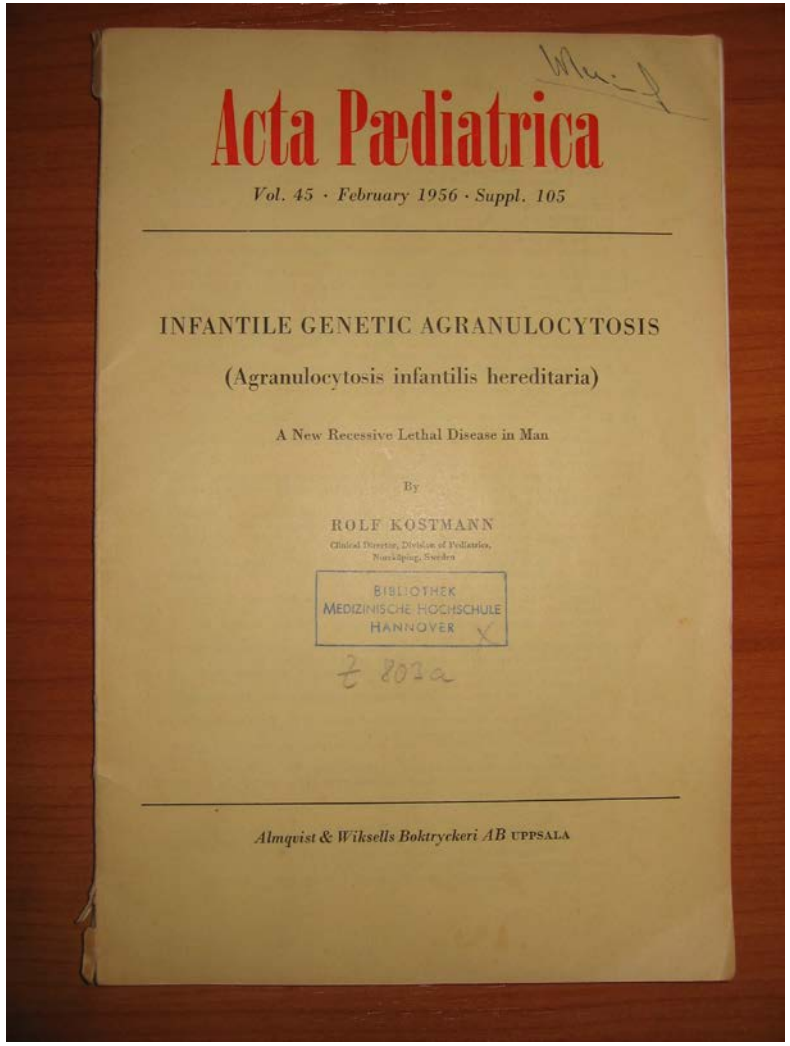
Severe congenital neutropenia



Acta Pædiatrica

Vol. 45 · February 1956 · Suppl. 105

INFANTILE GENETIC AGRANULOCYTOSIS

(Agranulocytosis infantilis hereditaria)

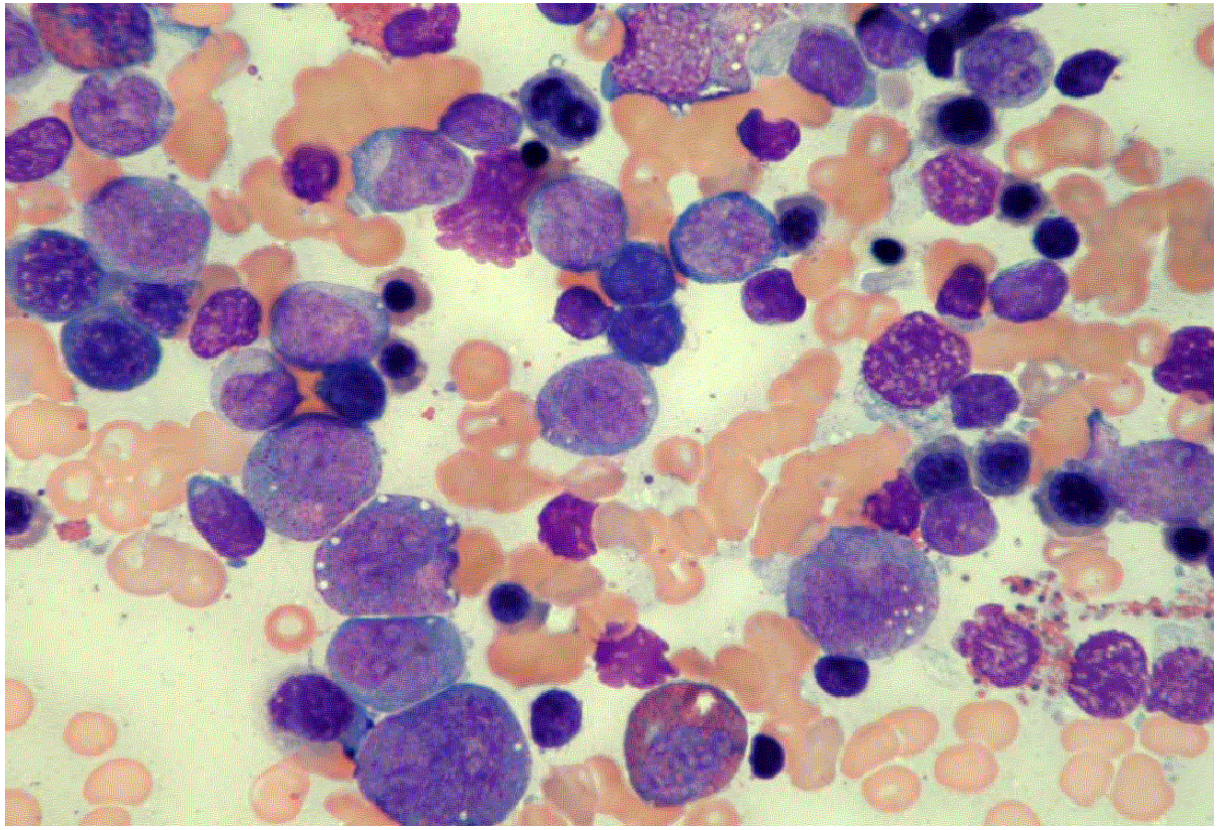
A New Recessive Lethal Disease in Man

By

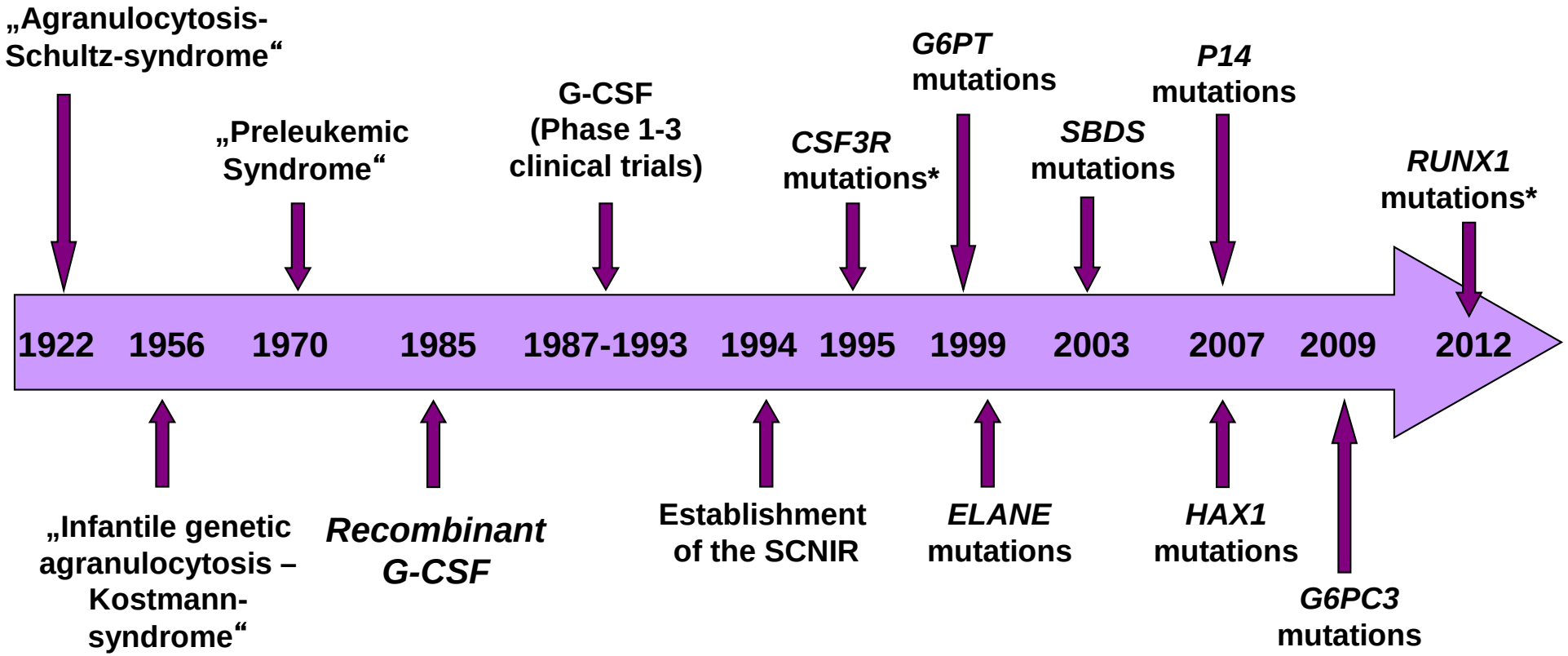
ROLF KOSTMANN

Clinical Director, Division of Pediatrics, Norrköping, Sweden

Almqvist & Wiksells Boktryckeri AB UPPSALA



Milestones of the history of congenital neutropenias

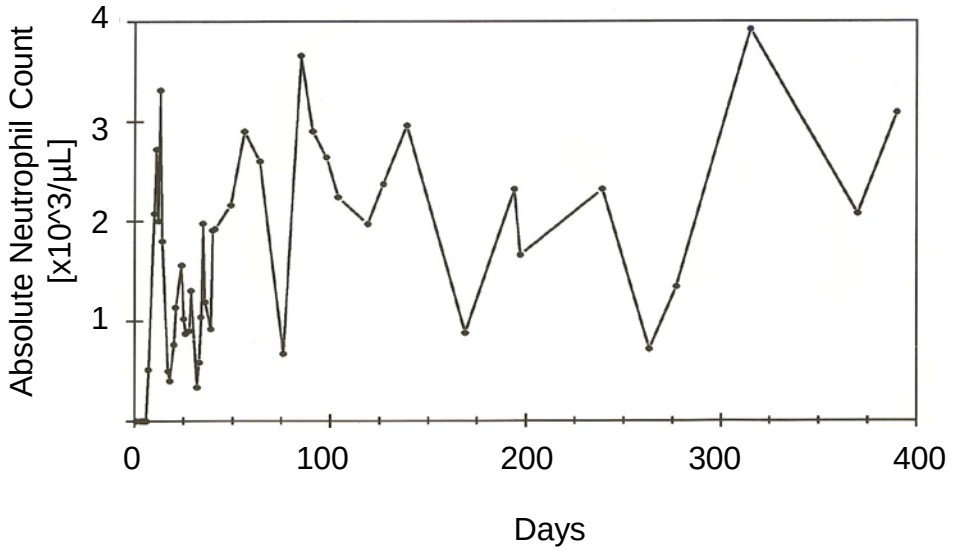


* = acquired mutations

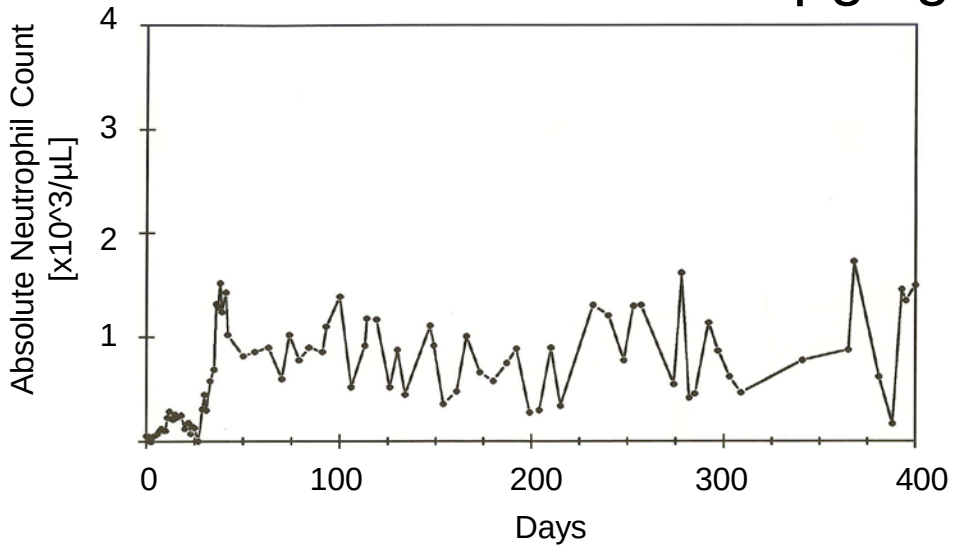
Severe congenital neutropenia

G-CSF Treatment

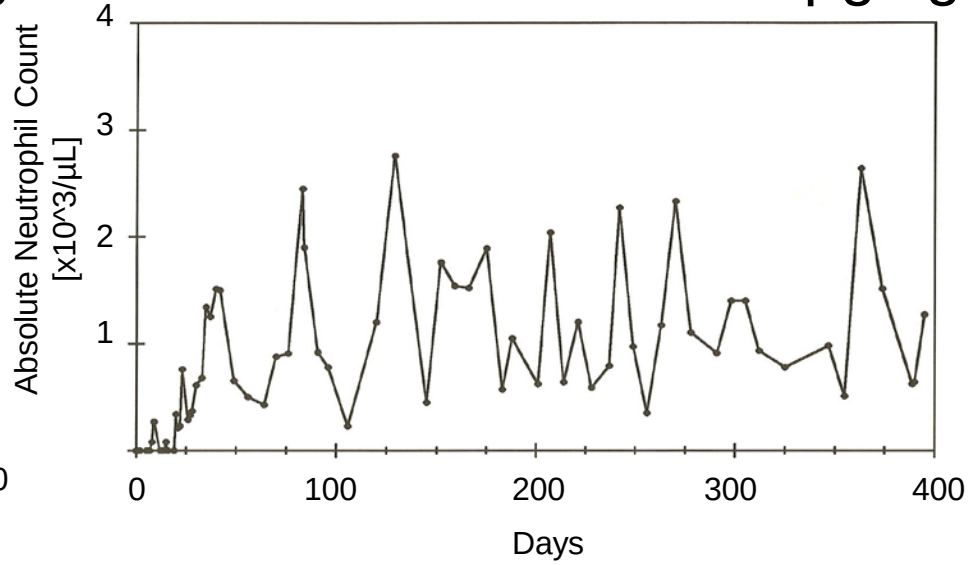
Patient 8716/27: 5 µg/kg/d



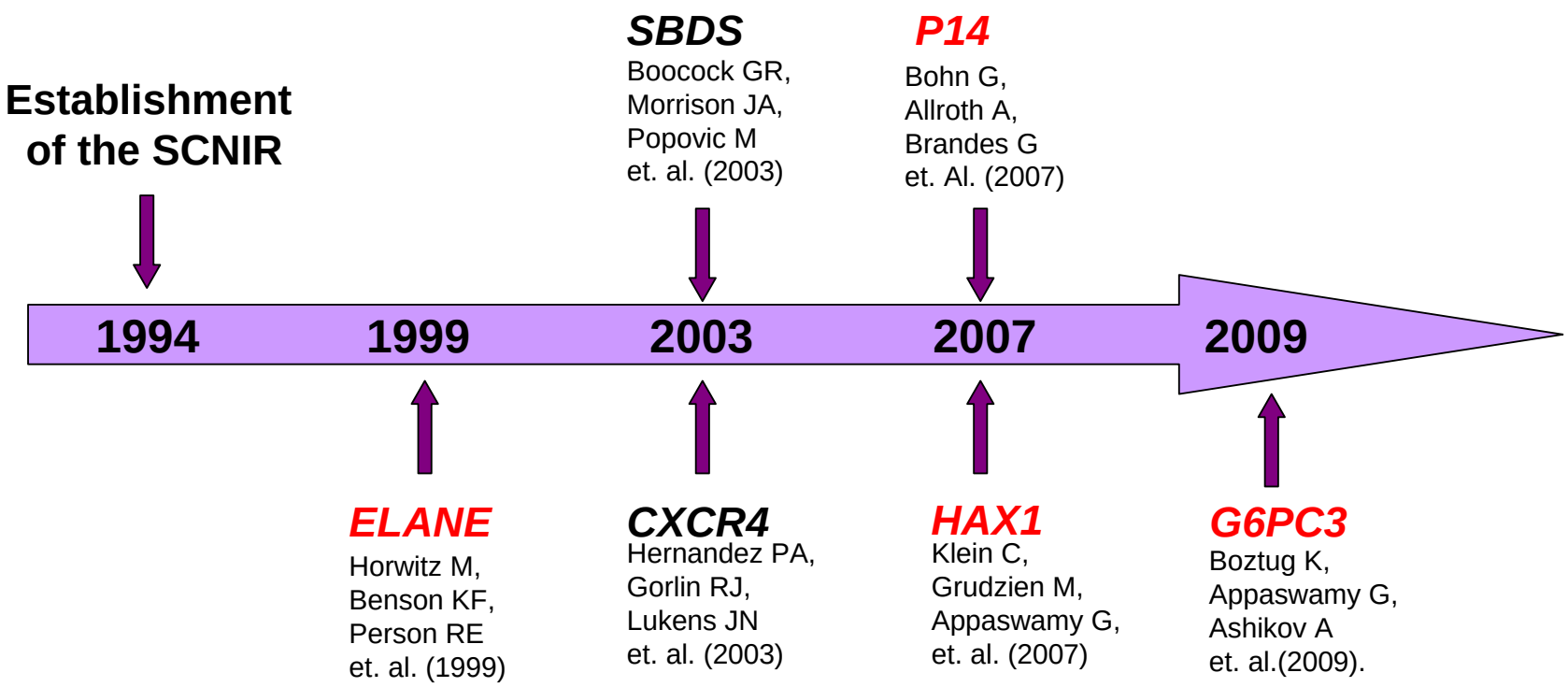
Patient 8716/01: 20 µg/kg/d



Patient 8716/27: 50 µg/kg/d



Identification of Neutropenia causing gene defects



Severe Chronic Neutropenia International Registry (SCNIR)

ELANE Mutations in Cyclic and Congenital Neutropenia

Linear Localization

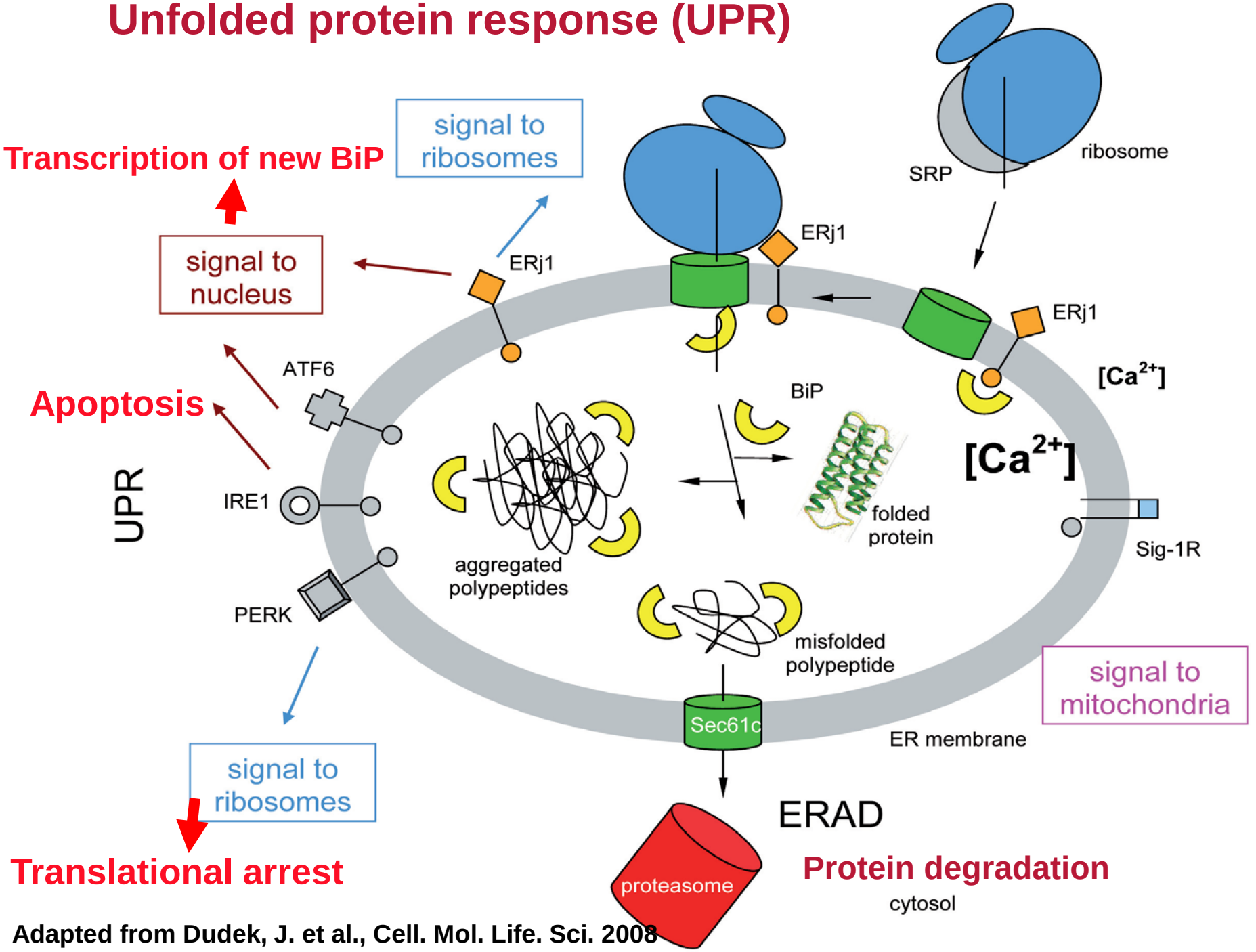
Congenital Neutropenia (CN)
189 patients, 29 AML/MDS

Cyclic Neutropenia (CyN)
118 patients,
0 MDS/AML

5' UTR	Exon 1	Exon 2	Exon 3	Intron III	Exon 4	Intron IV	Exon 5
1 patient	*3 patients*	*29 patients*	*29 patients*	*4 patients*	*63 patients*	*11 patients*	*49 patients*
	1 MDS/AML	*4 MDS/AML*	*4 MDS/AML*	*1 MDS/AML*	*7 MDS/AML*	*2 MDS/AML*	*10 MDS/AML*
c. -9 A>G	M1R[1]	F43L	A79fs[1]	R103L	L123H	IVS4 +1 G>A[1]	D201fs[1]
	M1T	V45M	R81P	R103P	S126L[1]	IVS4 +1 G>T	R220Q
		S46F	V83D	I118N	W156G	IVS4 +5 G>A	S202fs[1]
		C55S	L84P	I120F	W156R	IVS4 +6 3bp ins[1]	G221ter
		G56R[1]	G85E[1]	I120N[1]	S126W		G203R
		A57T	G85R	L121F	V174_C181del [1]		C223fs
		A57V[1]	V98_Q102del	L121H	A127D		L206fs[1]
		I60M	V101M[1]	IVS3 -8 C>A[1]	C181fs		C223ter[1]
		I60T[1]		IVS3 +2100 C>T	T128del		V207D
		A61G			V186I		S225ter
		A61V			I129del		C208G
		V65D[1]			V190fs[1]		G226R
		M66R			P139L		C208ter[1]
		S67W			R191S		Y228ter[1]
		C71F			C151S		G210V
		C71R			R193Q		D230fs[1]
		C71S			C151Y[3]		G210W
		C71Y			Q194ter		F232fs
					L152P[1]		G214E
					V197fs		A233fs
					A153P		G214R[3]
					F199fs		Q237fs
					M154R		G214ter
							N240del
							V219I
		10 patients	*3 patients*	*1 patient*	*16 patients*	*49 patients*	*39 patients*
		F43L	Q97L	IVS3 -2 A>C	S126L	IVS4 +1 G>A	L206F
		A61V	V101M		S126W	IVS4 +3 A>T	G214ter
			D117V		P139L	IVS4 +5 G>A	R220Q
					D174ins		Y228ter
					V186_D201del		W241G
					Q194ter		W241L
							W241ter

Dale D, Welte K, et al.,Curr Opin Hematol. 2015;22:3-11

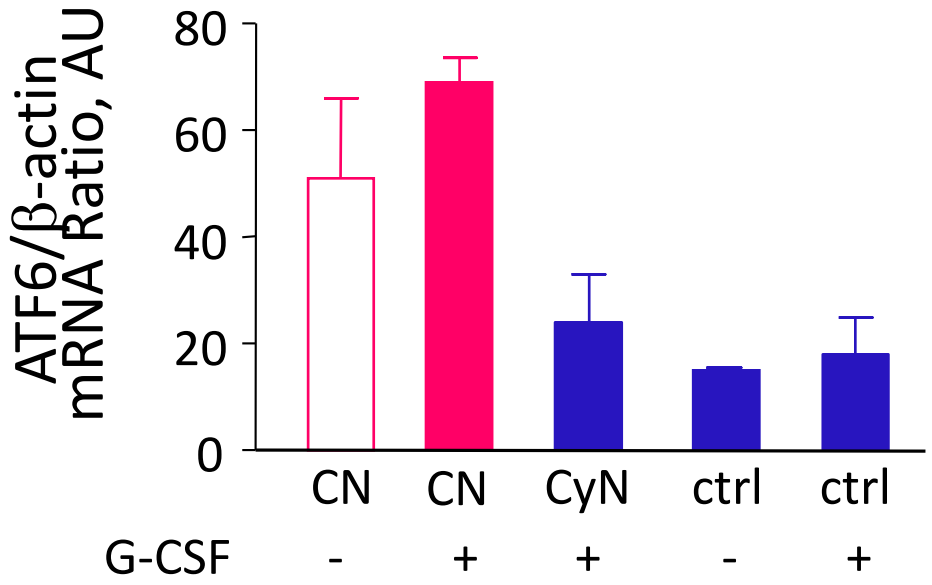
Unfolded protein response (UPR)

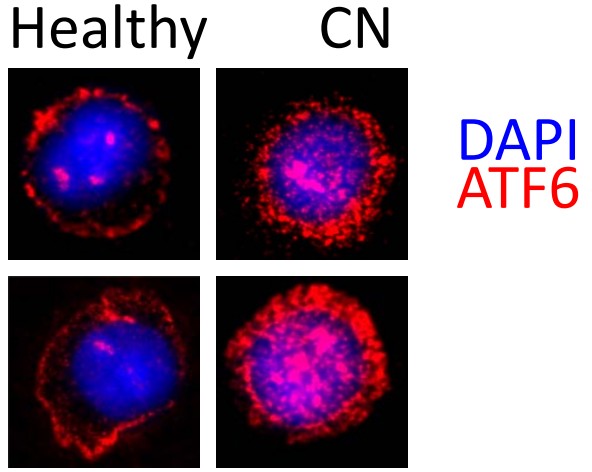


Adapted from Dudek, J. et al., Cell. Mol. Life. Sci. 2008

ATF6 is upregulated in myeloid cells of CN, but not CyN patients

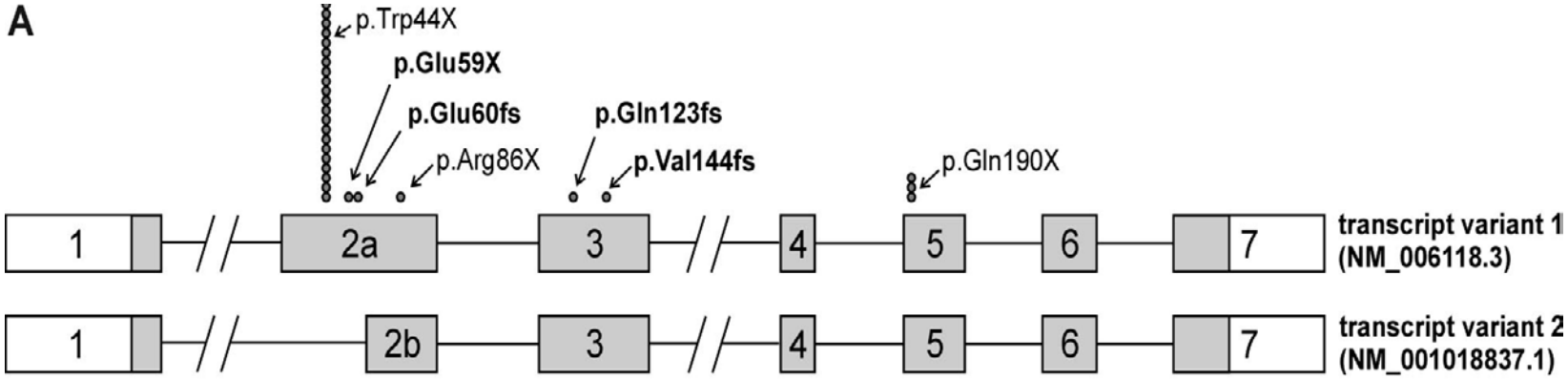
CD33^{+} bone marrow cells





Nustede R., et al., BJH 2016

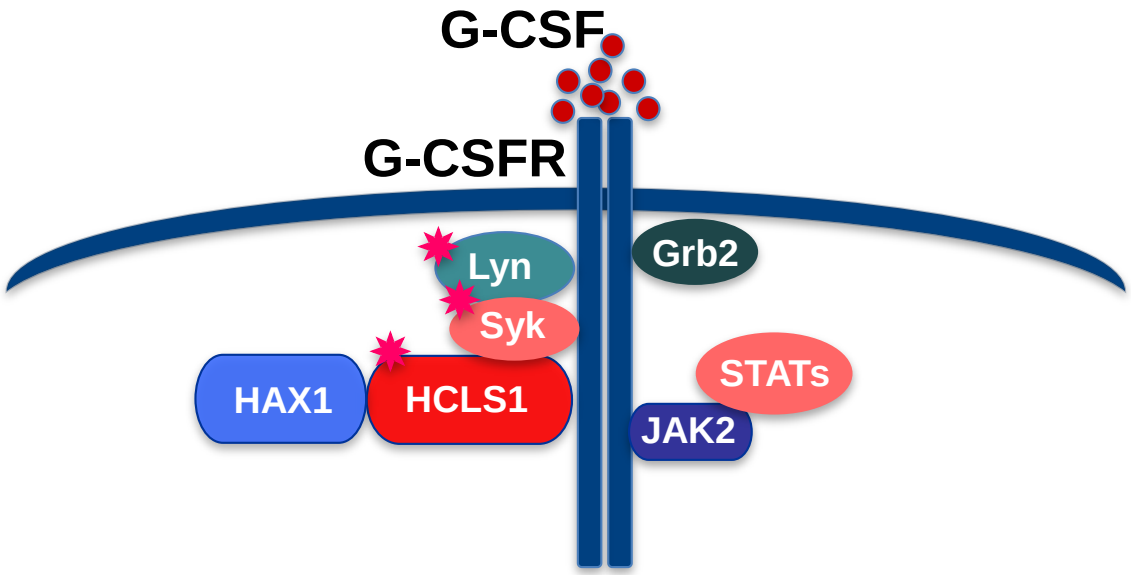
HAX transcript variants



Mutations affecting both isoforms are associated with neutropenia and a neurological phenotype:
Isoform 2 is critical for neuronal functions,
Mutations affecting isoform 1 only (e.g. Trp44X) are associated with neutropenia only.

Klein, C., et al., Nat Gen 2007
Germeshausen M., et al., Blood 2008
Carlsson G., et al., J Intern Med 2008

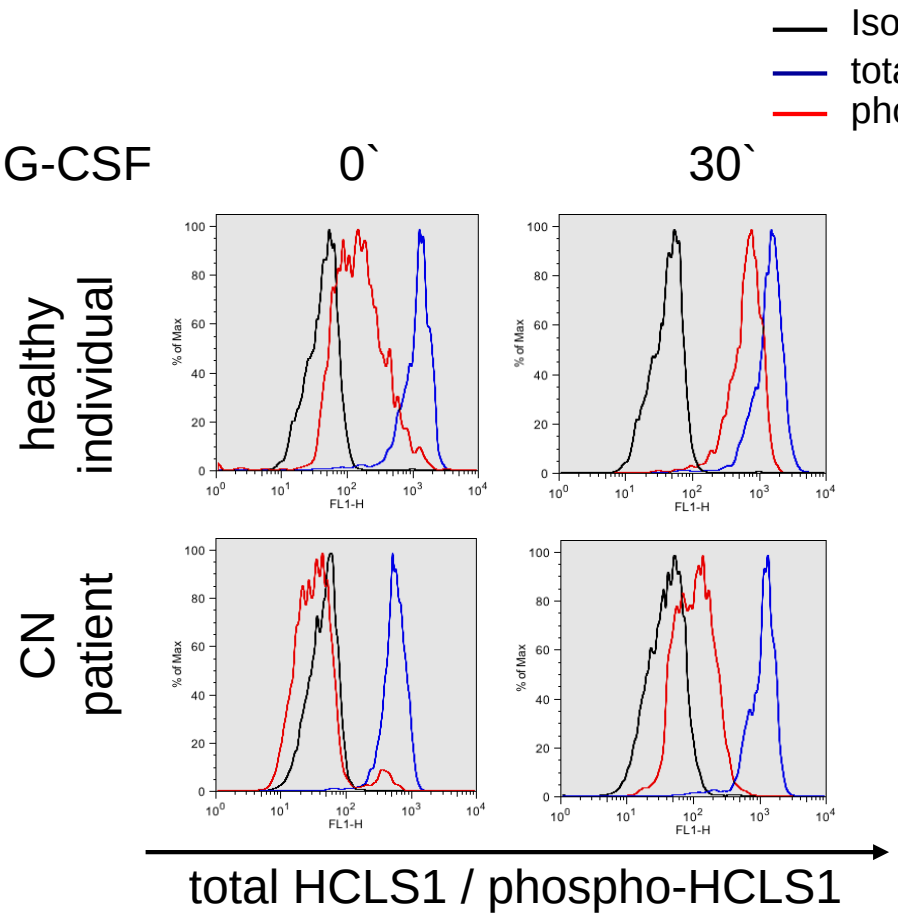
HCLS1 is phosphorylated by Lyn and Syk upon G-CSF stimulation

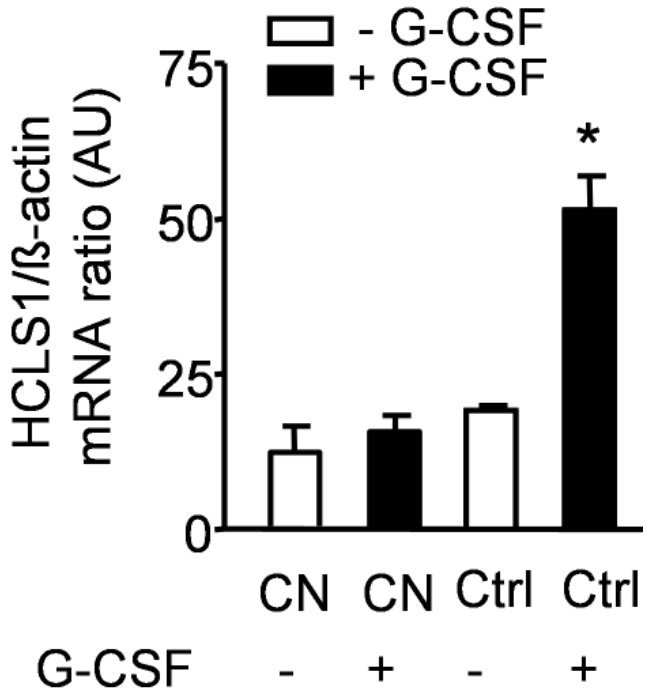


HCLS1 is a **H**ematopoietic **C**ell specific **L**yn **S**ubstrate 1

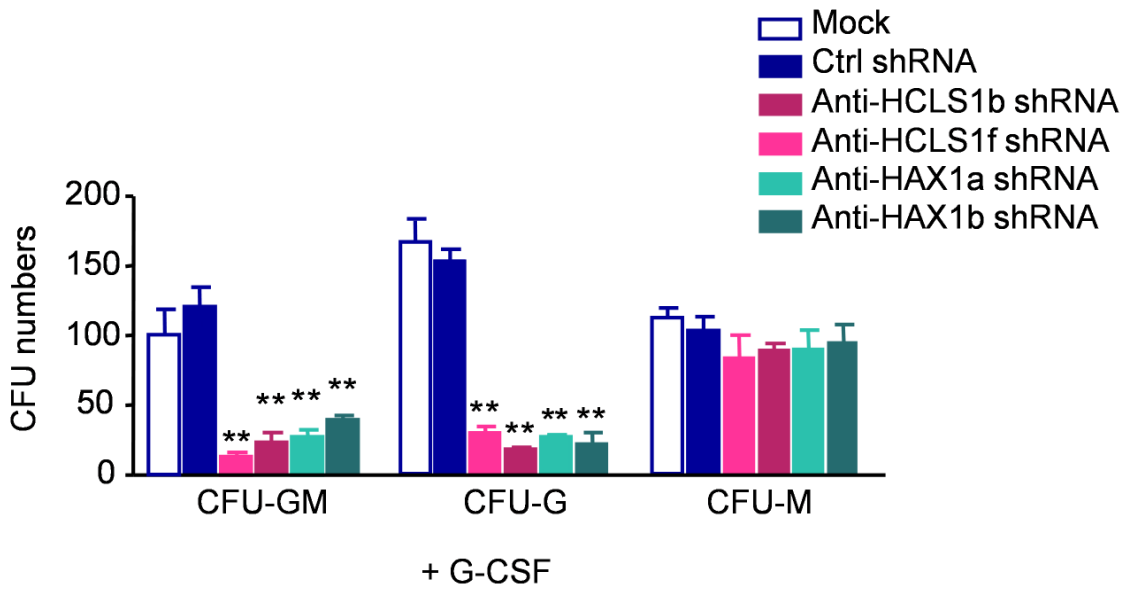
HAX1 is a **H**CLS1 **A**ssociated protein **X 1**

G-CSF failed to phosphorylate HCLS1 in hematopoietic cells of CN patients harboring HAX1 mutations



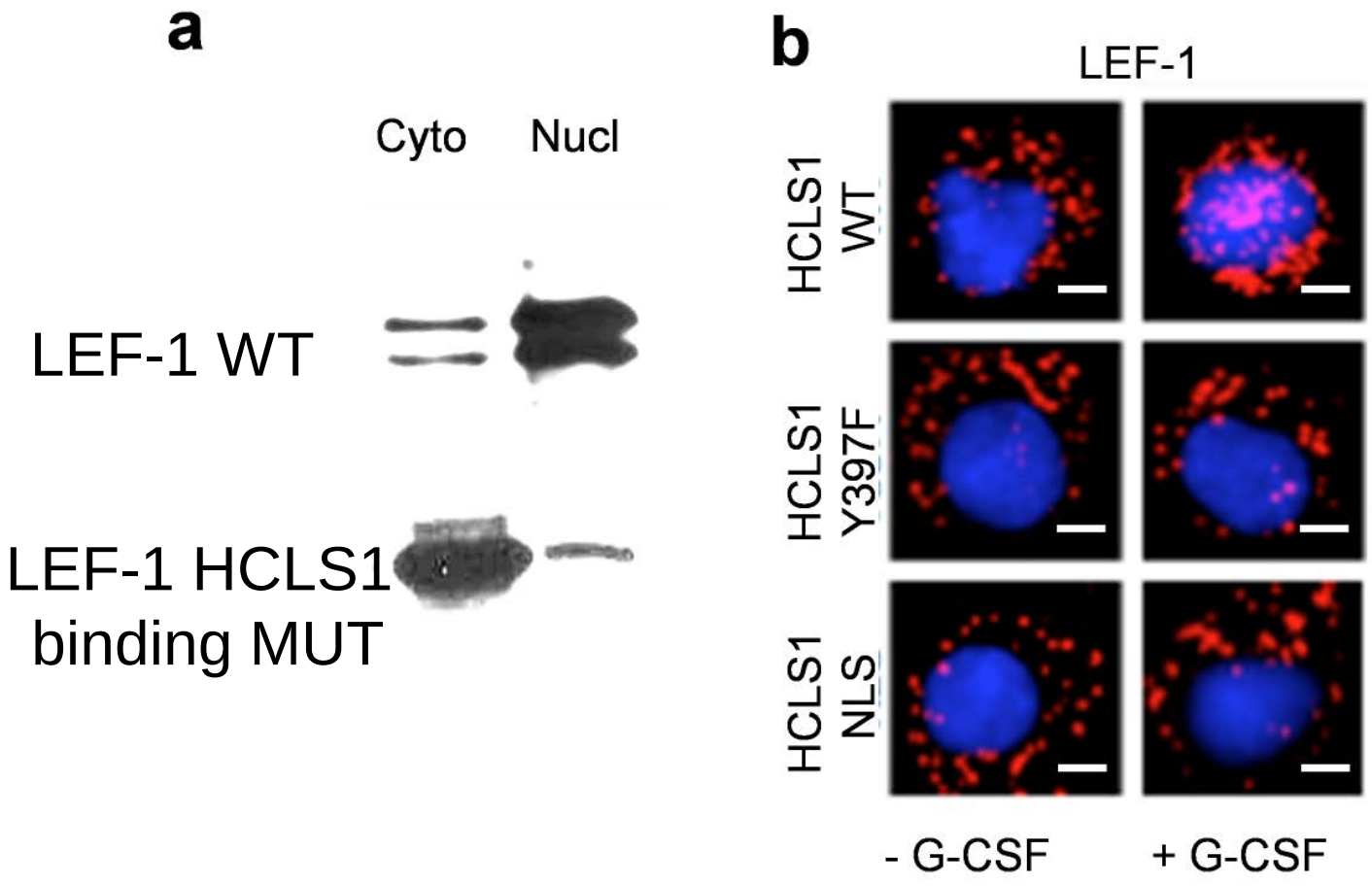


HCLS1 is essential for myeloid differentiation

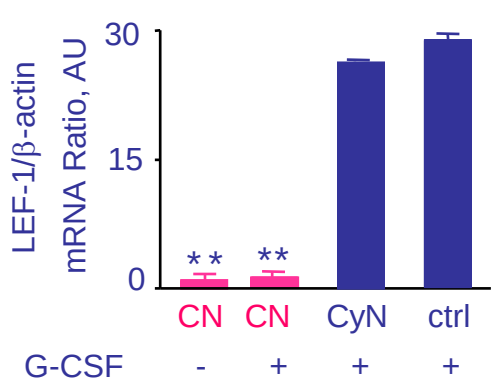


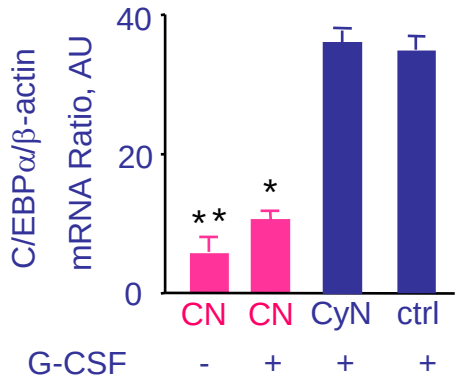
Skokowa, J., et al., Nat Med 2012

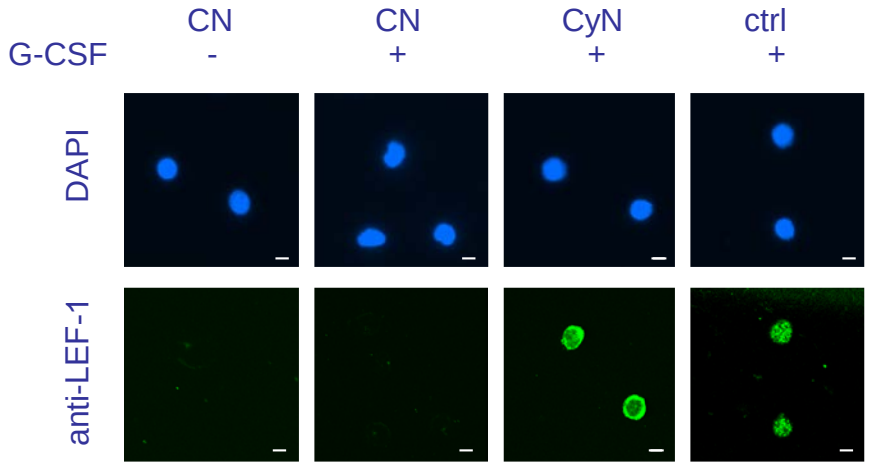
HCLS1 is involved in the nuclear transport of LEF-1 protein

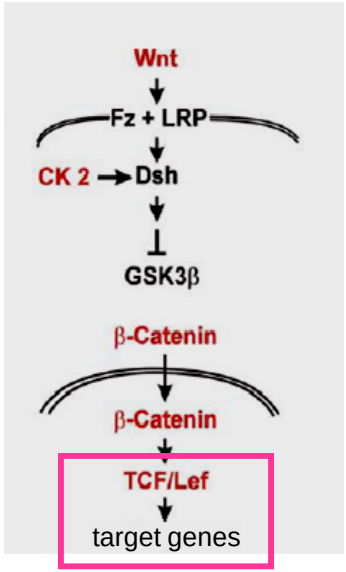


LEF-1 and its target gene C/EBPα expression are downregulated in ELA2 – and HAX1 mutated CN patients





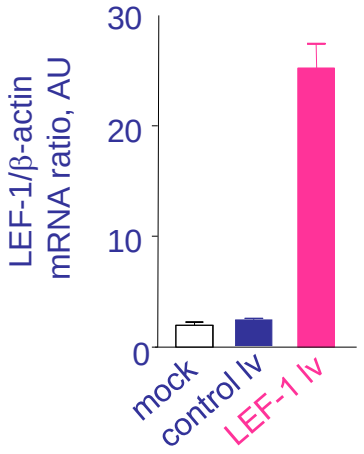


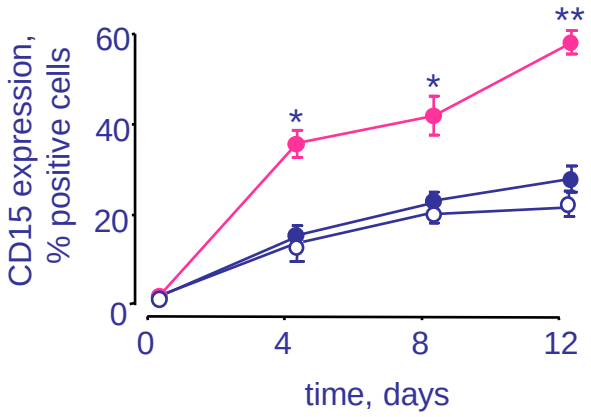


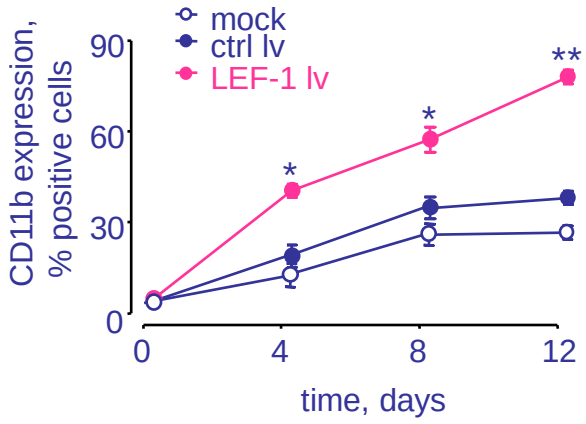
CN: congenital neutropenia; CyN: cyclic neutropenia;

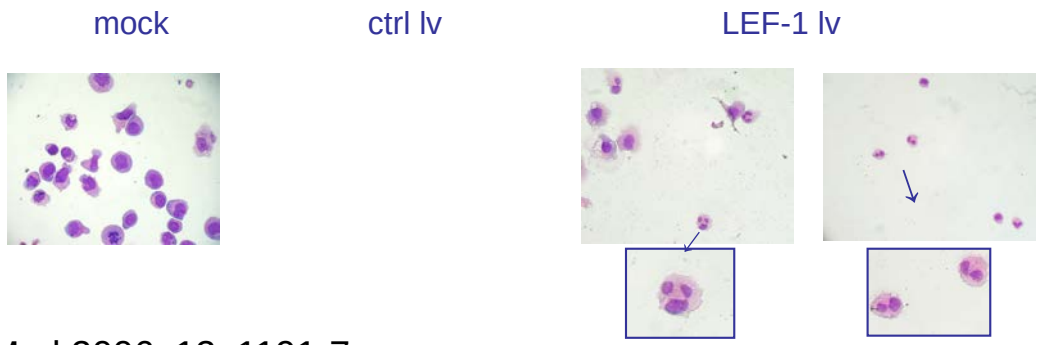
Skokowa, J., et al., Nat Med 2006; 12: 1191-7

Restoration of defective LEF-1 expression promotes granulocytic differentiation of CD34$^+$ progenitors of CN patients



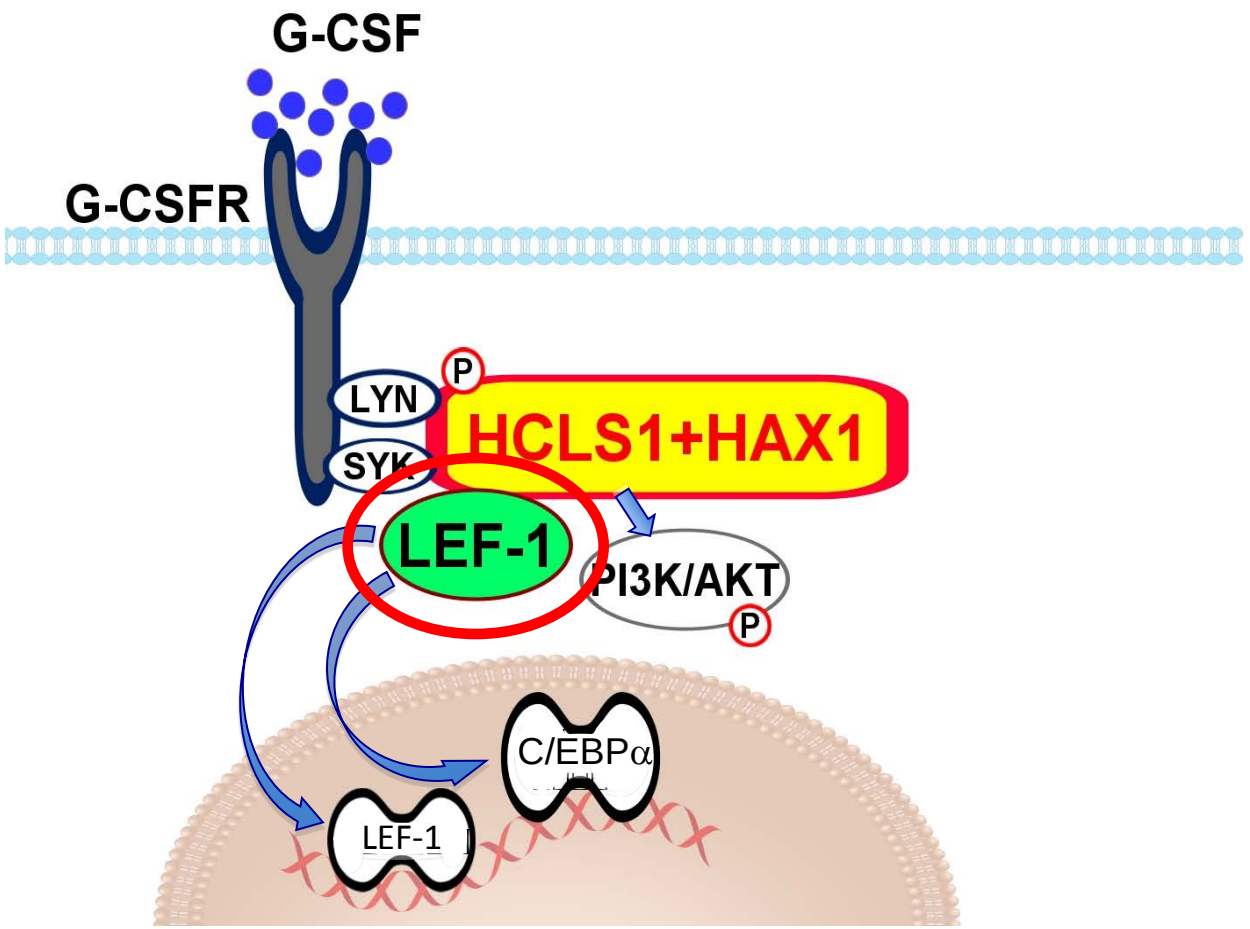






Skokowa, J., et al., Nat Med 2006; 12: 1191-7

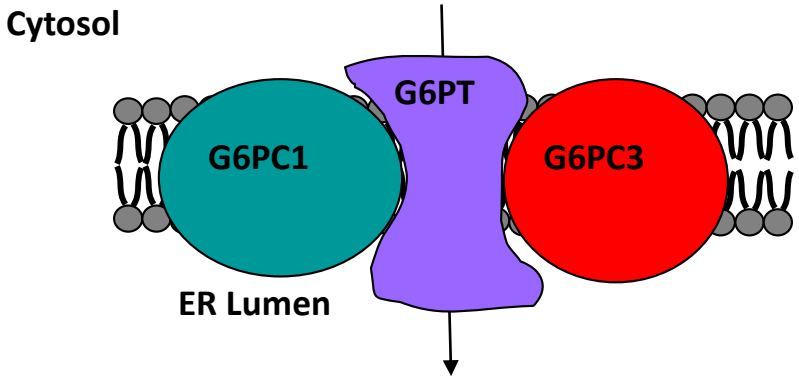
HCLS1 interacts with LEF-1 transcription factor inducing its nuclear translocation and activation upon G-CSF treatment

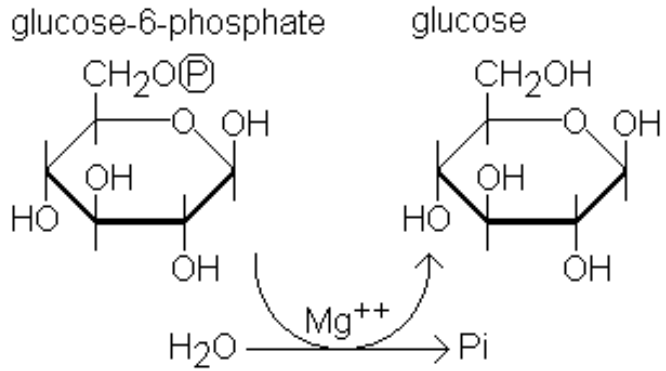


Skokowa J. et al., Nature Medicine, 2012

Glucose-6-Phosphatase Komplex

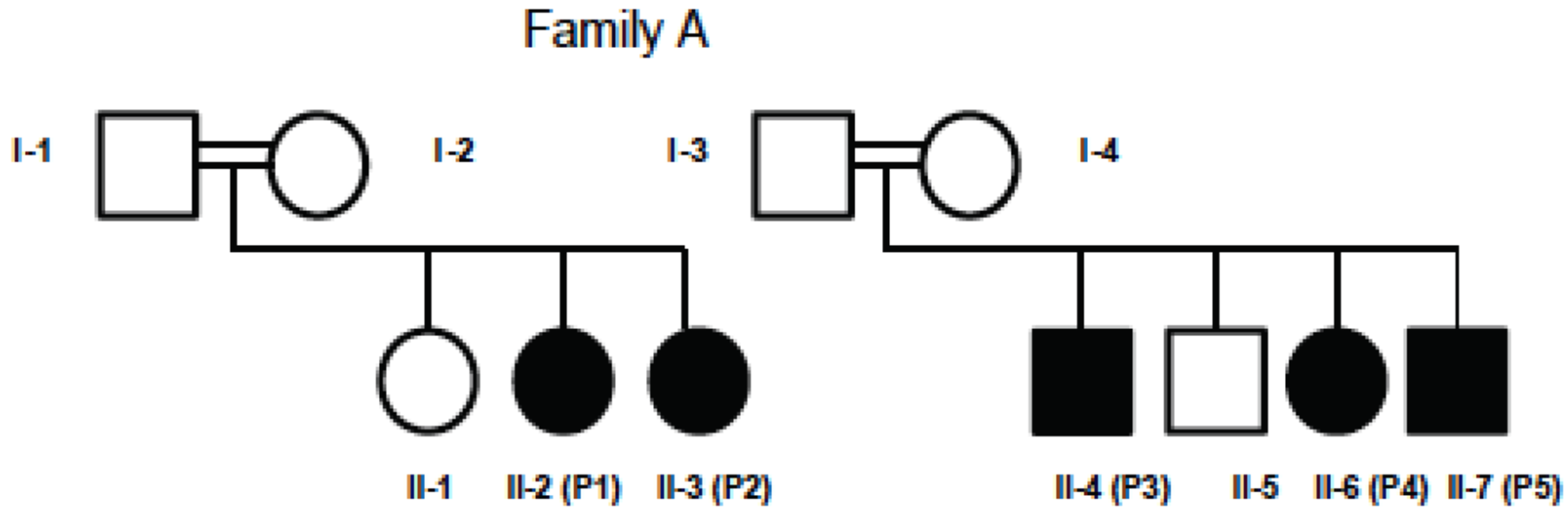
Disease	Gene	Expression	Phenotype
GSD1a	G6PC1	Liver, kidney, intestine	GSD
GSD1b	G6PT	ubiquitous	GSD + CN
G6PC3-deficiency	G6PC3	ubiquitous	CN





JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia
Boztug K., et al., *Nature Genetics* **46**, 1021–1027 (2014)

JAGN1-mutant granulocytes are characterized by ultrastructural defects, absence of secretory vesicles and aberrant N-glycosylation of multiple proteins, and increased apoptosis.



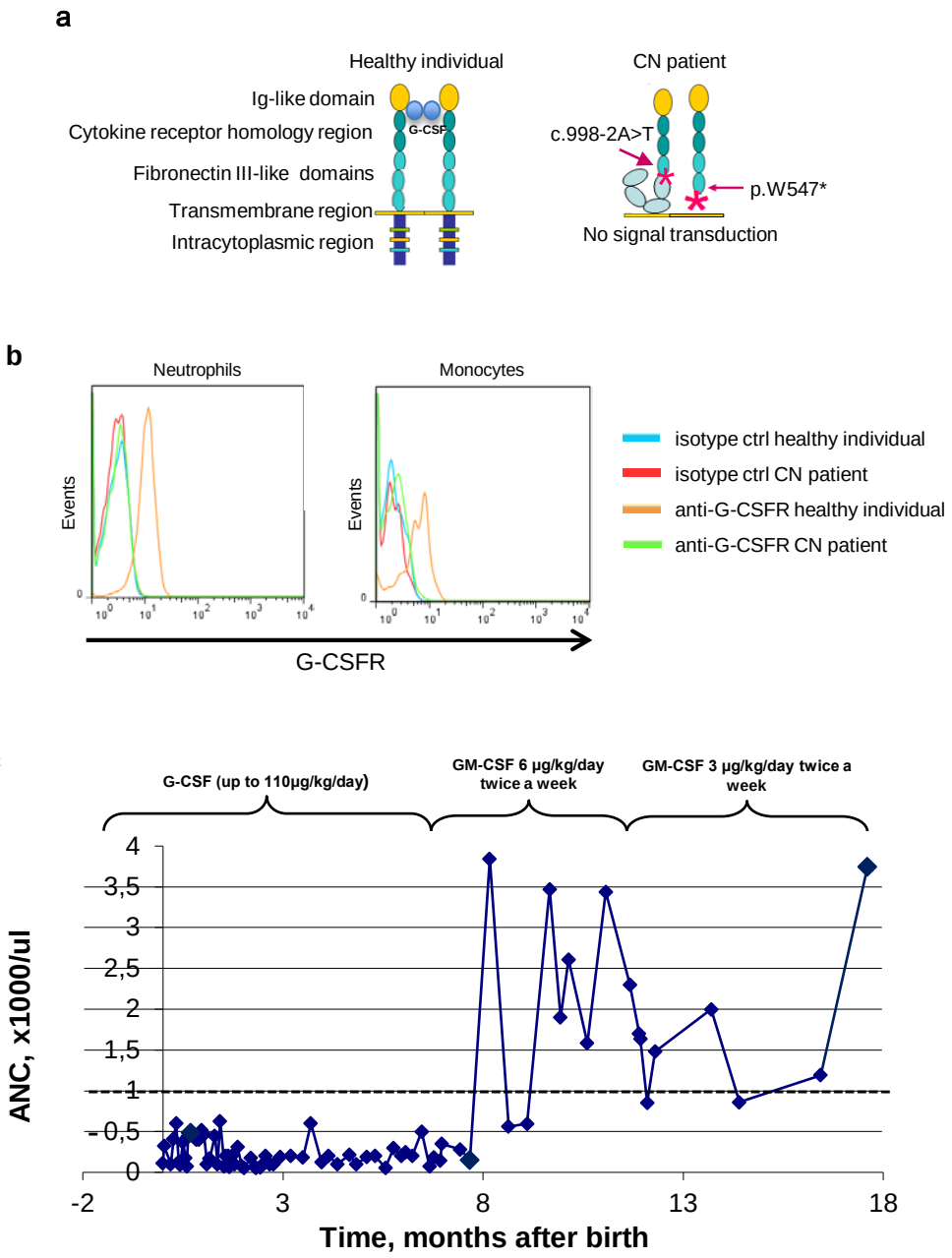
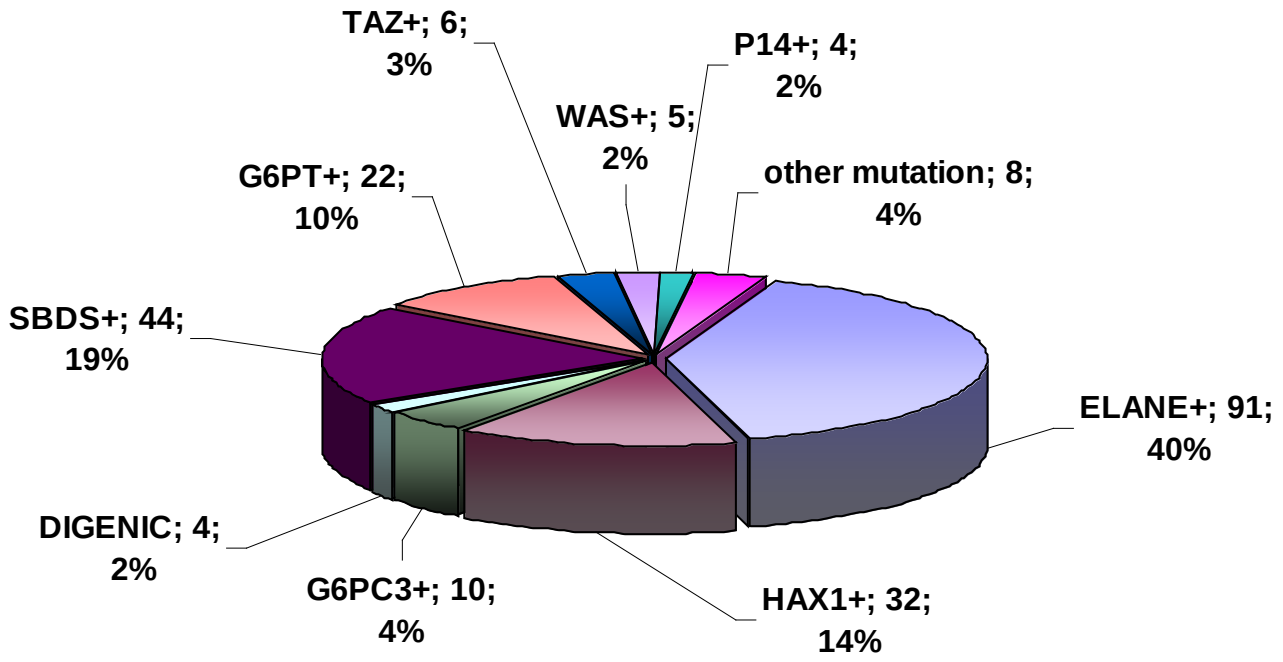


Figure 1



Distribution of gene mutations in 226 European congenital neutropenia patients

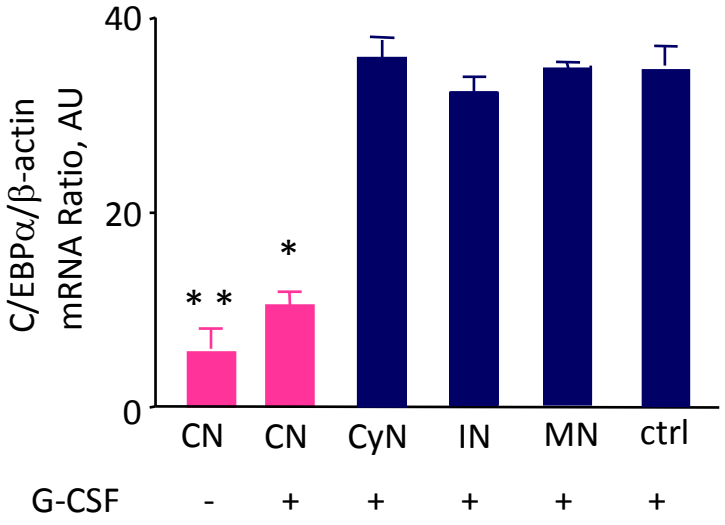
Neutropenia causing mutations

- Most of cases of SCN are attributable to ELANE mutations, but there are
- mutations in genes affecting G-CSF signaling (CSF3R, HAX1)
- genes affecting glucose homeostasis (SLC37A4, G6PC3),
- lysosomal function (LYST, RAB27A, ROBLD3/p14, AP3B1, VPS13B, TCIRG1),
- ribosomal proteins (SBDS, RMRP), mitochondrial proteins (HAX1, TAZ),
- immune functions (STK4, GFI1, CXCR4), and X-linked (WAS)
- ultrastructural defects, absence of secretory vesicles and

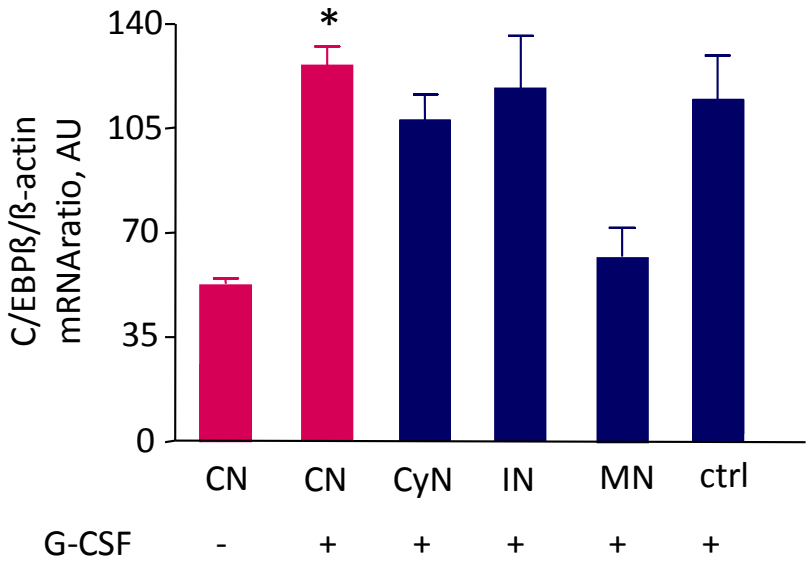
How does G-CSF induce granulopoiesis (overcome senescence) in CN, if both LEF-1 and HCLS1 are severely downregulated?

How does G-CSF induce granulopoiesis in CN?

LEF-1 dependent **steady-state** granulopoiesis

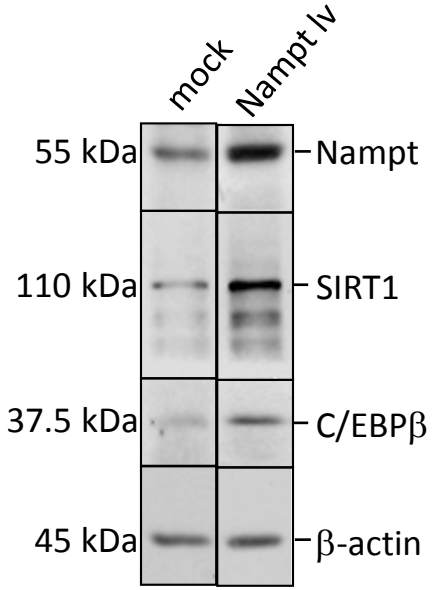


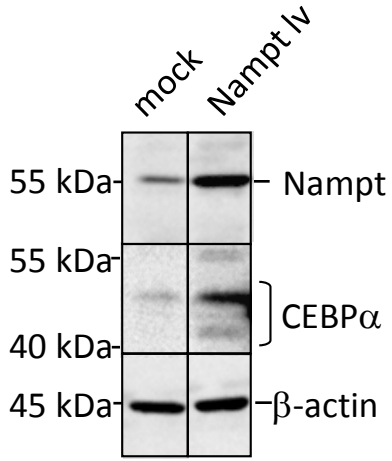
LEF-1 independent **emergency** granulopoiesis

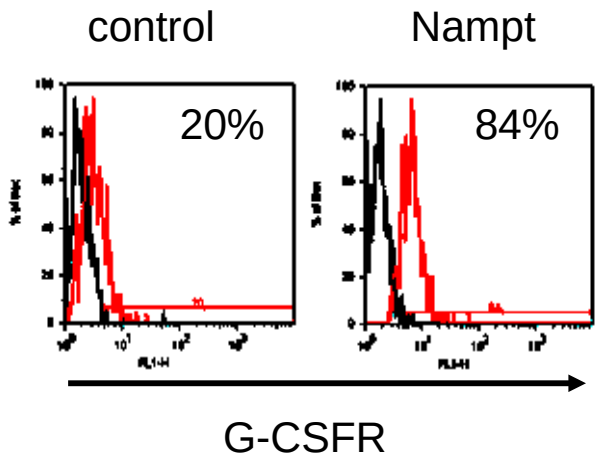


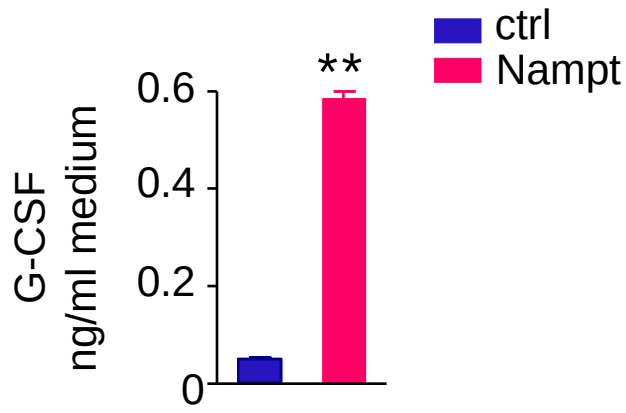
G-CSF induces C/EBPß in CN!

Nampt triggers myeloid differentiation of CD34^{+} cells

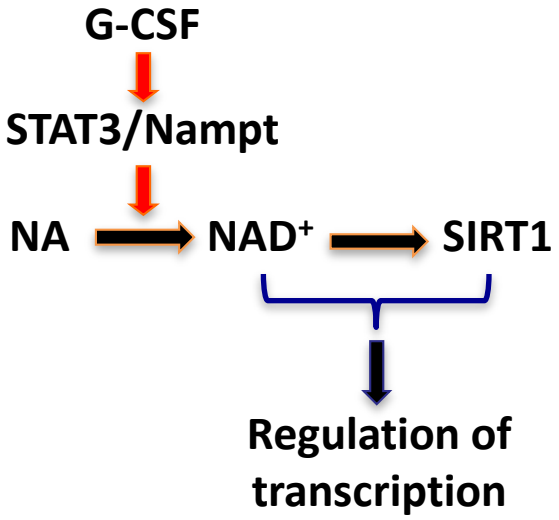


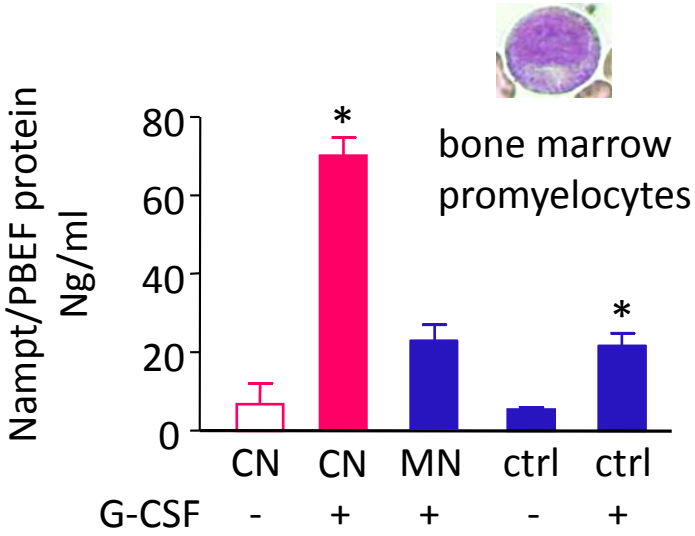


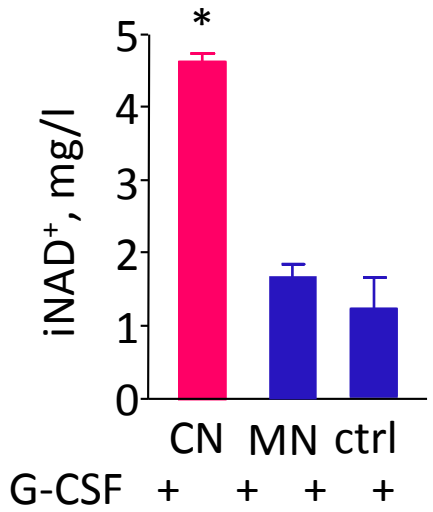


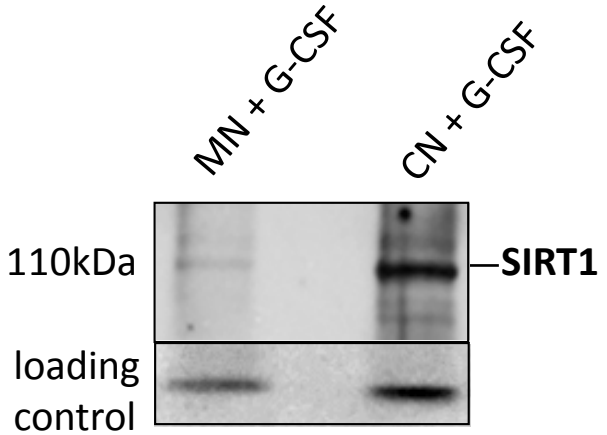


G-CSF induces Nampt/PBEF and NAD+ in myeloid progenitors from CN patients



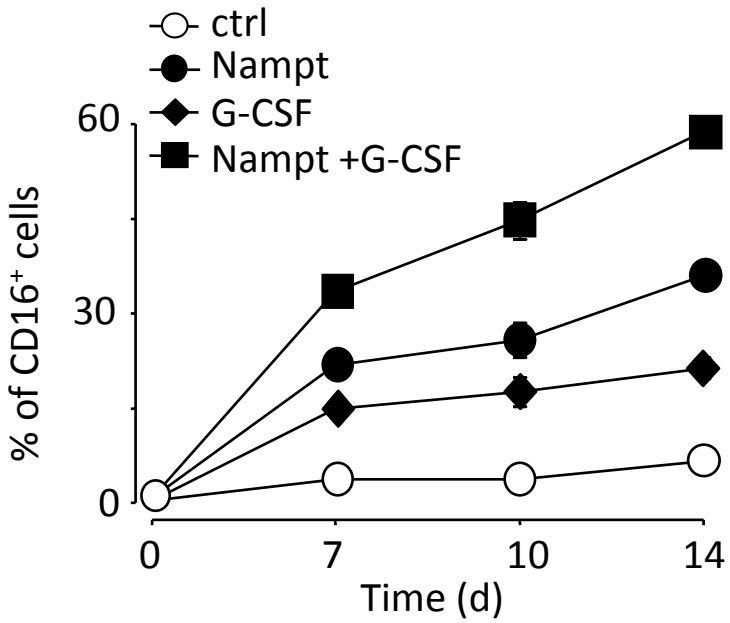


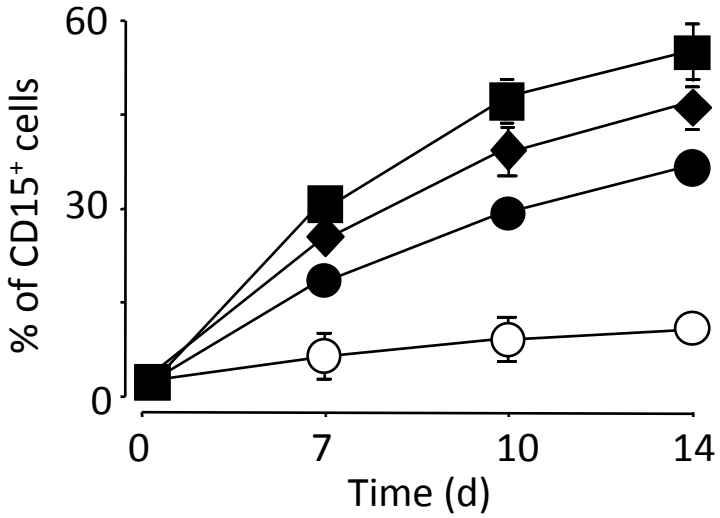


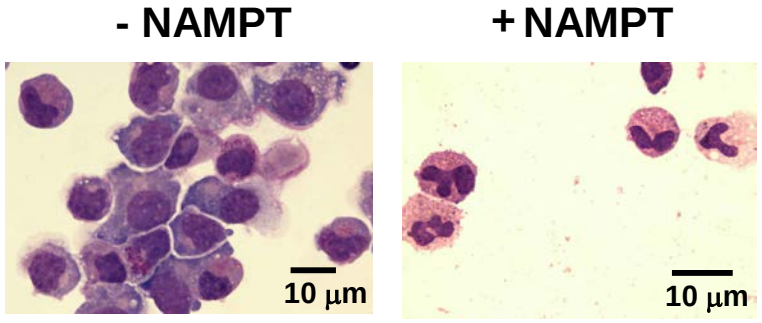


Skokowa, J., et al., Nat Med 2009; 15: 151- 8

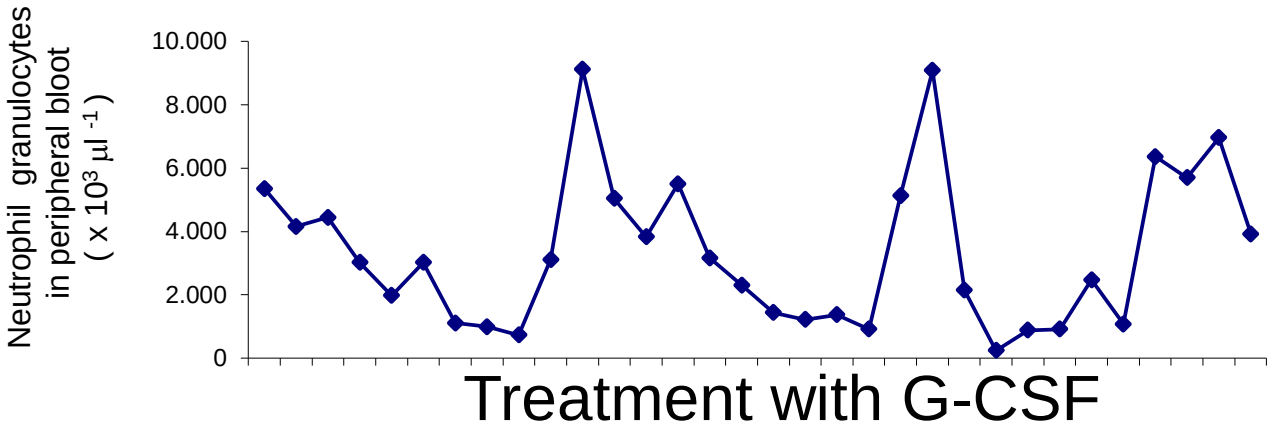
Nampt triggers myeloid differentiation of CD34$^+$ cells



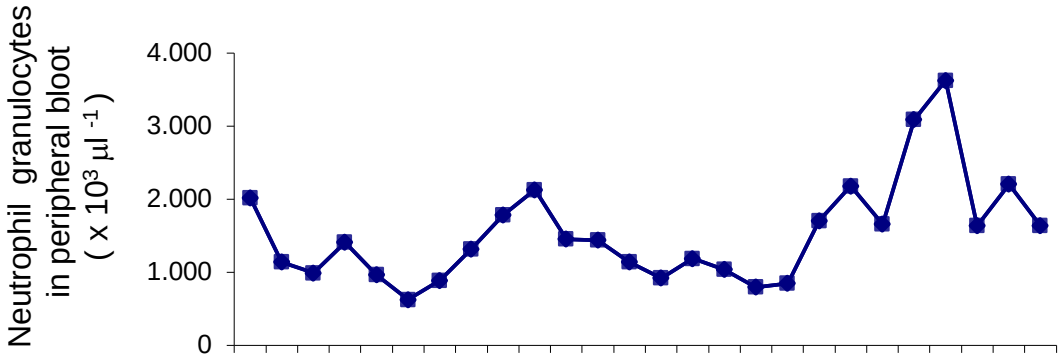




Vitamin B3 treatment of patient with cyclic neutropenia

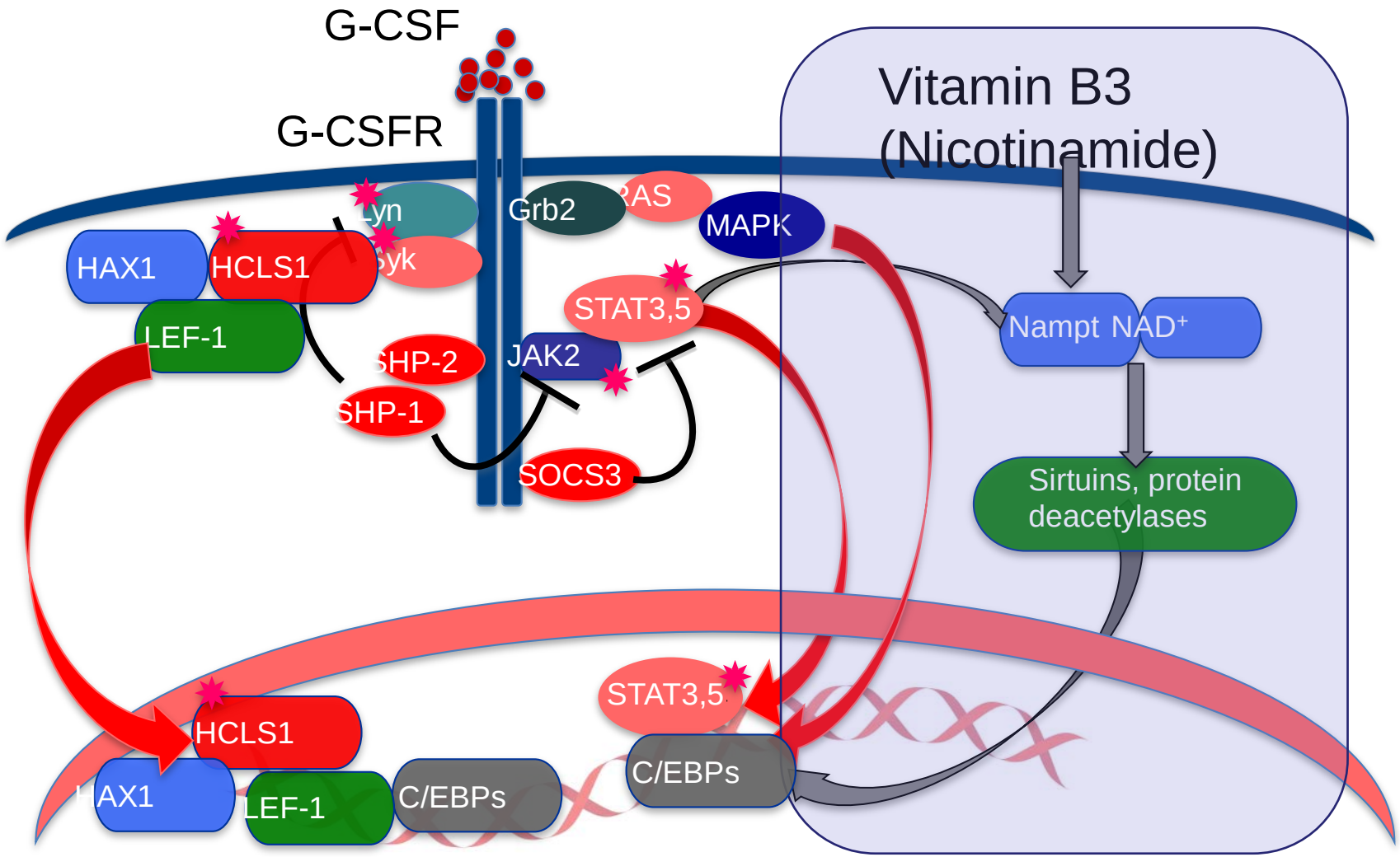


Treatment with G-CSF



Treatment with Vitamin B3 without G-CSF

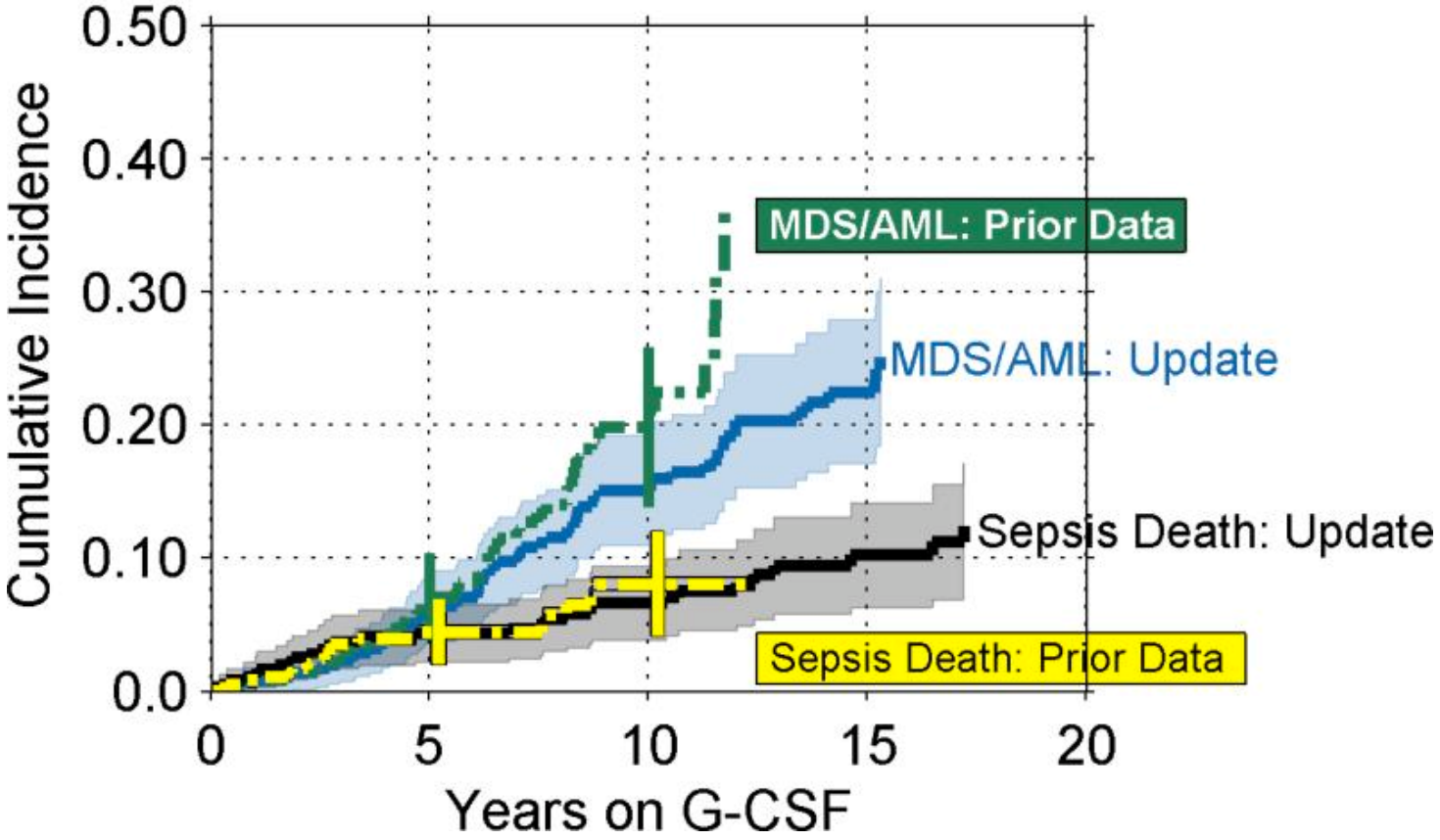
G-CSF signaling pathways



Nampt: Skokowa J, et al, Nat. Med 2009

Risk of leukemia in CN patients

First reports on leukemias in CN:
Miller RW, (Period 1960-66). Pediat Res **1969**
Gilman PA, et al., Blood **1970**

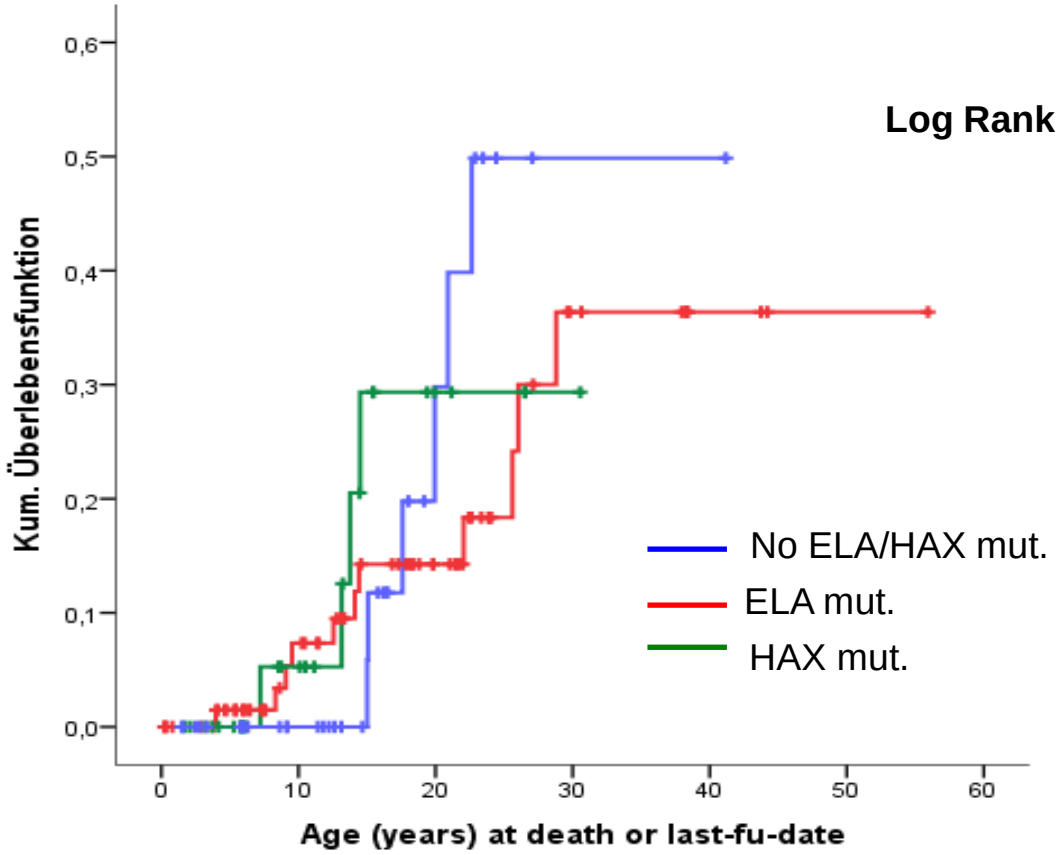


G-CSF Treatment by Neutropenia-Genotype

Neutropenia Code	No Leukemia (n)	Median G-CSF dose (µg/kg/d)	Leukemia (n)	Median G-CSF dose (µg/kg/d)
ELANE-CN	72	4,9	11	18,7
HAX1-CN	25	3,5	6	7,05
ELANEneg/HAX1neg	19	11,7	6	15,05
neg tested	15	4,43	1	4,86
WAS	3	3,23	2	3,09
SDS	6	1,72	1	4,3
CN not tested	44	5,69	6	5,22
GSD1B	19	3,21	1	3,0
CyC not tested	24	1,53	2	10,76

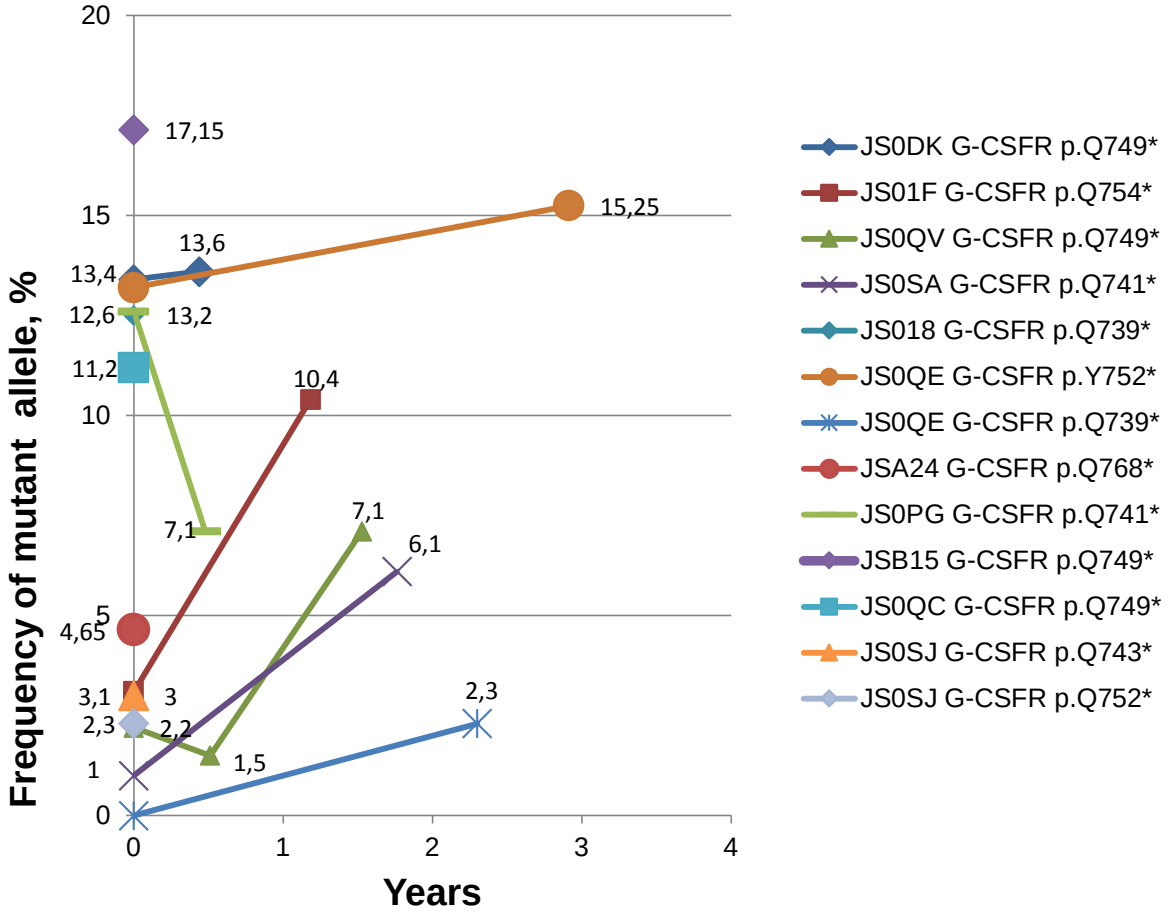
* Median G-CSF Dose for all Congenital Patients **4,85** µg/kg/d
and for all Cyclic Patients **1,6** µg/kg/d

Congenital Neutropenia
Incidence of Leukemia
CI at 30 Years by Genetic Subtype

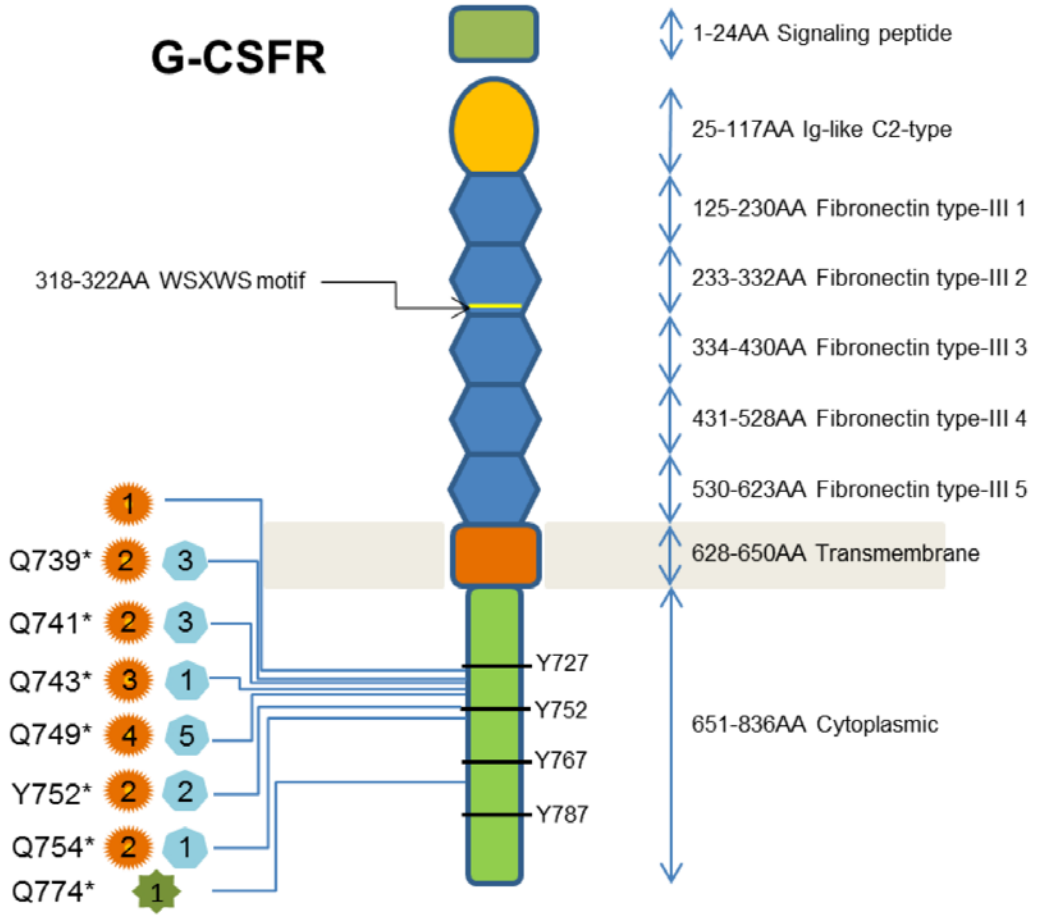


Events/N	6/35
Events/N	11/74
Events/N	4/31

VAFs of *CSF3R* mutant clones in CN and CN/AML patients

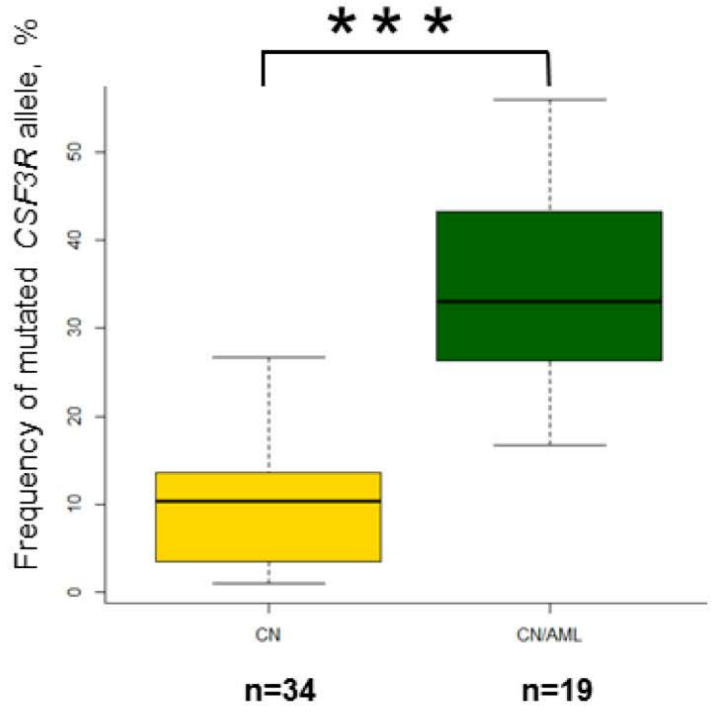


CSF3R mutations



VAFs of CSF3R mutant clones

in CN and CN/AML patients.



Leukemia-associated mutations in 31 CN/AML patients

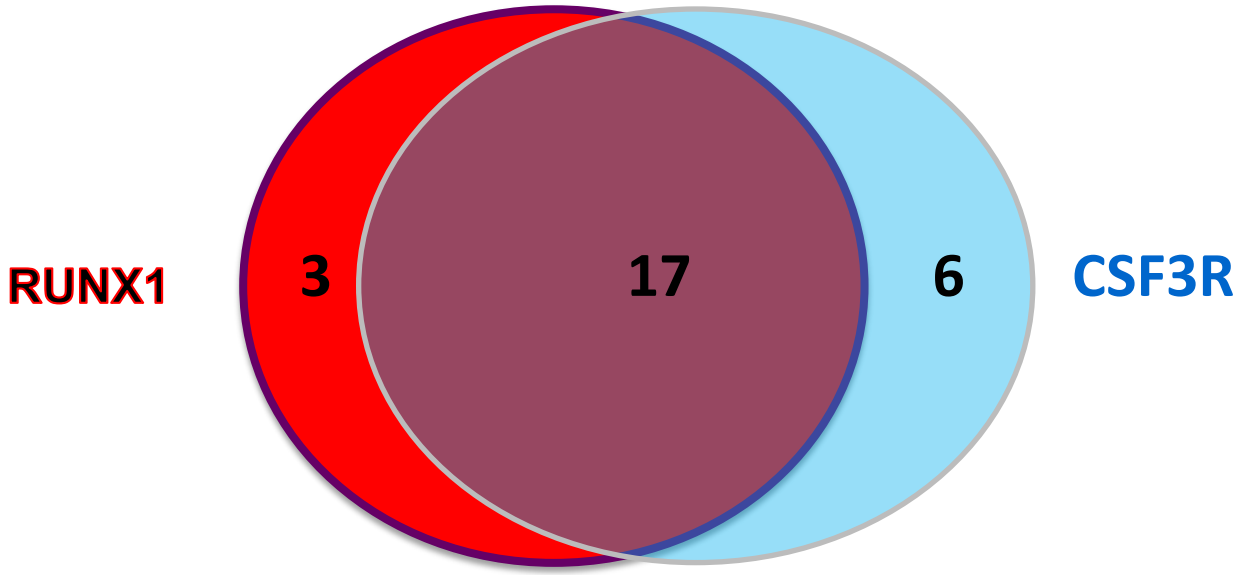
Targeted deep sequencing

23 (74 %) *CSF3R*
20 (64,5 %) *RUNX1*

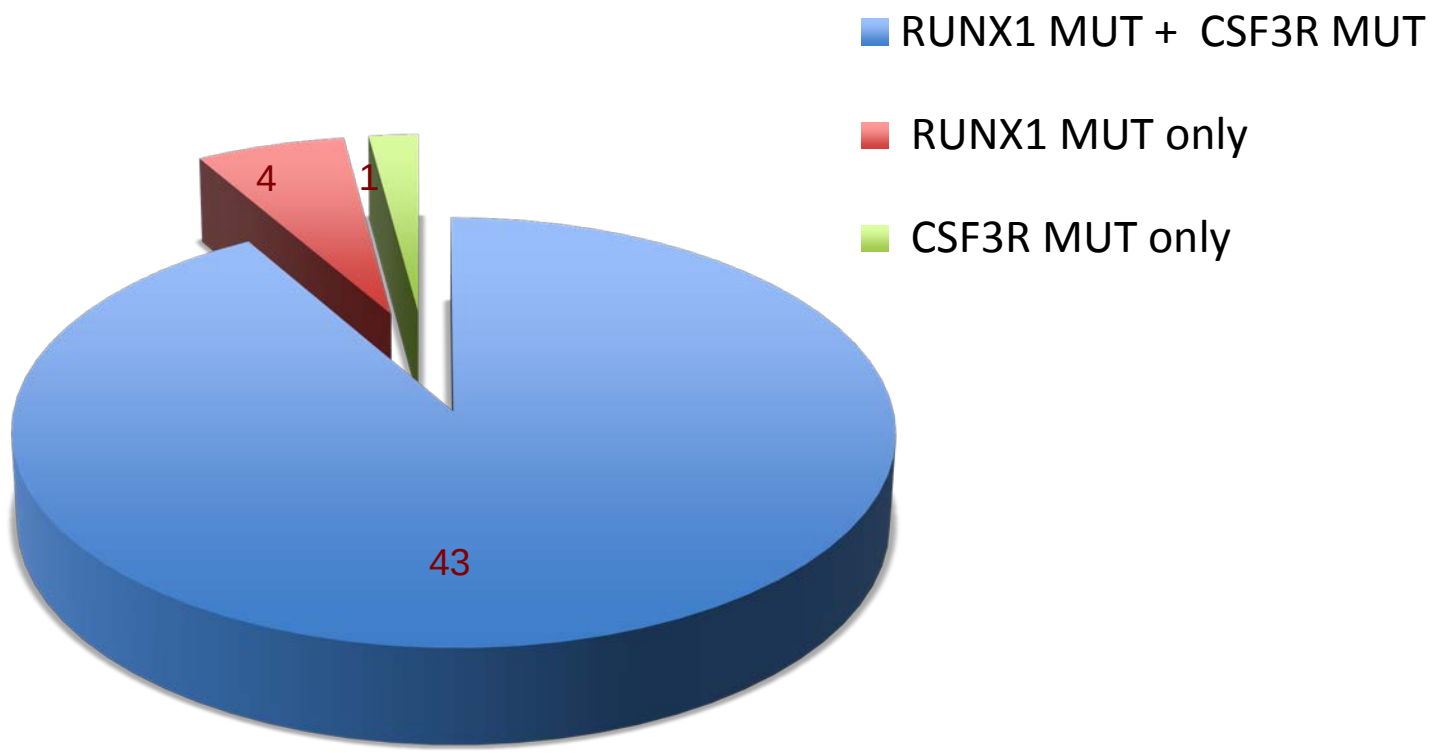
2 *FLT3-ITD*
4 *EP300*
2 *SUZ12*
1 *CREBB*
1 *CBL*
1 *NRAS*

!!! Neg. for: *CEBPA, DNMT3A, IDH1, IDH2, NPM1, TET2*

High frequency of cooperating *RUNX1* and *CSF3R* mutations in 31 CN/AML patients



Segregation of *RUNX1* and *CSF3R* mutations in blasts of CN/AML patient

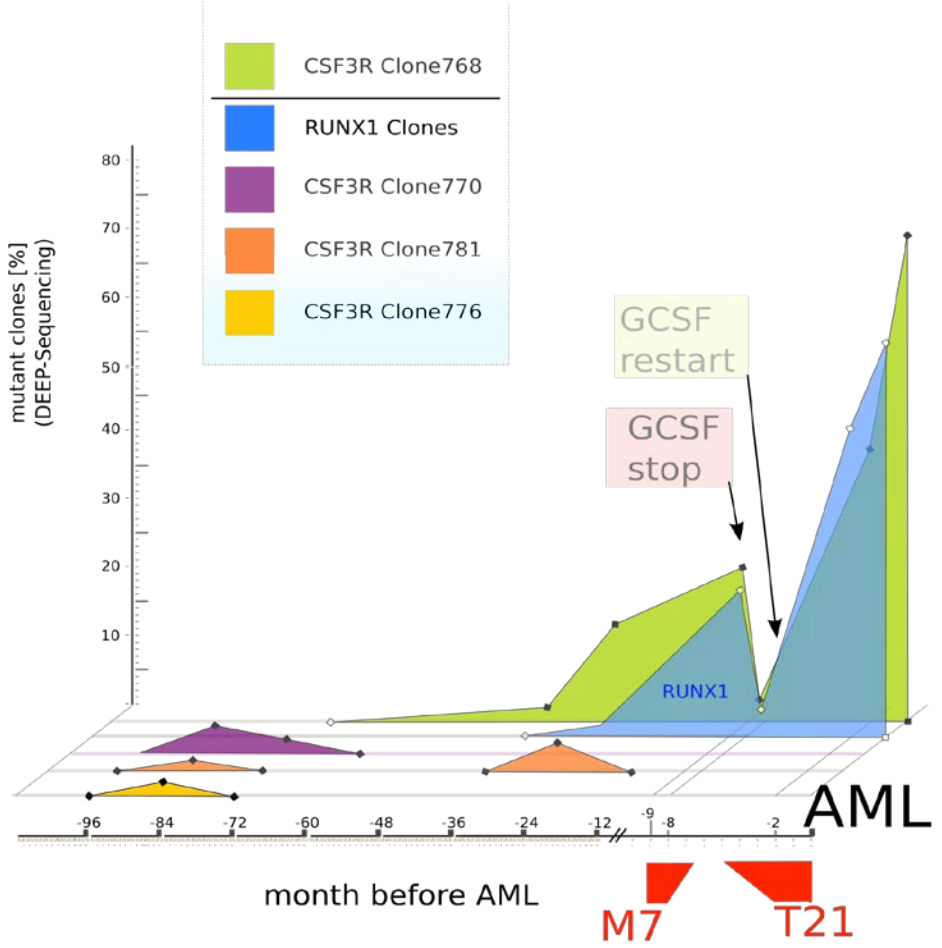


N=48

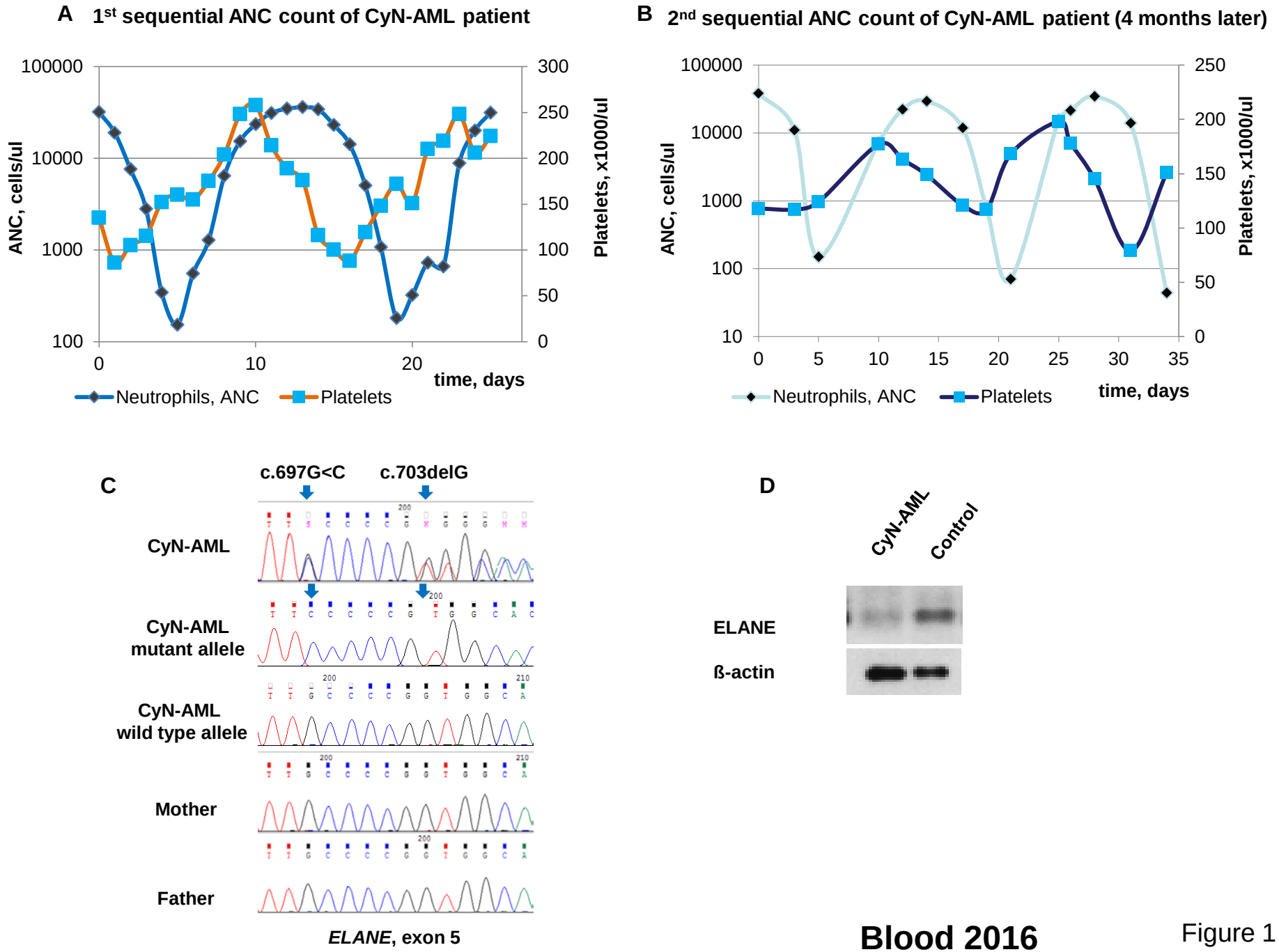
First detection of *CSF3R*- and *Runx1* mutations in months prior to AML

Patient	*CSF3R* mut	*Runx1* mut
# 6	-192	-36
# 7	-36	-12
# 10	-36	- 1
# 14	-24	- 8
# 16	-60	-4
# 19	-36	-9

G-CSF treatment in combination with mutations in *CSF3R* and *RUNX1* are leukemogenic



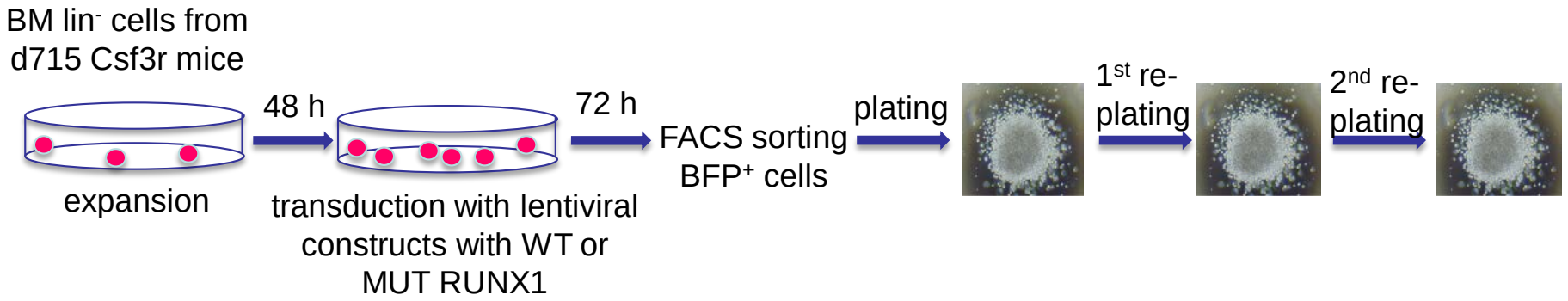
Skokowa et al., EHA 2014 Presidential Symposium

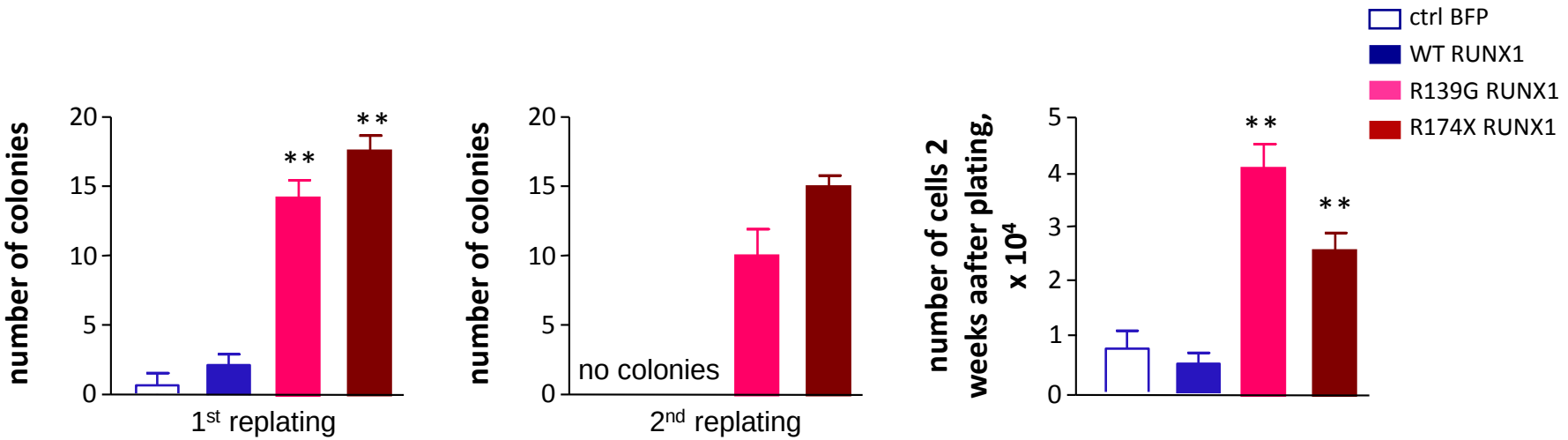


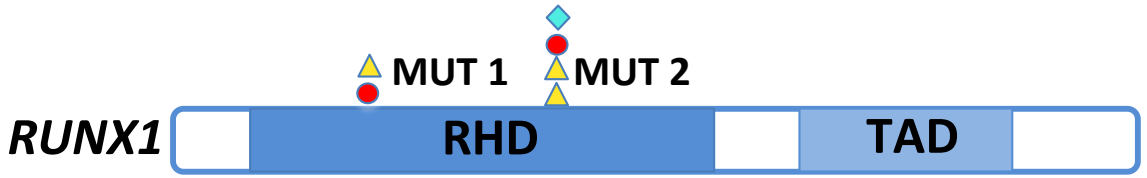
Blood 2016

Figure 1

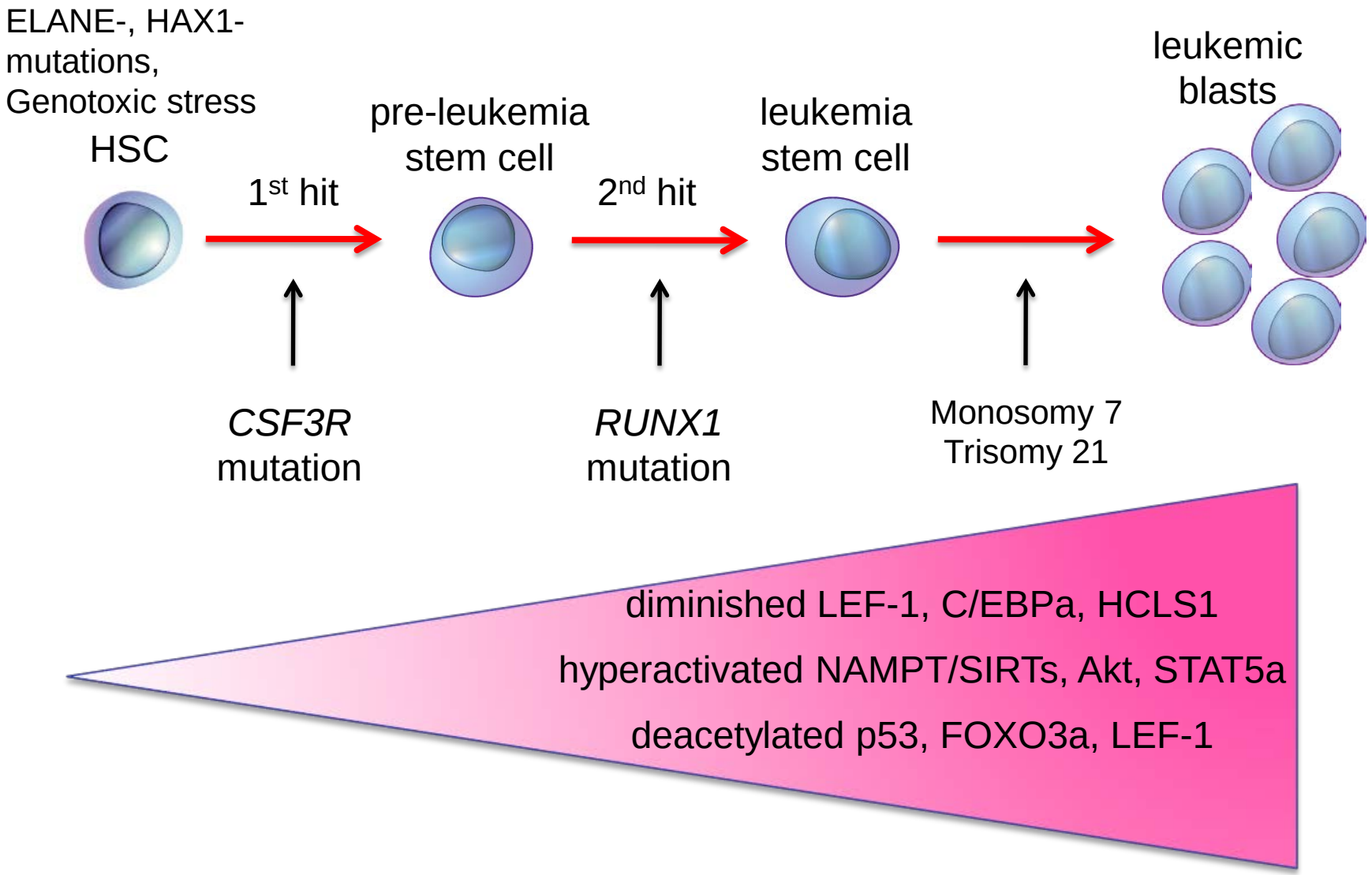
Mutated RUNX1 enhanced clonogenic capacity of lin$^-$ cells from d715 Csf3r mice



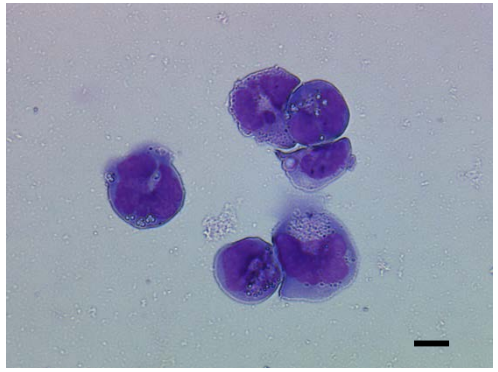




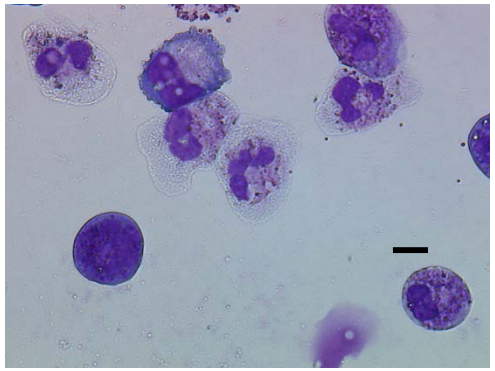
The two-hit hypothesis of leukemogenesis in CN



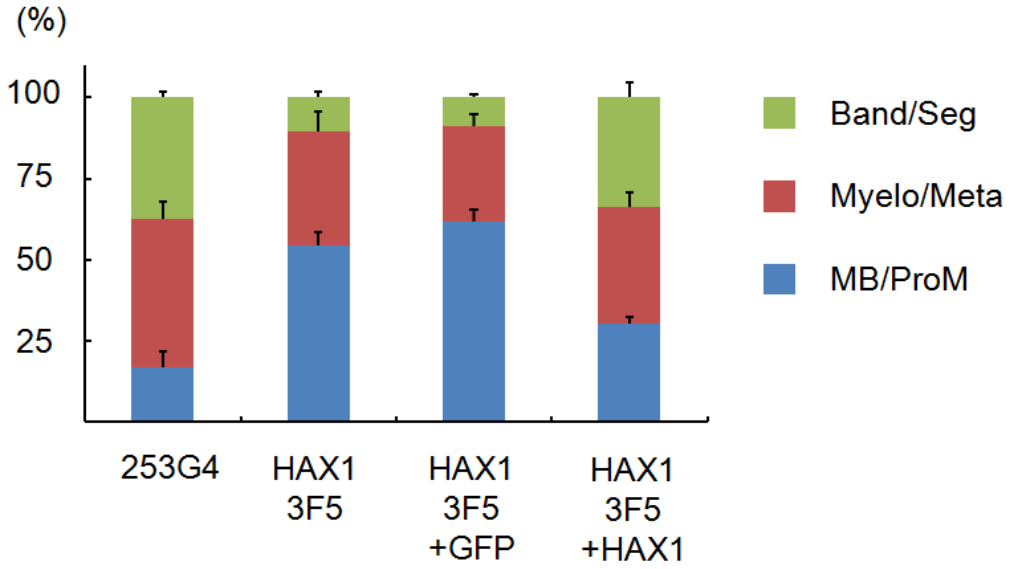
Improvement of maturation arrest after genetic correction



HAX1 3F5
+GFP

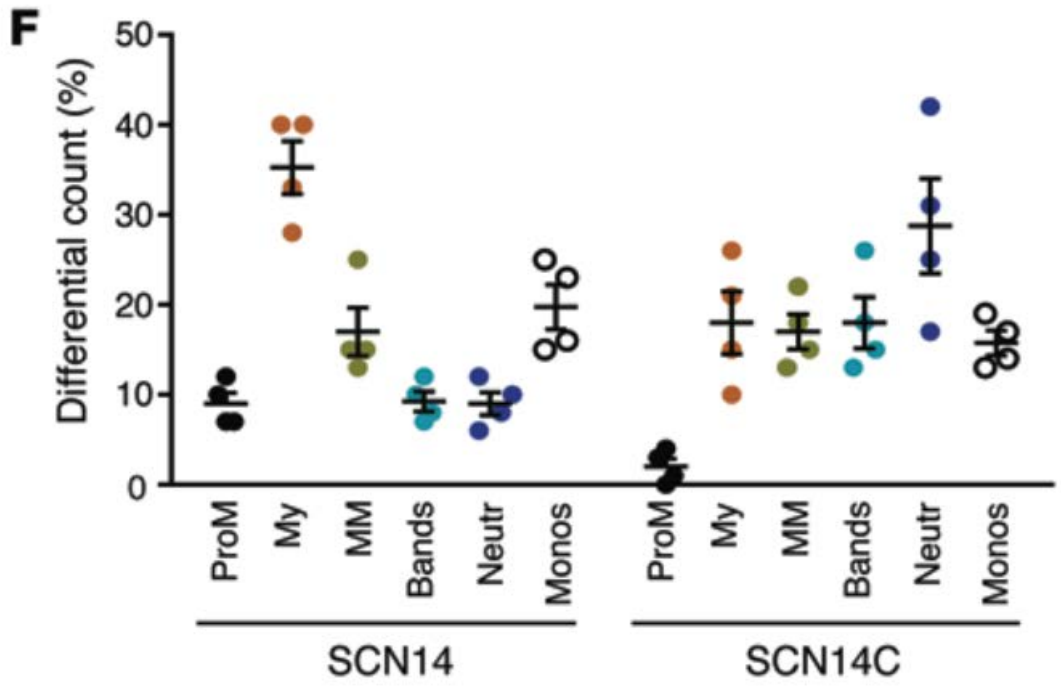


HAX1 3F5
+HAX1



Morishima T et al. Haematologica. 2014; 99:19-27.

Correction of ELANE mutations in iPSCs from a patient with congenital neutropenia by CRISP/Cas9 technology



Nayak RC, et al. JCI 2015

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