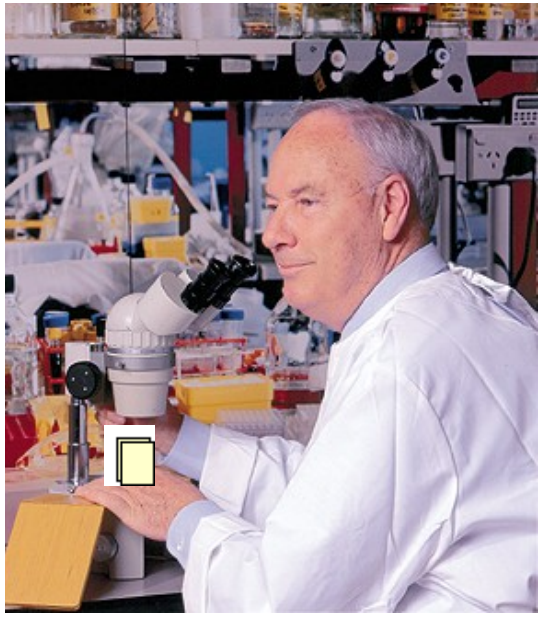
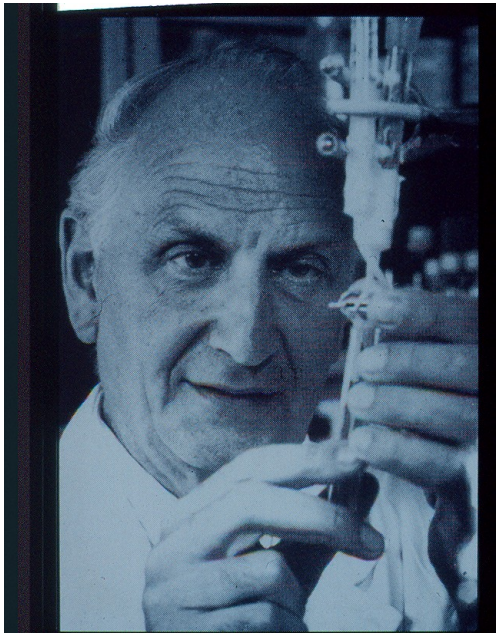
G-CSF – a wonderful molecule

Prof. Dr. med. Karl Welte

Medizinische Hochschule
Hannover



1964



Donald Metcalf

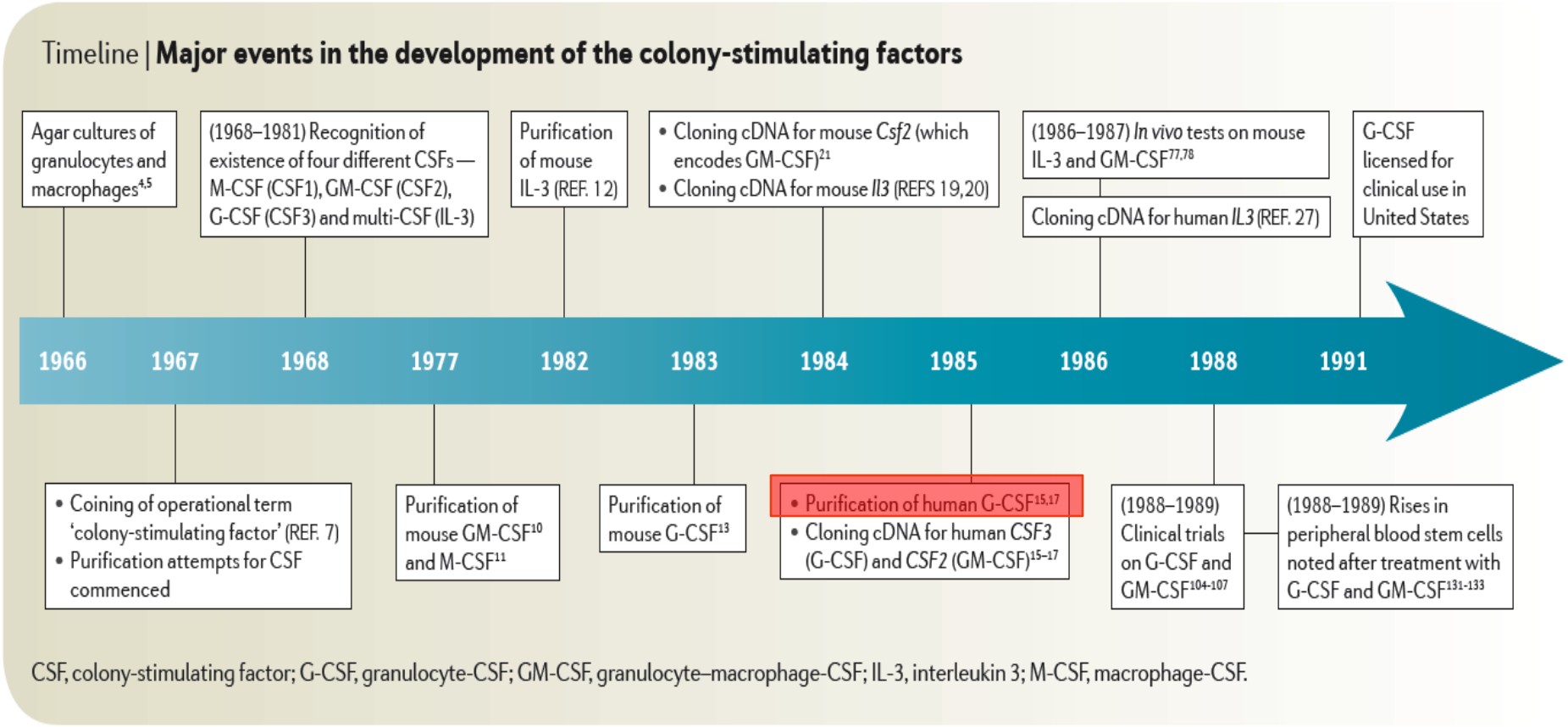
The growth of mouse bone marrow cells in vitro. Aust J Exp Bio Med Sci 1966

Leo Sachs

The cloning of normal mast cells in tissue cultures. J.Cell.Physiol. 1965

The early 1960s

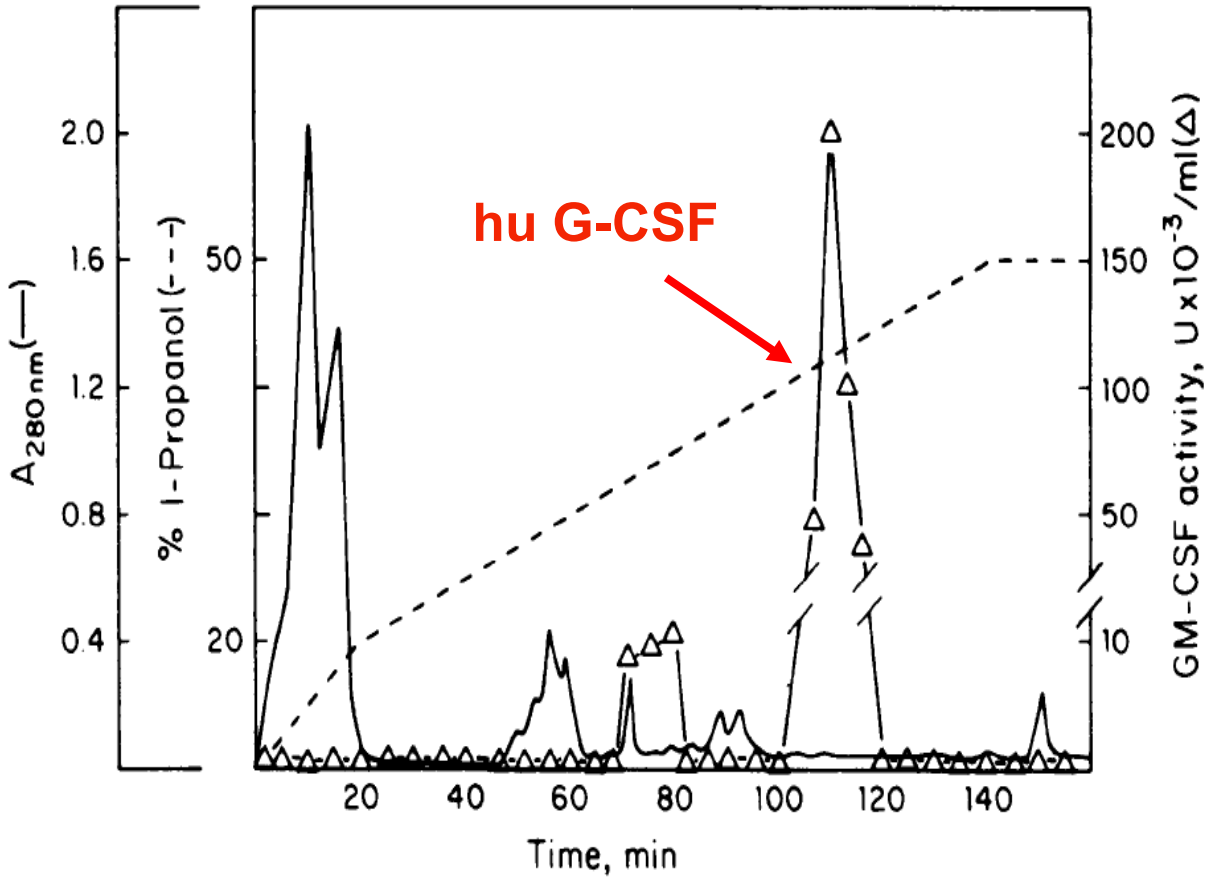
Metcalf, D., Nature Review Cancer, 2010

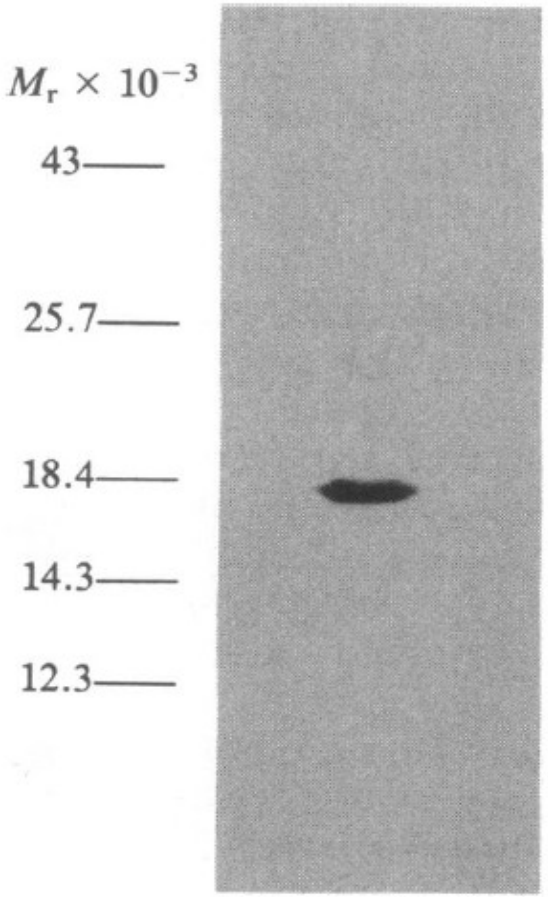


CSF, colony-stimulating factor; G-CSF, granulocyte-CSF; GM-CSF, granulocyte–macrophage-CSF; IL-3, interleukin 3; M-CSF, macrophage-CSF.

Purification of G-CSF

(40 L supernatant of the 5637 cell line: 5 µg G-CSF)





Welte, K., et al., PNAS, 1985

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1526–1530, March 1985
Medical Sciences

Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor

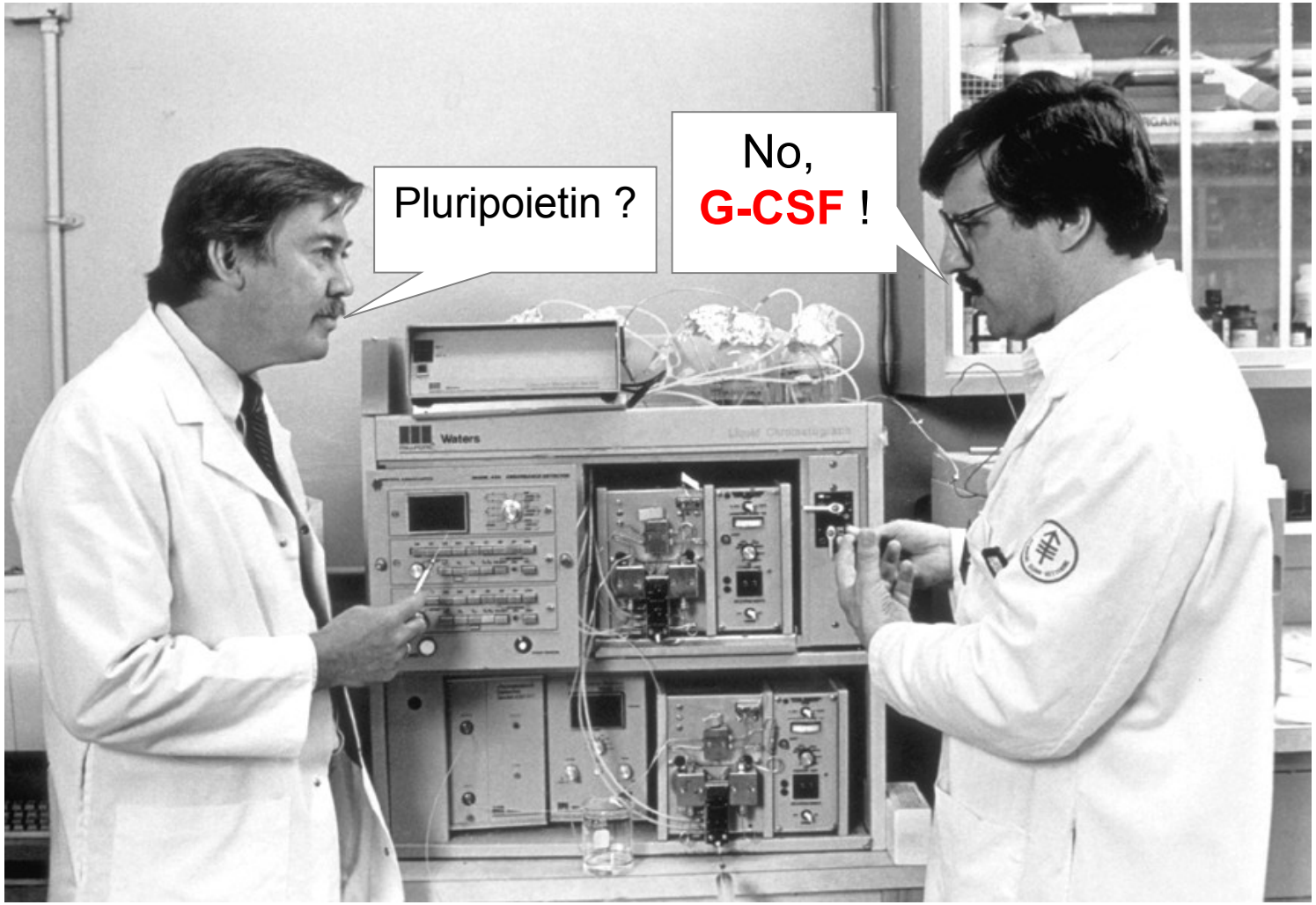
(protein purification/hematopoietic stem cells)

KARL WELTE, ERICH PLATZER, LI LU, JANICE L. GABRILOVE, ESTER LEVI, ROLAND MERTELSMANN, AND MALCOLM A. S. MOORE

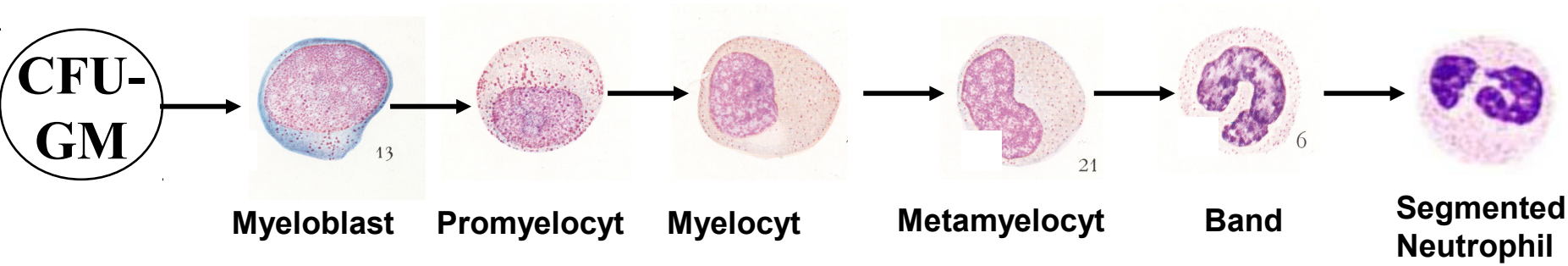
Laboratory of Developmental Hematopoiesis, and Laboratory of Molecular Hematology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Communicated by Lewis Thomas, October 24, 1984

MSKCC New York, G-CSF-Laboratory, 1984



Effects of G-CSF on Myelopoiesis



Proliferation → **Anti-apoptosis**

Differentiation →

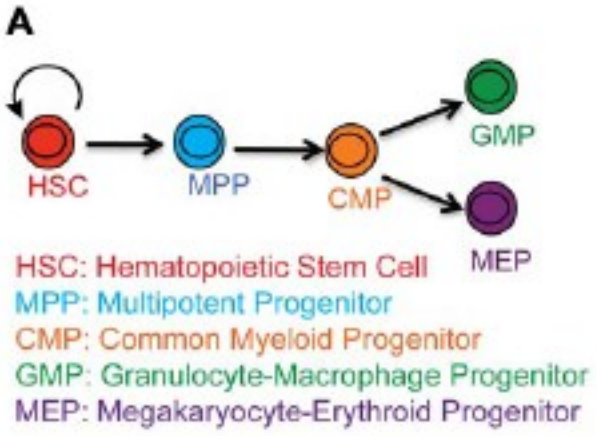
Table 2. Comparison of CFU-GEMM and BFU-E activities of pluripotent CSF (GM-CSF activity, 500 u/ml)

	CFU-GEMM			BFU-E		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Medium*	0.3 ± 0.3	0	0	42 ± 6	17 ± 3	17 ± 2
LCM†	7 ± 1	3 ± 0	3.3 ± 0.3	67 ± 1	65 ± 3	34 ± 3
PP-CSF‡	7.7 ± 2.1	4 ± 0.8	2.3 ± 0.9	85 ± 6	31 ± 1	28 ± 2

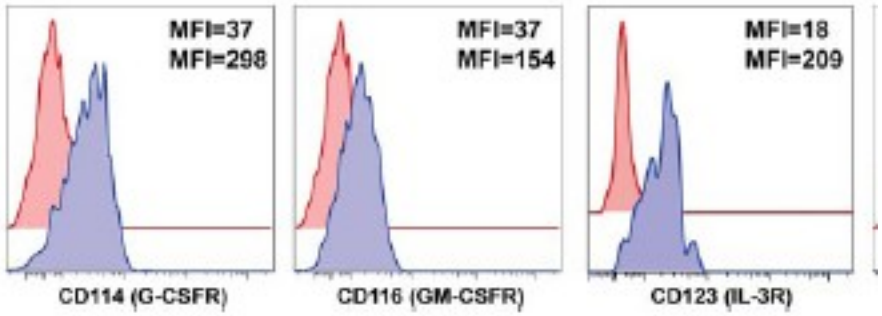
Target cells were 5×10^4 per ml of low density, nonadherent, and T-cell-depleted normal human BM cells. Experiment 3 was done in the absence of hemin. The number of colonies is shown as the mean ± SEM.

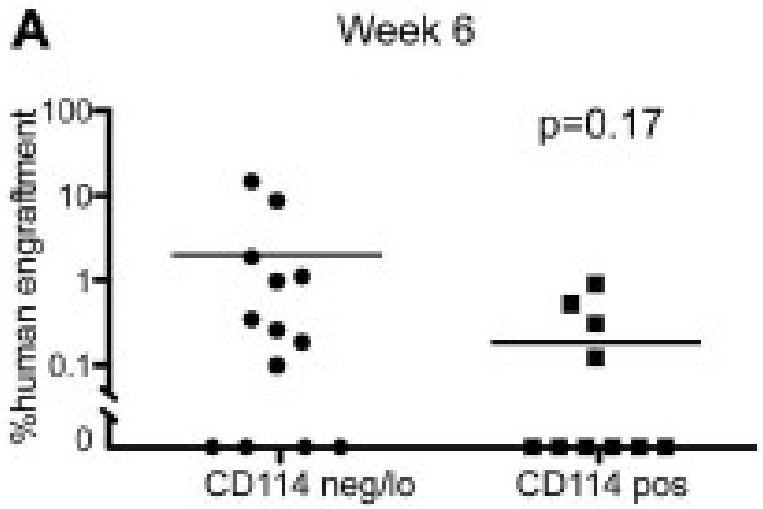
*Iscove's modified Dulbecco medium plus 30% FCS.

Gibbs KD, ..., Weissman, IL, et al., Blood 2011



HSC





Engraftment of G-CSFR (CD114) positive cells in NSG-mice

AMINO ACID SEQUENCE REPORT

Sample Number: 062184A Sample submitted by: K.W./L.S.

Sample: Pluripactin

Laboratory: _____ Phone Ext.: _____

Sequence analyzed by: PL/RE/CG

Amount of sample sequenced: 100 % Pmoles _____ µg
nmoles _____ mg

Total cycles run 30

Sequencing method: ABI 470A / Beatice

Pretreatment at protein laboratory: _____

Sequence identified: T-P-L-G-P-A-S-(S)-L-P-Q-X-M-
(M)-X-K-(R)-X-X-(R)-(L)-X-

Recombinant Human Granulocyte Colony-Stimulating Factor: Effects on Normal and Leukemic Myeloid Cells

LAWRENCE M. SOUZA, THOMAS C. BOONE, JANICE GABRILOVE, POR H. LAI, KRISZTINA M. ZSEBO, DOUGLAS C. MURDOCK, VICKI R. CHAZIN, JOAN BRUSZEWSKI, HSIENG LU, KENNETH K. CHEN, JEAN BARENDT, ERICH PLATZER, MALCOLM A. S. MOORE, ROLAND MERTELSMANN, KARL WELTE

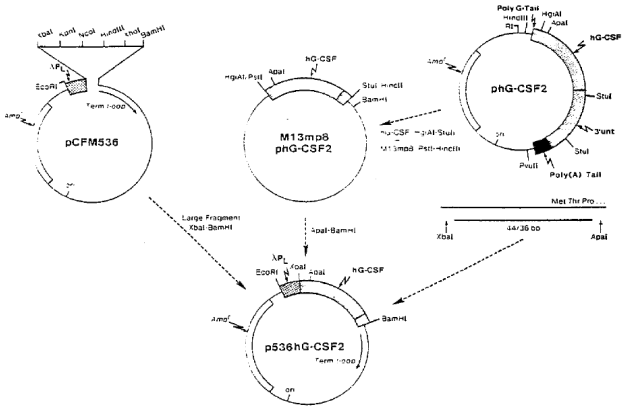
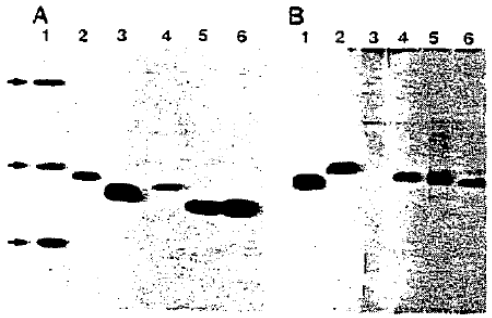


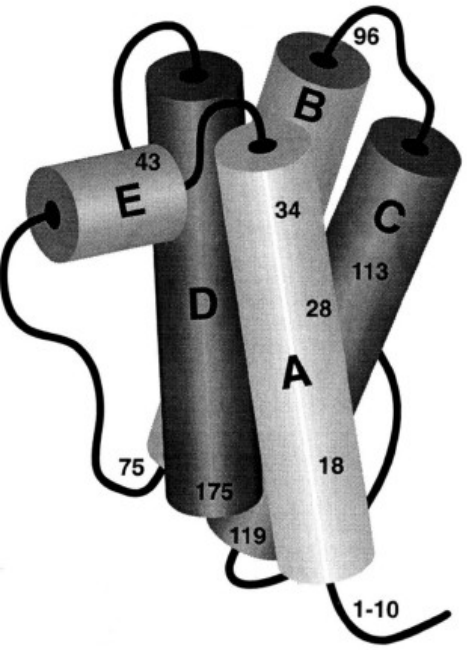
Fig. 3. Schematic of the constructions used to generate an *E. coli* expression vector for production of nonglycosylated phG-CSF.

cDNA



Protein

Souza et al., Science, 1986



Welte, K., et al., PNAS 1985
Souza, L., et al., Science 1986

Biochemical Characteristics of G-CSF:

174 amino acids
Glycosylation: yes
Molecular weight: 19.6 kD

Filgrastim (E.Coli):
175 amino acids
Glycosylation: no
MW18.7 kD,

Nagata, S., et al., Nature 1986,
G-CSF splice form : 177 amino acids

Poster in Wilsede 1984

Modern Trends in Human Leukemia VI

R Neth, RC Gallo, MF Greaves, et al.

Biological activities of a human pluripotent hemopoietic colony-stimulating factor.

Platzer E, Welte K, Lu L, Gabrilove JL, Yung YP, Nathan CF, Mertelsmann R, Moore MA.

Haematol Blood Transfus. 1985;29:418-22.

United States Patent [19]

Welte et al.

[54] **HUMAN PLURIPOTENT HEMATOPOIETIC COLONY STIMULATING FACTOR**

[75] Inventors: **Karl Welte**, New York, N.Y.; **Erich Platzer**, Spardorf, Germany; **Janice L. Gabrilove**, New York, N.Y.; **Roland Mertelsmann**, Chappaqua, N.Y.; **Malcolm A. S. Moore**, Larchmont, N.Y.

[73] Assignee: **Sloan-Kettering Institute for Cancer Research**, New York, N.Y.

[21] Appl. No.: **280,582**

[22] Filed: **Jul. 26, 1994**

Related U.S. Application Data

United States Patent [19]

Souza

[11]

[45]

[54] **HUMAN PLURIPOTENT GRANULOCYTE COLONY-STIMULATING FACTOR**

[75] Inventor: **Lawrence M. Souza**, Thousand Oaks, Calif.

[73] Assignee: **Amgen Inc.**, Thousand Oaks, Calif.

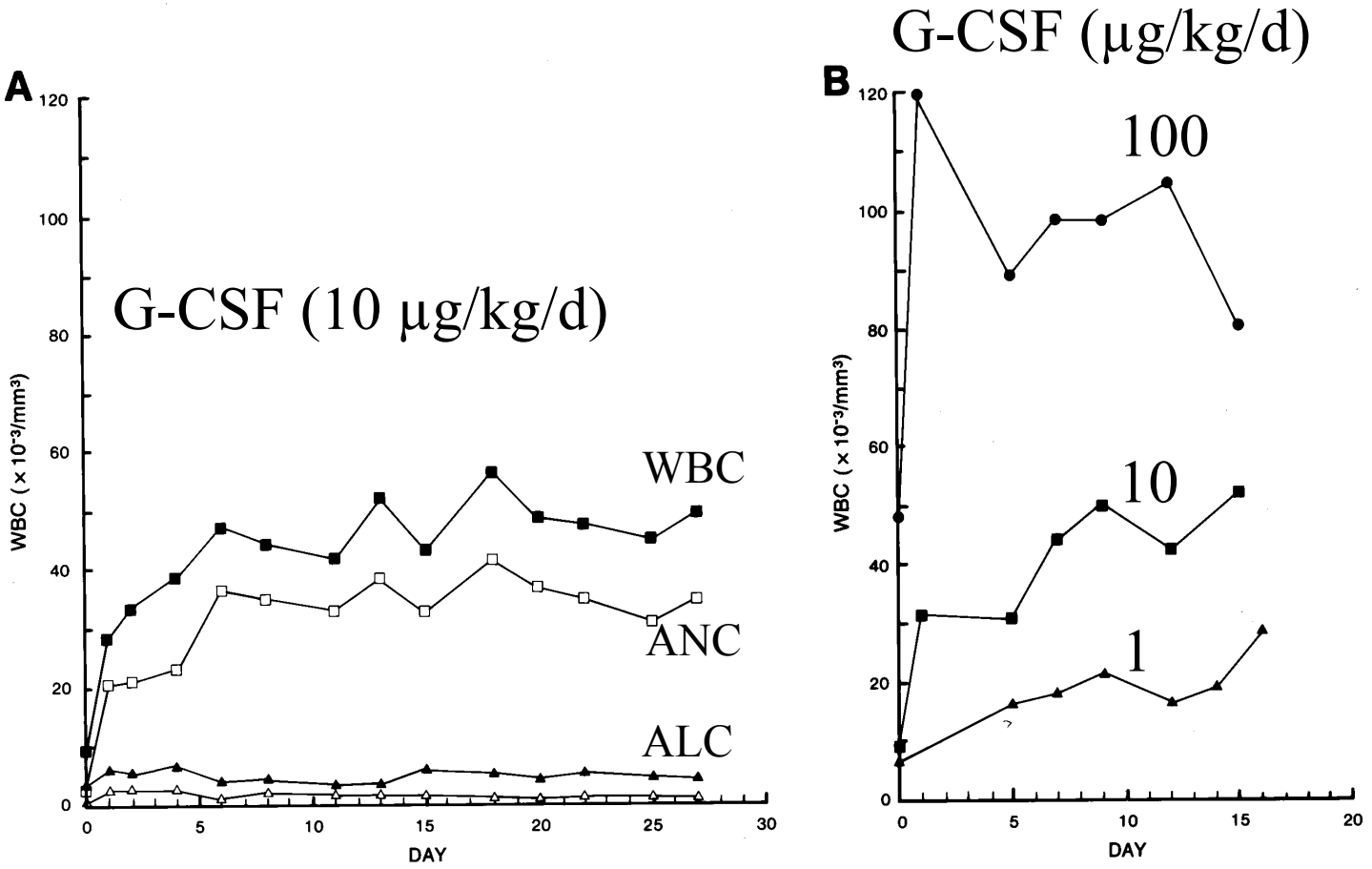
[21] Appl. No.: **459,298**

[22] Filed: **Jun. 2, 1995**

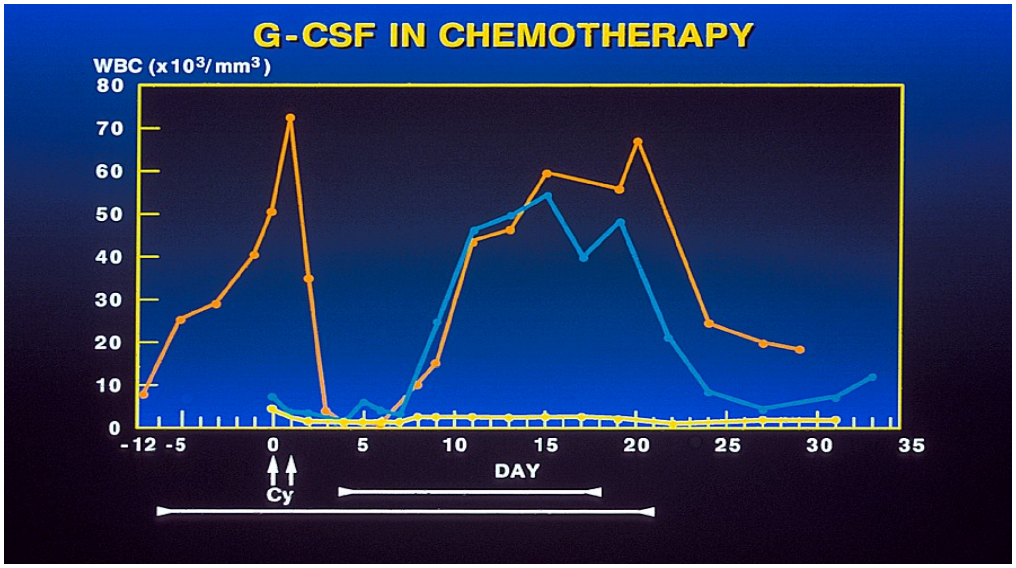
Related U.S. Application Data

G-CSF in Primates

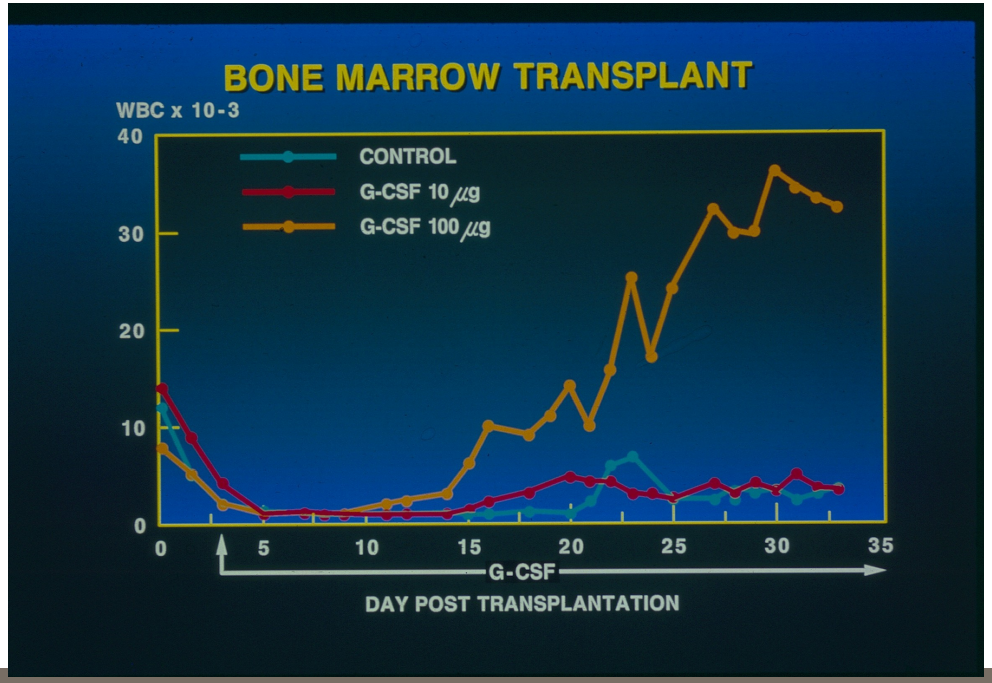
G-CSF in Primates



Welte, et al., J. Exp. Med. 165, 941, 1987



G-CSF in Primates

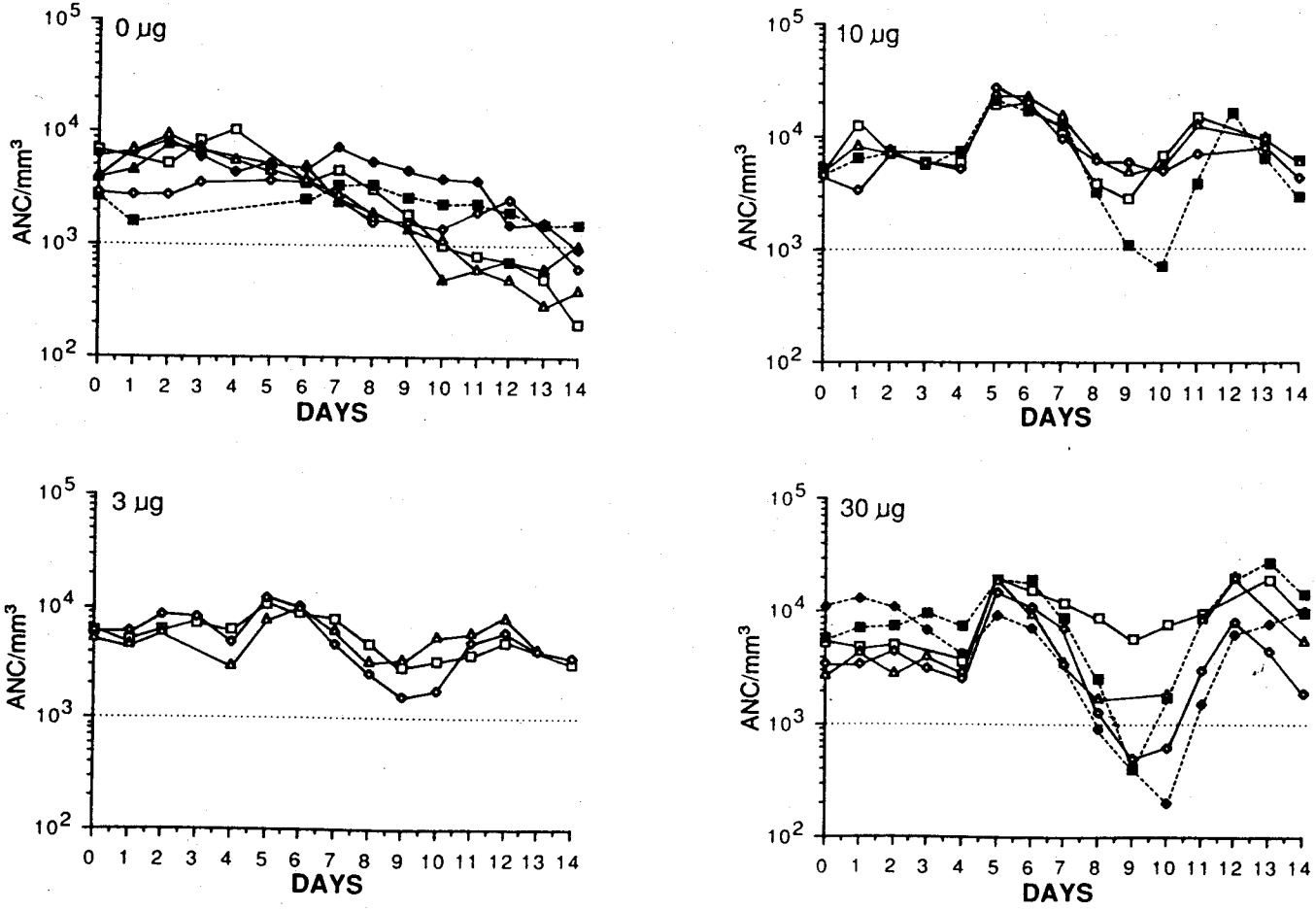


Welte, K., et al., J Exp Med 1987

N Engl J Med 1988

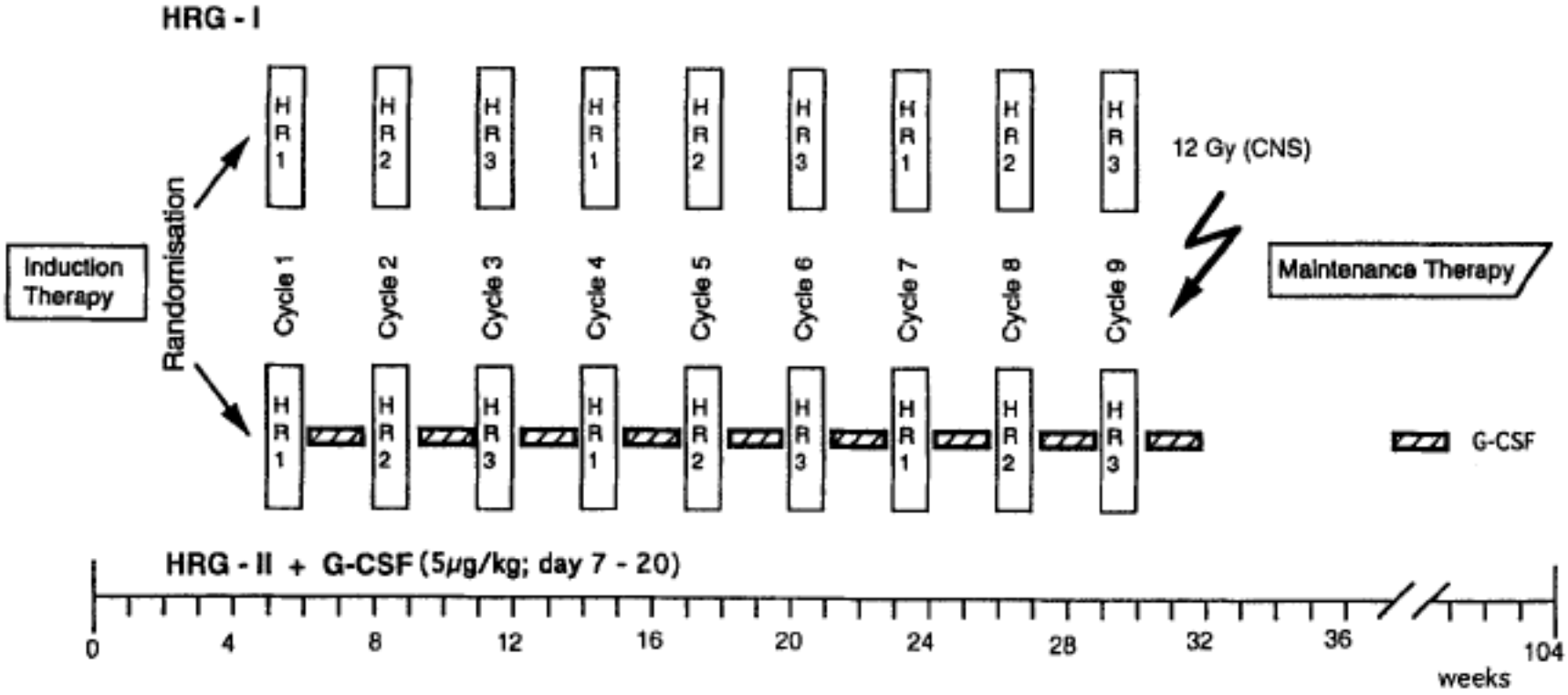
EFFECT OF GRANULOCYTE COLONY-STIMULATING FACTOR ON NEUTROPENIA AND ASSOCIATED MORBIDITY DUE TO CHEMOTHERAPY FOR TRANSITIONAL-CELL CARCINOMA OF THE UROTHELIUM

JANICE L. GABRILOVE, M.D., ANN JAKUBOWSKI, M.D., PH.D., HOWARD SCHER, M.D., CORA STERNBERG, M.D., GEORGE WONG, PH.D., JOHN GROUS, M.D., ALAN YAGODA, M.D., KATHERINE FAIN, MALCOLM A.S. MOORE, PH.D., BAYARD CLARKSON, M.D., HERBERT F. OETTGEN, M.D., KIRBY ALTON, PH.D., KARL WELTE, M.D., AND LAWRENCE SOUZA, PH.D.



G-CSF in Childhood ALL (BFM2000):

Does administration of more chemotherapy in time lead to increased survival ?



Welte, K., et al. Blood 1996

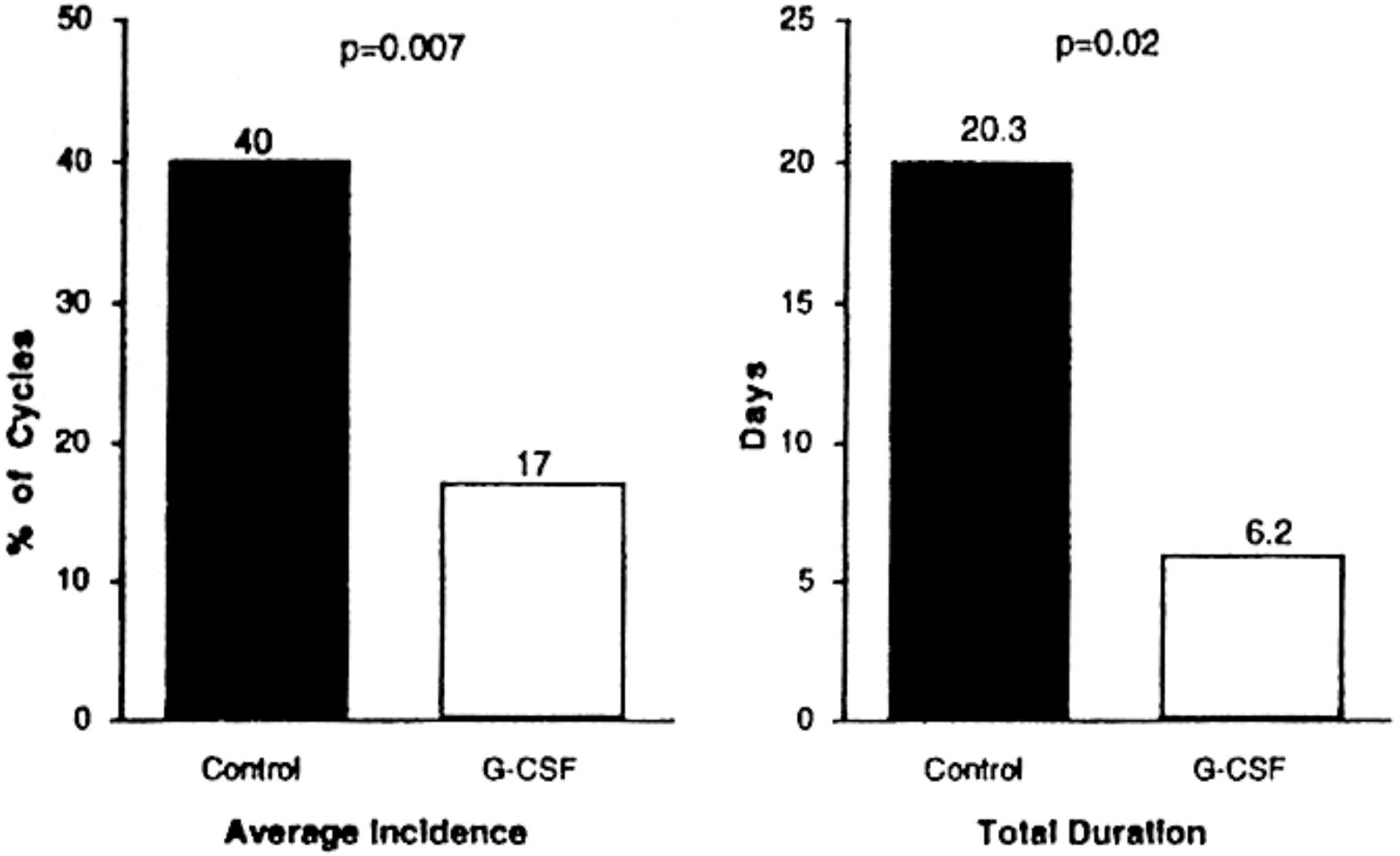
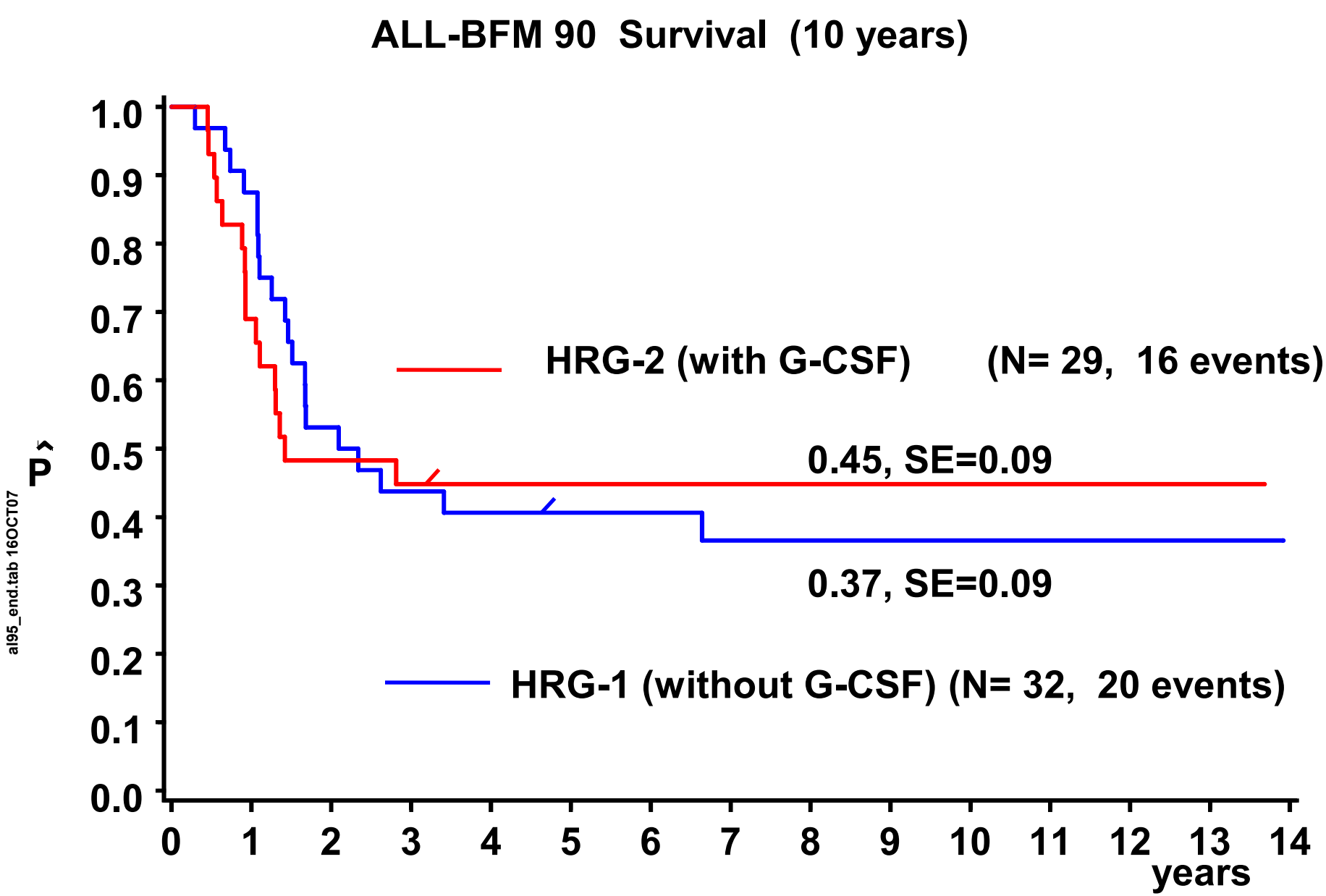
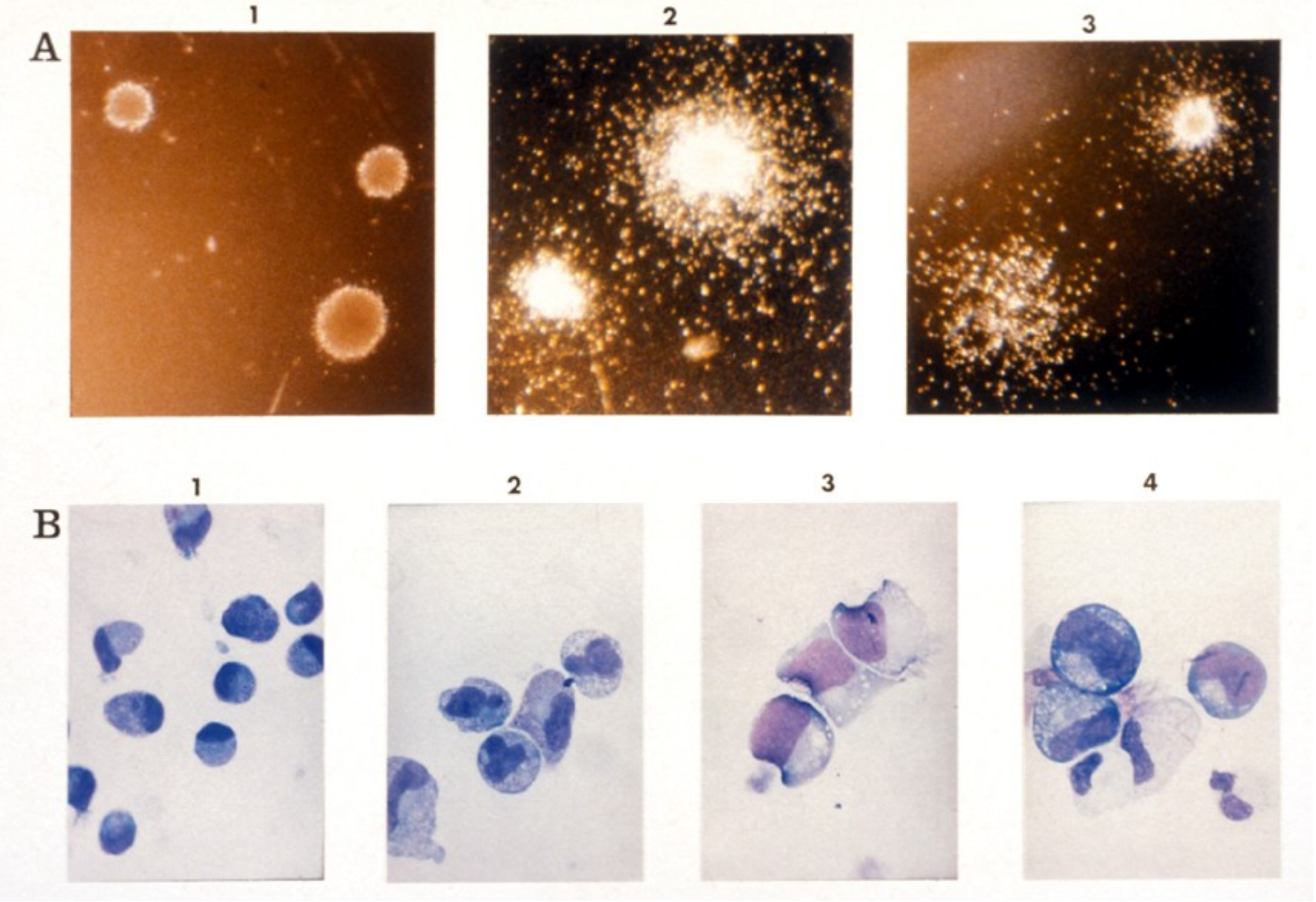
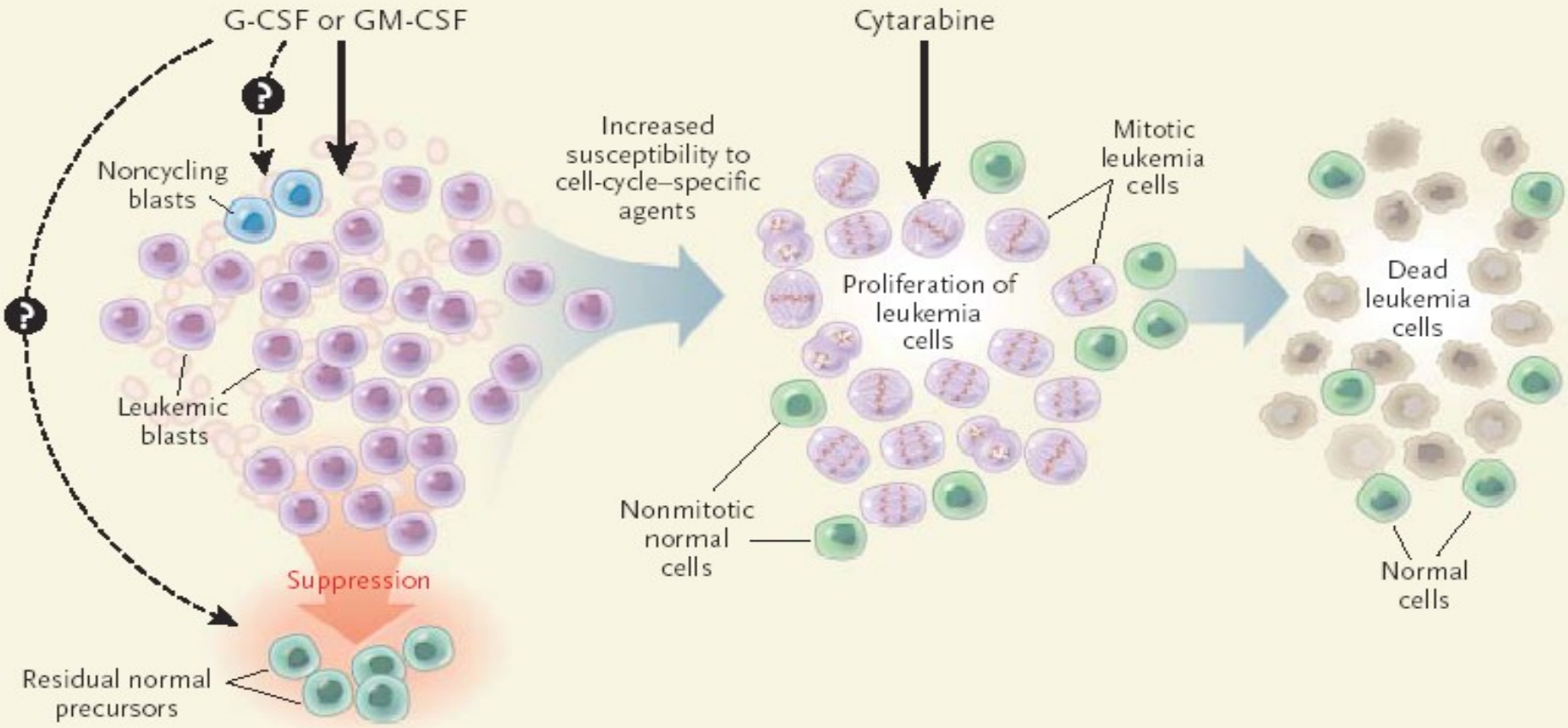


Fig 2. Incidence and duration of febrile neutropenic episodes (ANC $< 0.5 \times 10^9$/L and oral temperature $\geq 38.5^{\circ}C$) during the study period in the control group and r-metHuG-CSF group.


ALL-BFM 90 Survival (10 years)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
$\hat{P}$
al95_end.tab 16OCT07
HRG-2 (with G-CSF) (N= 29, 16 events)
0.45, SE=0.09
0.37, SE=0.09
HRG-1 (without G-CSF) (N= 32, 20 events)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
years



Souza and Welte, Science 1986



Principle Underlying the Concurrent Use of Chemotherapy and Growth Factors That Stimulate Cell Division.

The concurrent use of growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) could enhance the cytotoxic effect of agents such as cytarabine on leukemia cells. Little is known about the effects of these growth factors on the subpopulation of putative leukemia stem cells, which may have a relatively low rate of proliferation, or the residual normal hematopoietic precursors.

Ch. Schiffer, N Engl J Med 349, 727, 2003

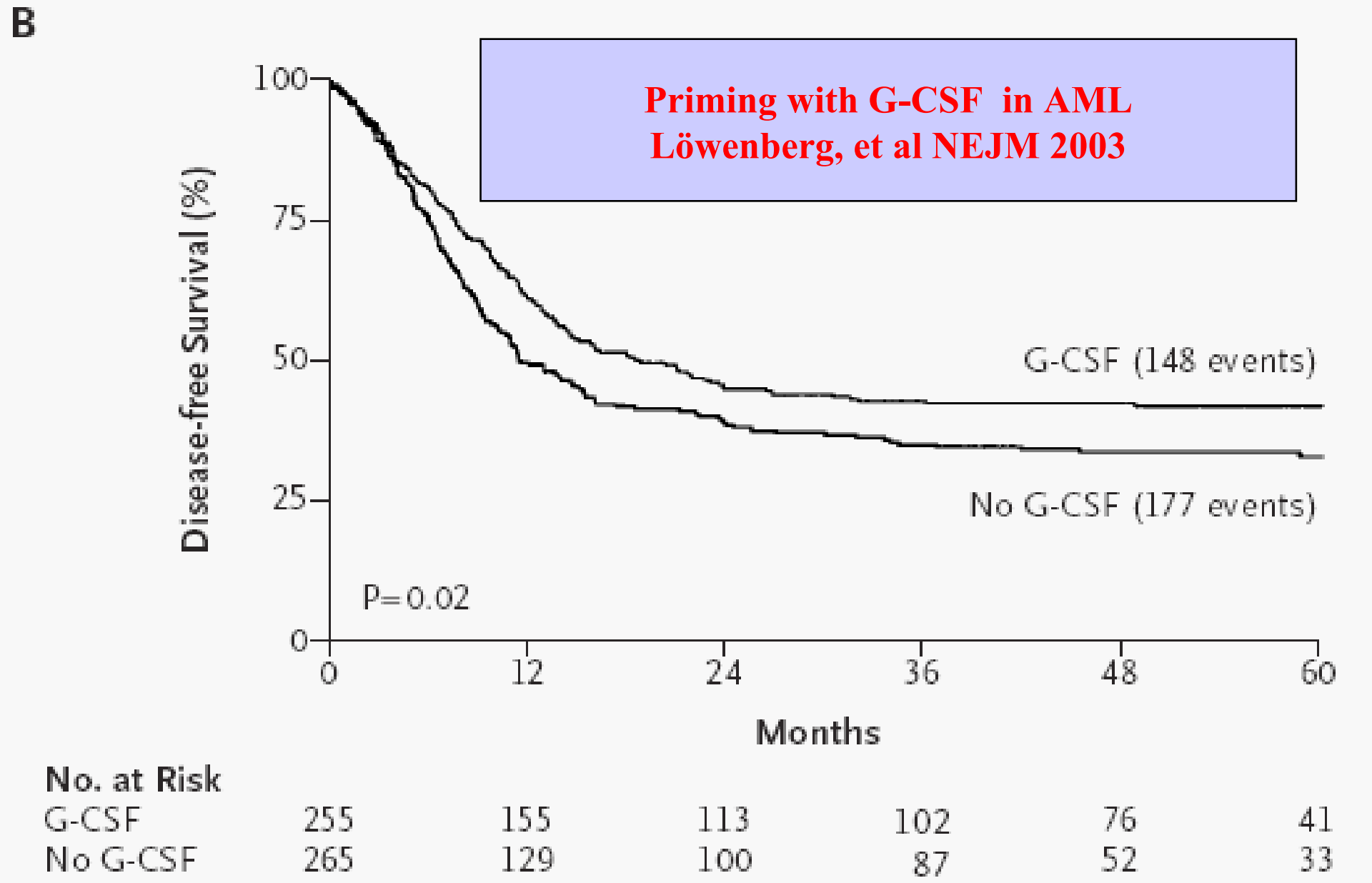
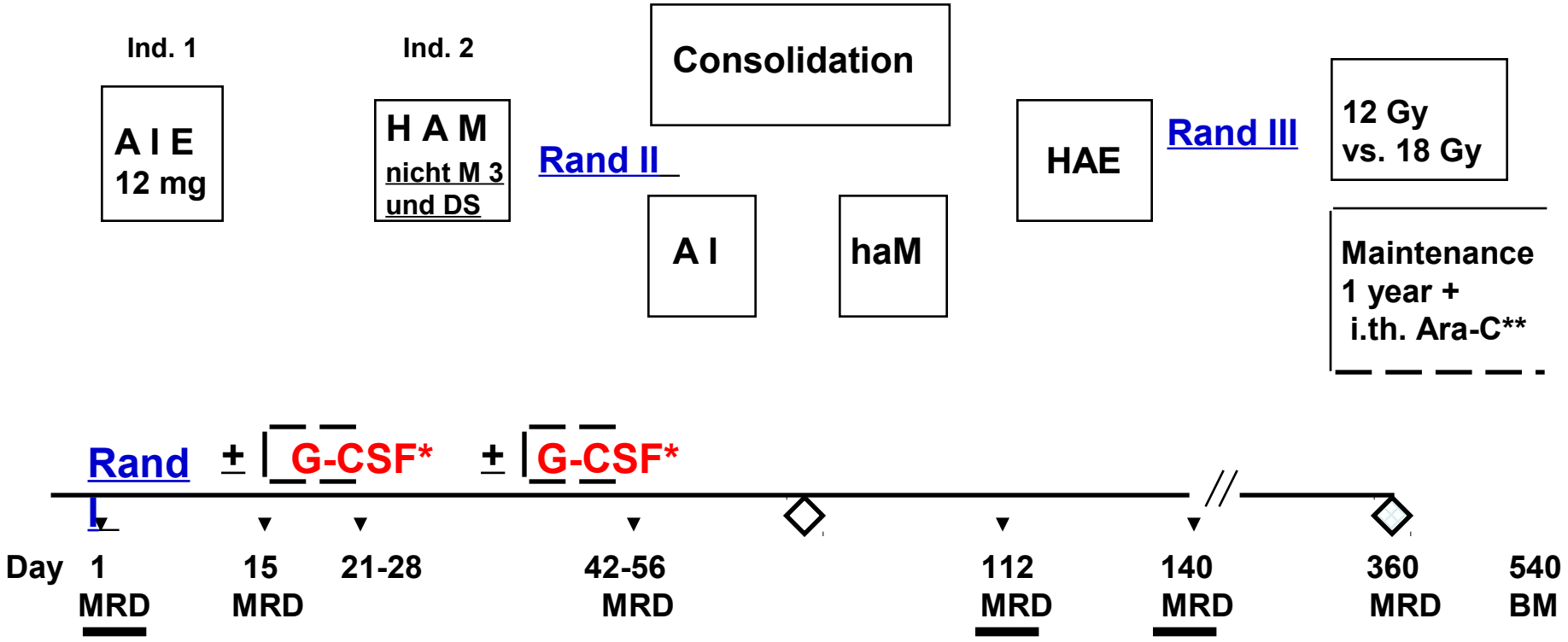


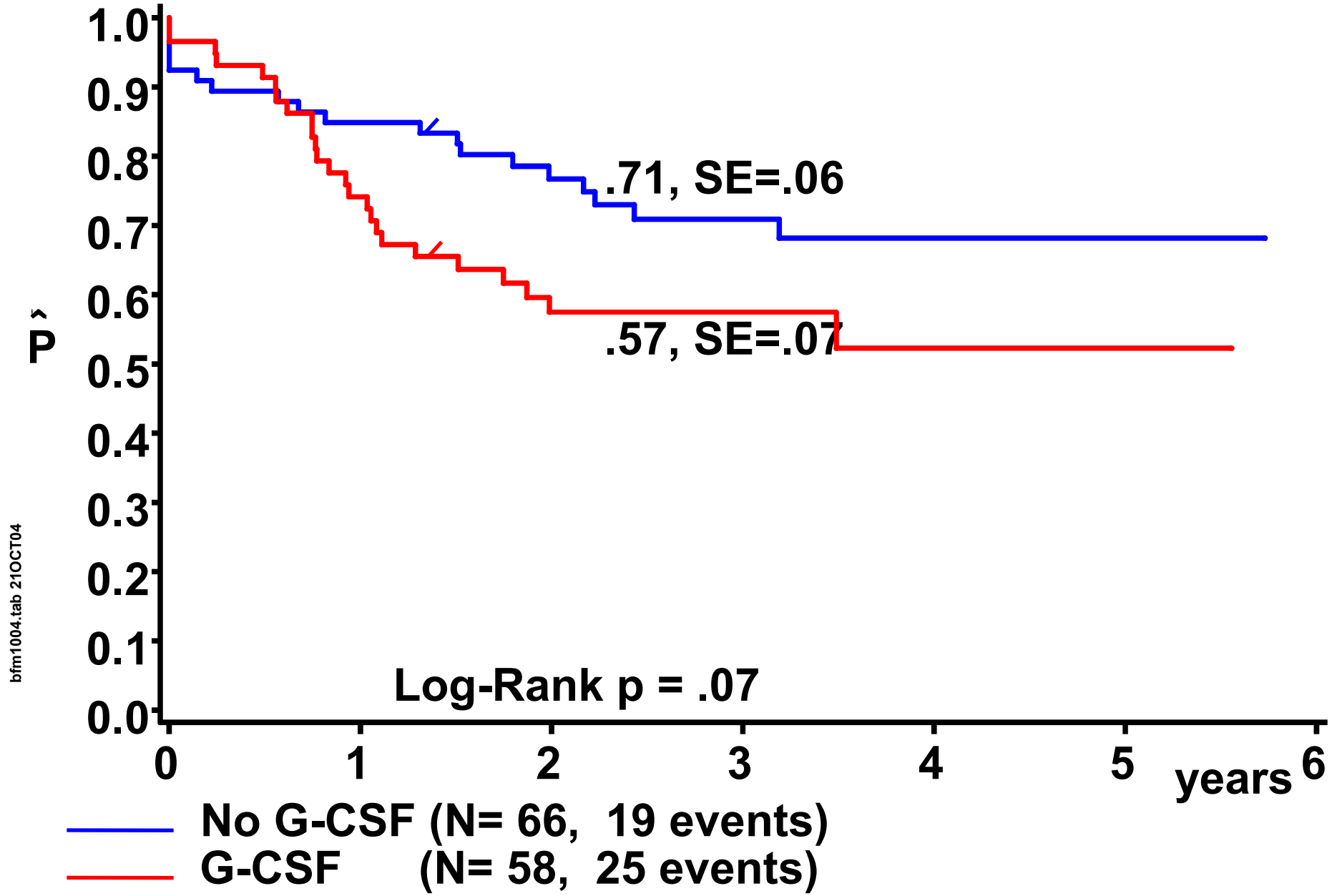
Figure 2. Cumulative Rate of Overall Survival (Panel A) and Disease-free Survival (Panel B), According to the Assigned Treatment.

AML-BFM 98

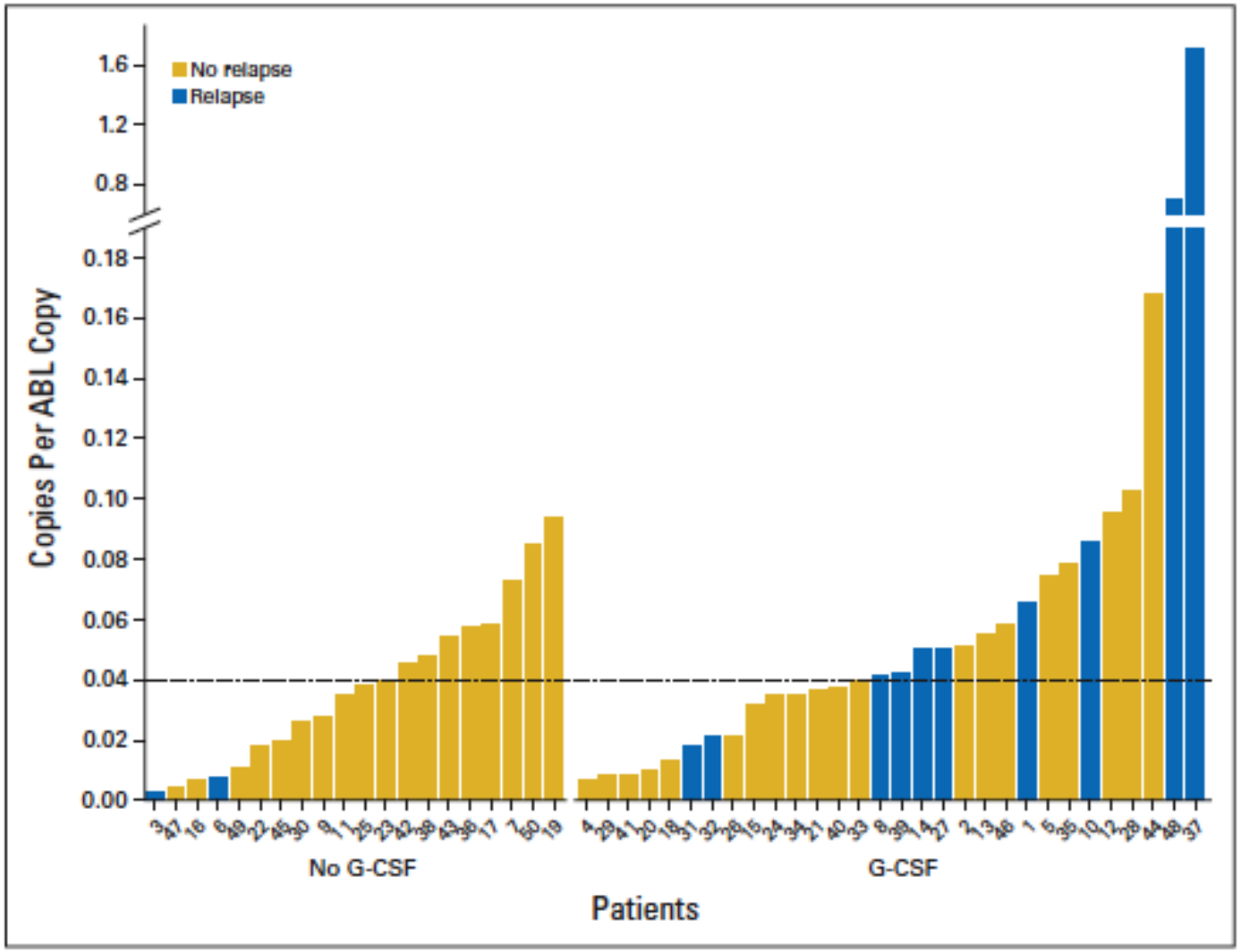


* G-CSF 5-8 days or until ANC $>500/mm^3$ except patients with > 5% Blasts on day 15

AML-BFM 98 Standard risk patients (without Down-S.): EFS (3 years)



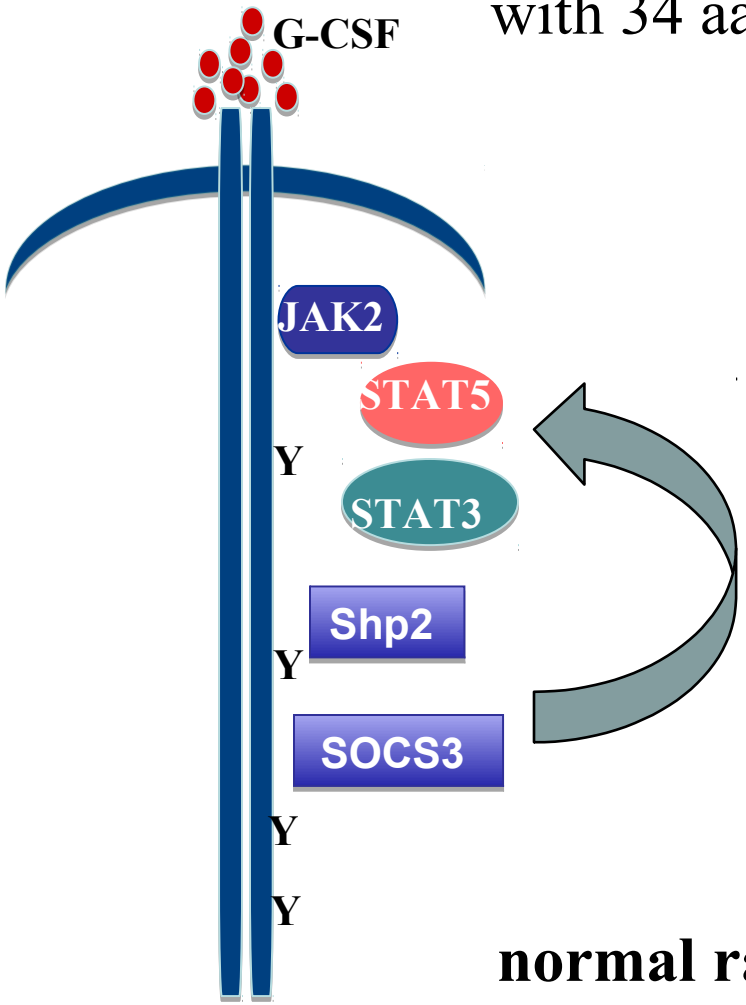
Ehlers S., et al., J Clin Oncol 2010

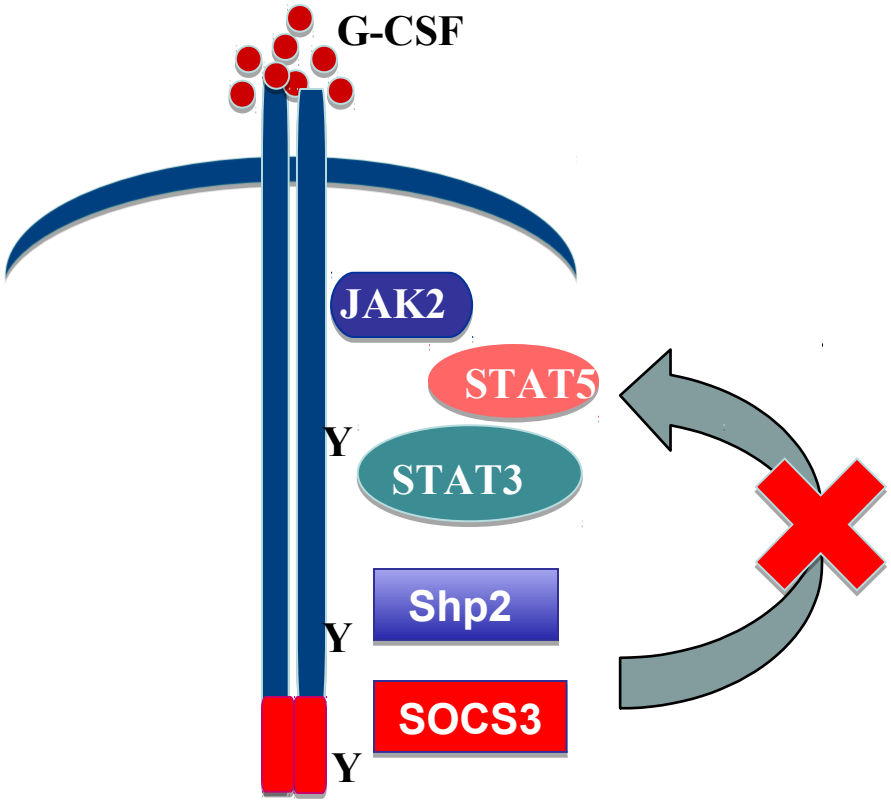


G-CSFR isoform I

G-CSFR isoform IV

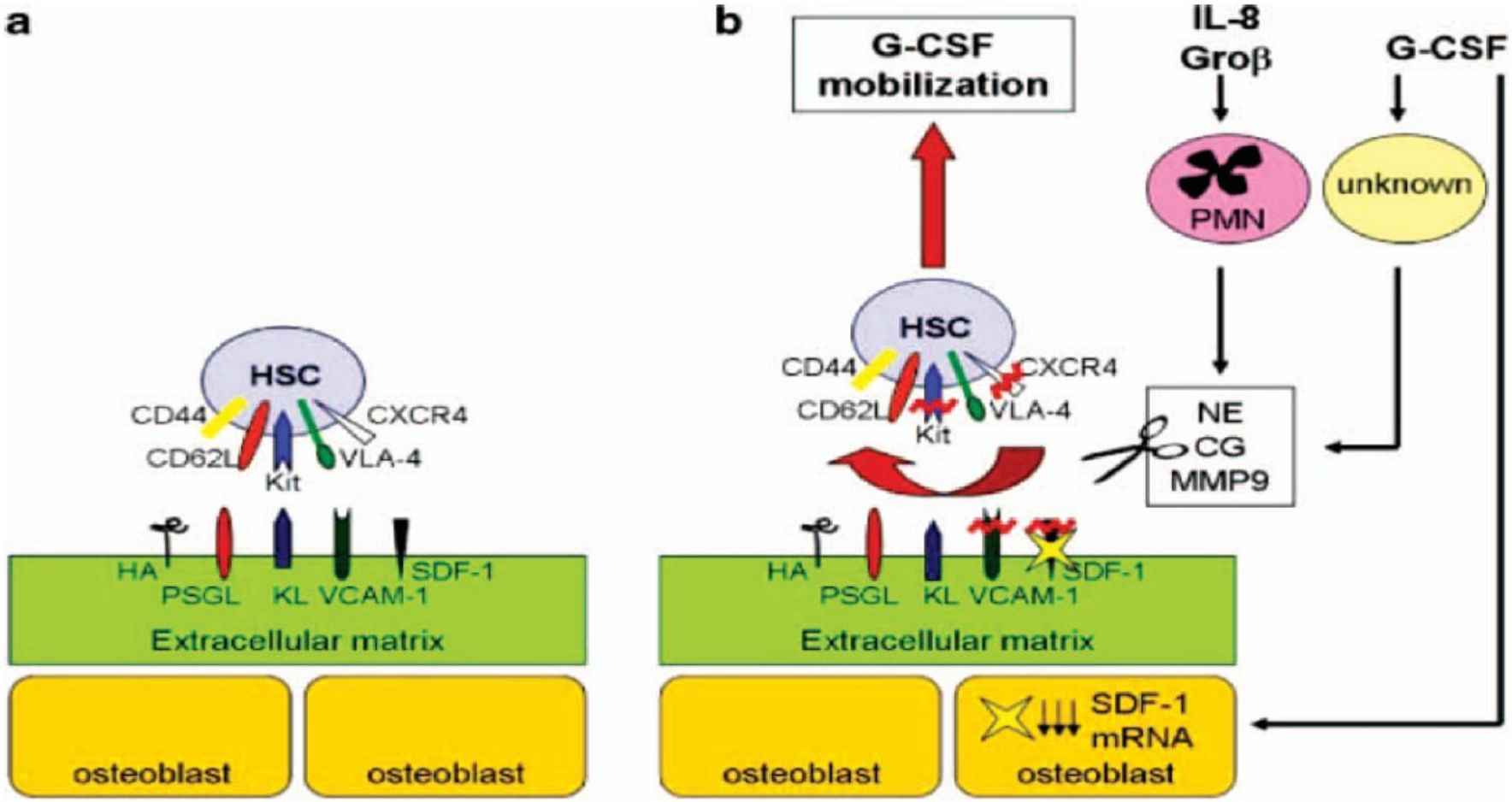
The isoform IV replaces the carboxy-terminal 87 aa with 34 aa of **novel sequence**

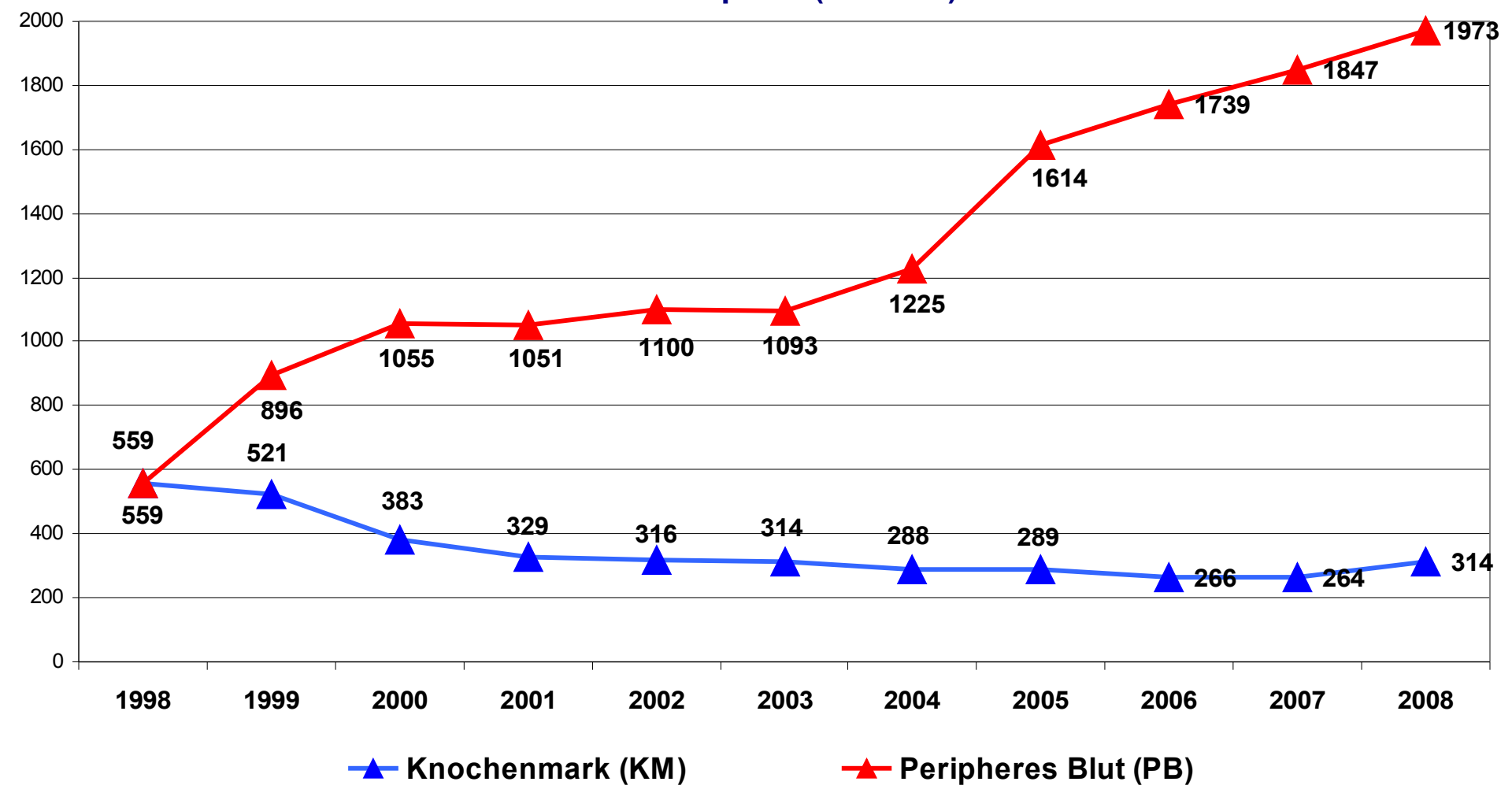




normal ratio 200 : 1

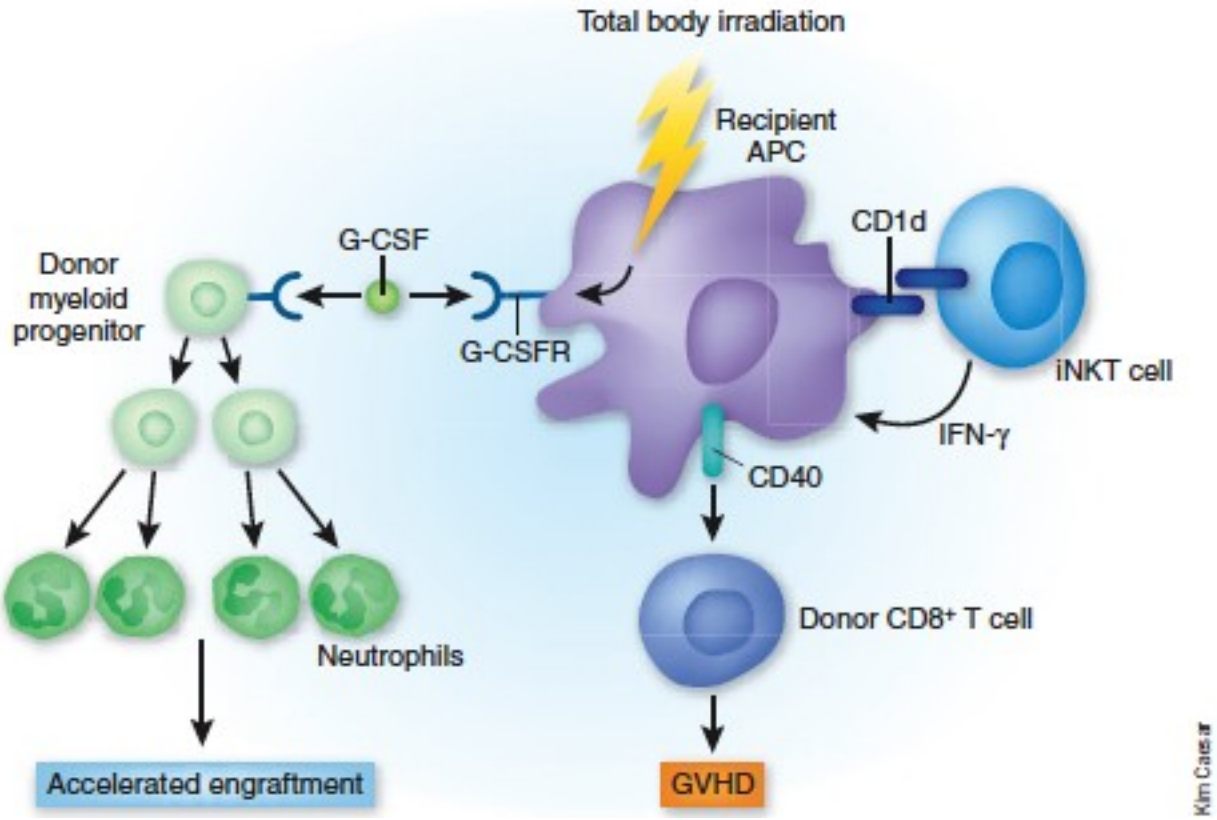
Mobilization of hematopoietic stem cells by G-CSF





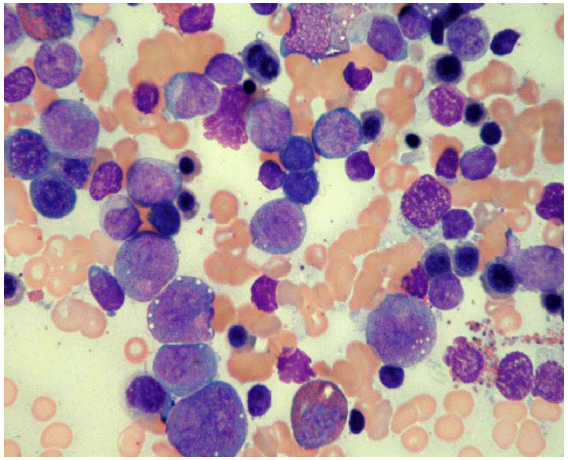
Paradigm shift of SCT by G-CSF mobilization (Germany 1998 – 2008)

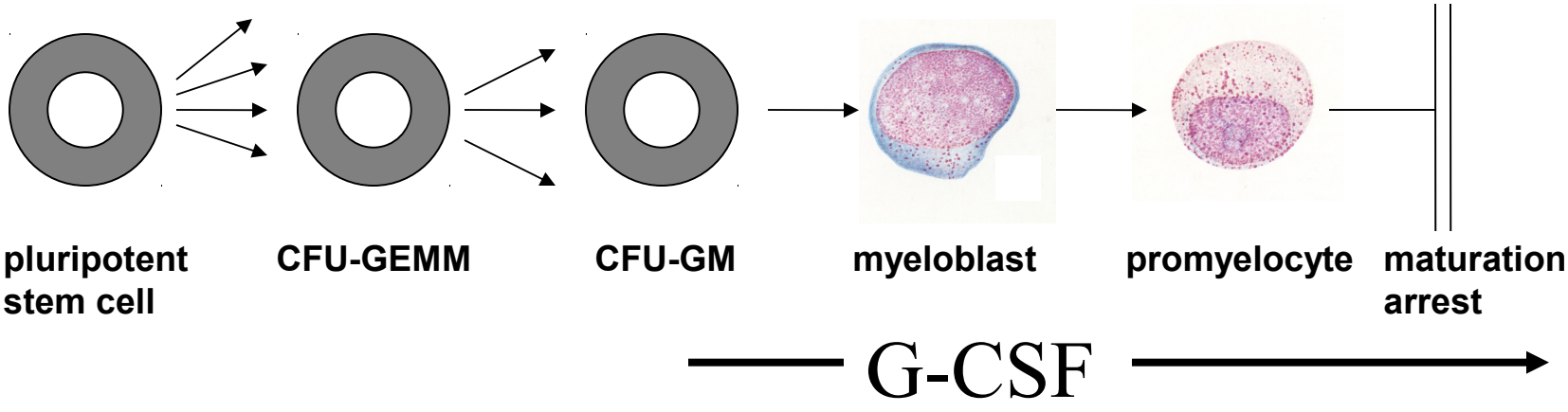
Choosing between GVHD and delayed engraftment



Transplantation`s balancing act !

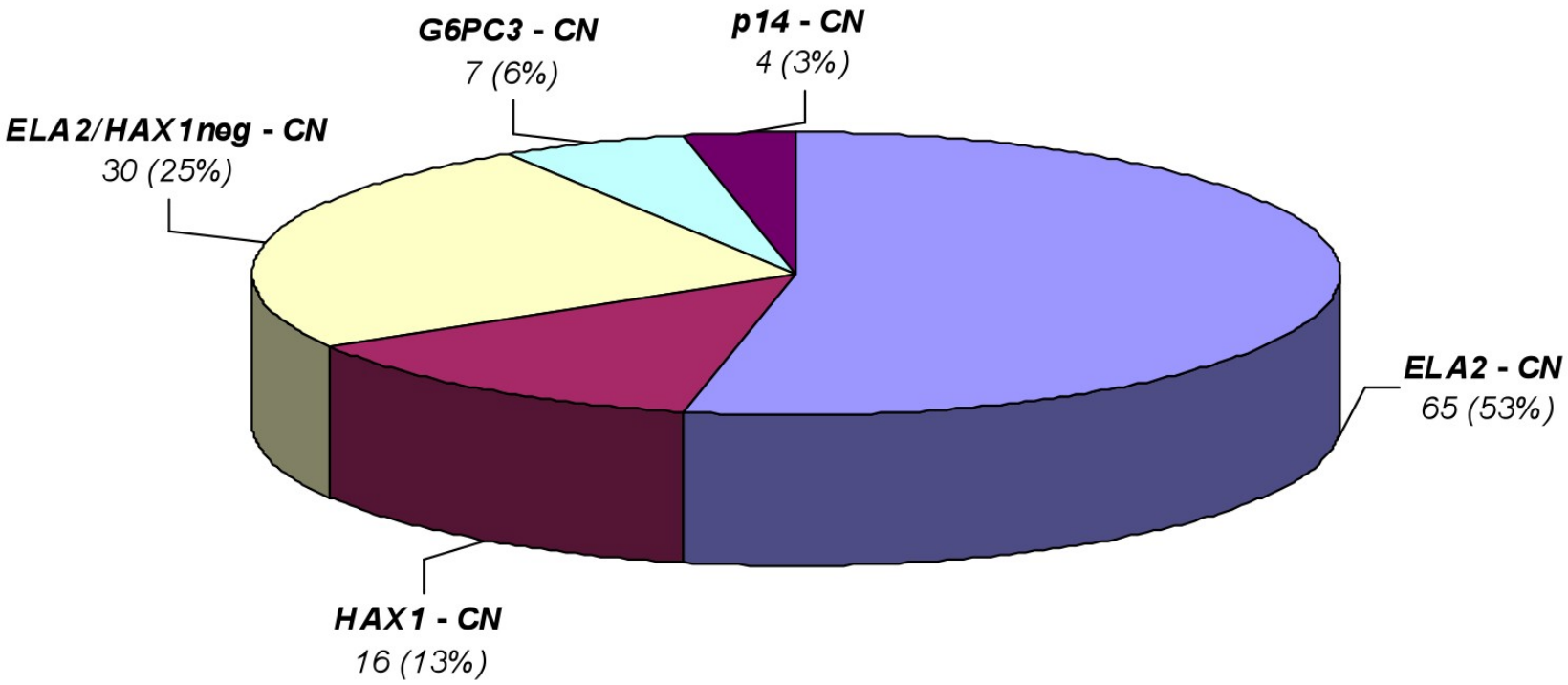
Martin, PJ, Nature Medicine 2009
Morris, ES, Nature Medicine 2009





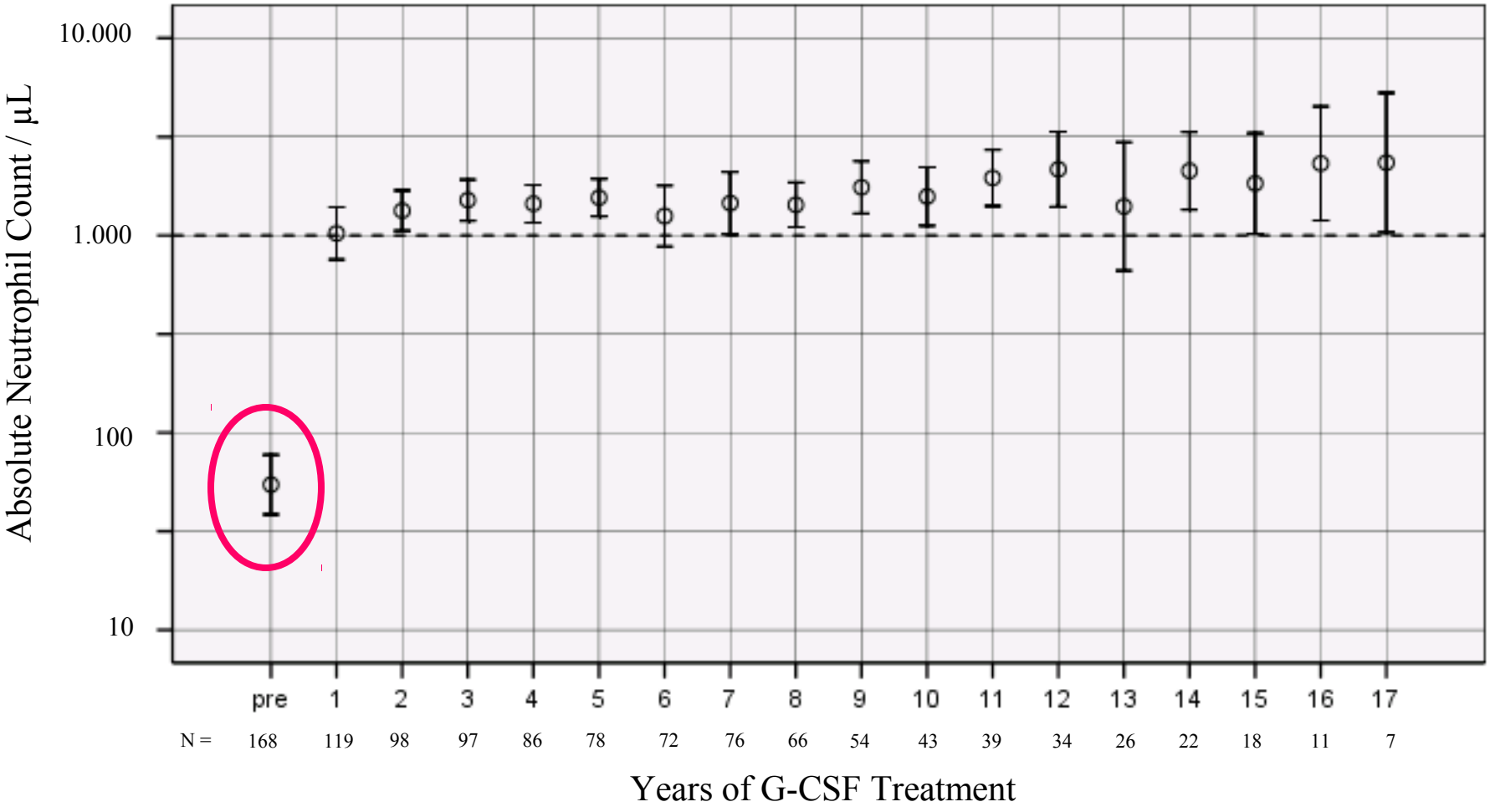
Severe congenital neutropenia

Genetic distribution in 122 patients with CN

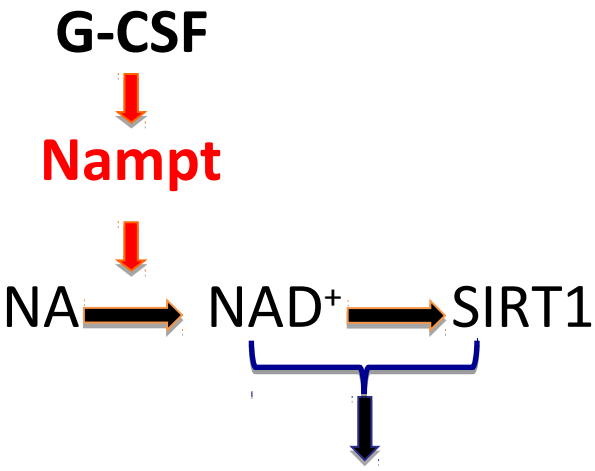


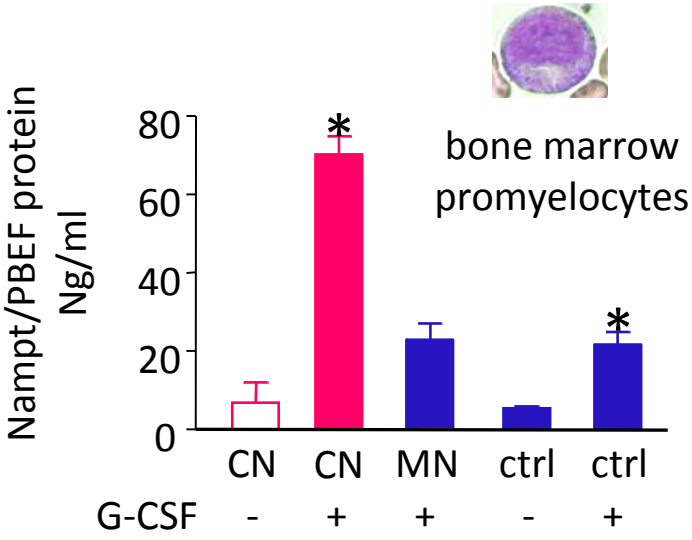
Severe Chronic Neutropenia Registry, Europe

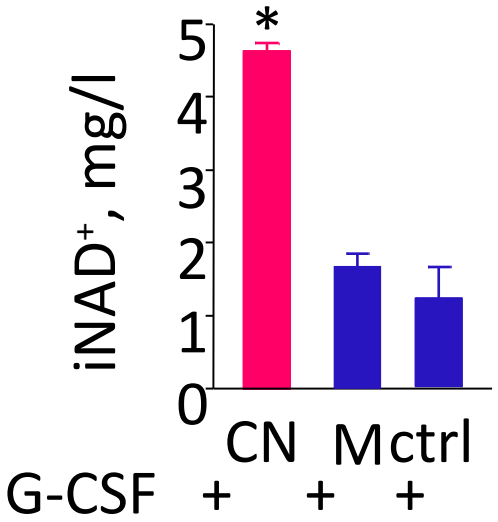
Long Term Course of Median Absolute Neutrophil Count in Patients with Severe Congenital Neutropenia

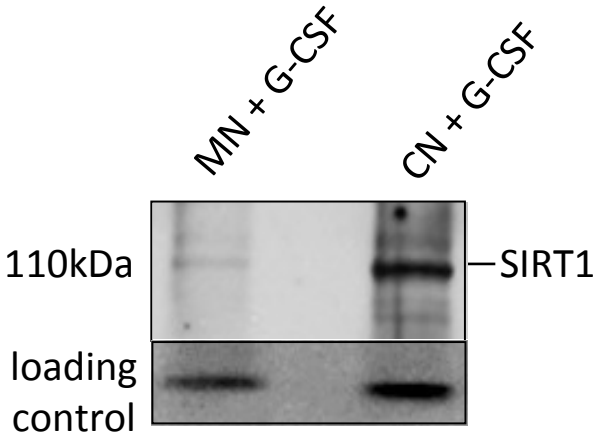


G-CSF induces Nampt and NAD⁺ in myeloid progenitors from CN patients



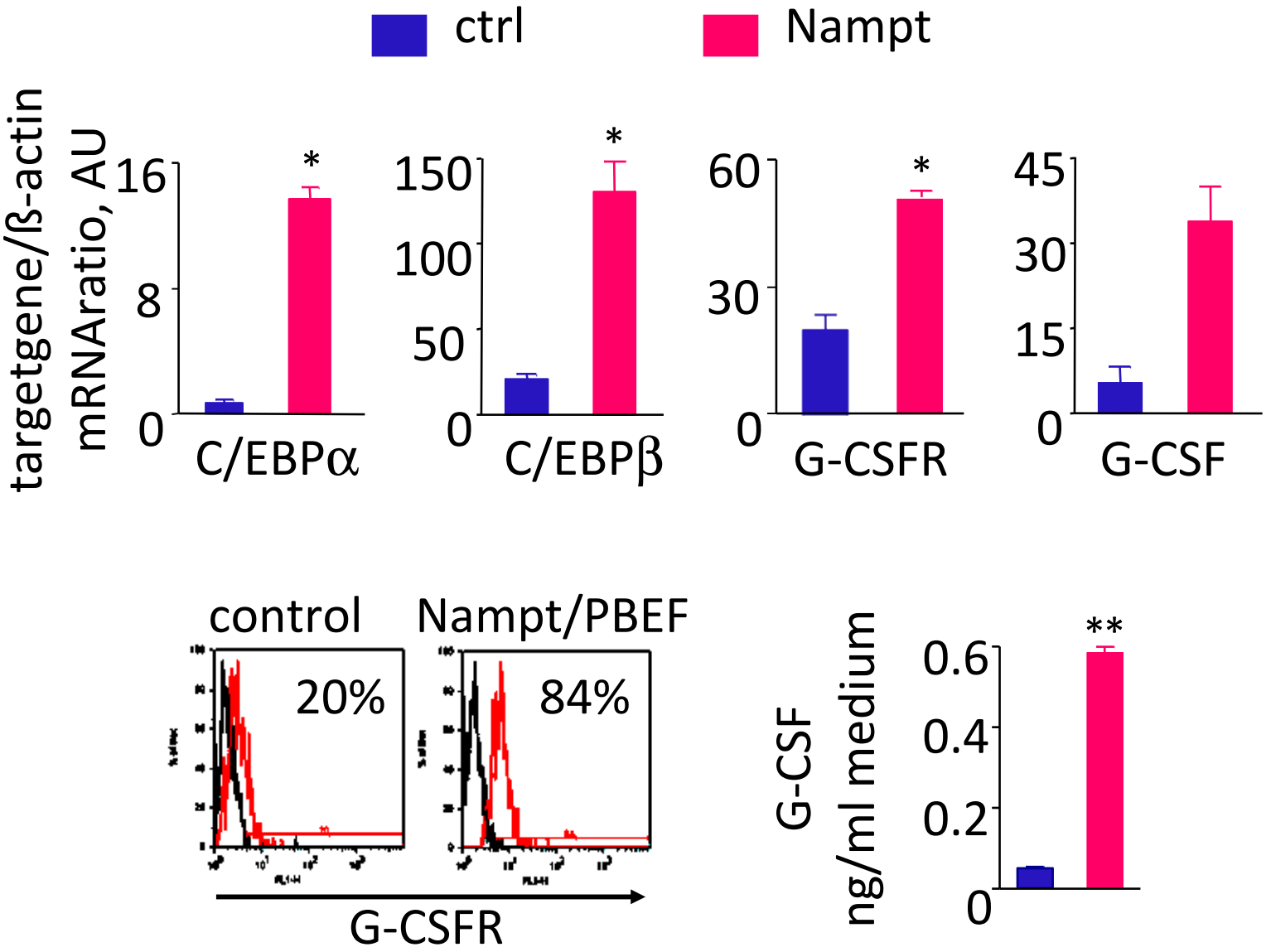




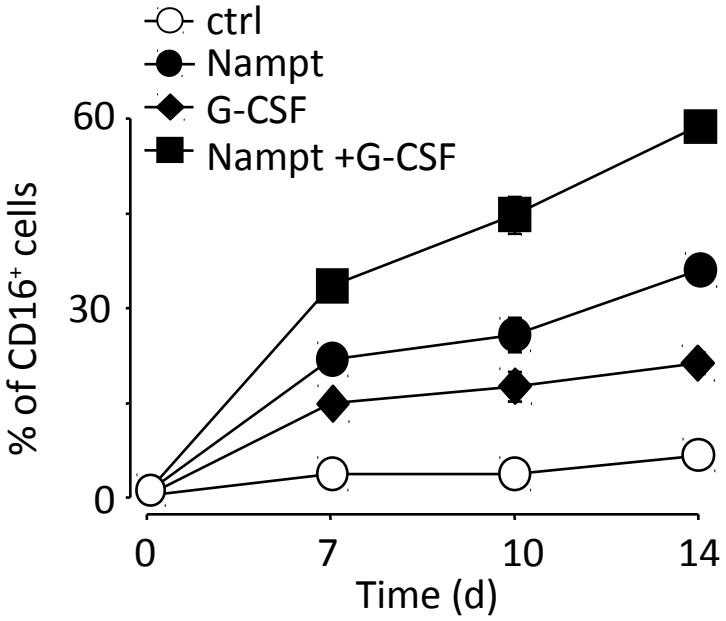


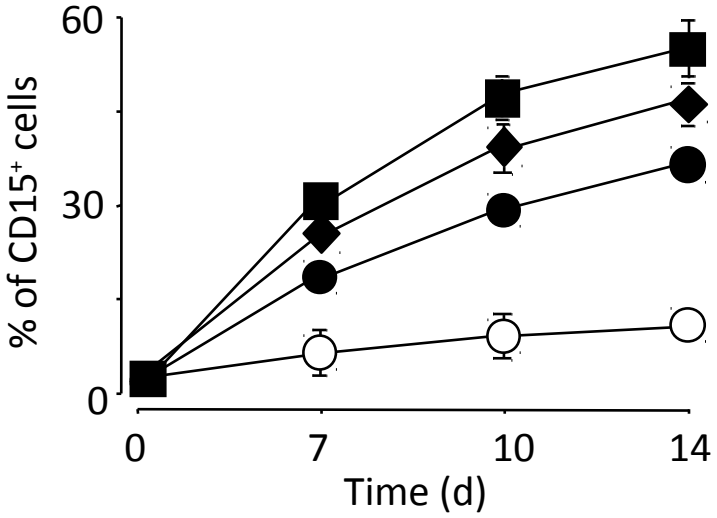
Skokowa, J., et al., Nat Med 2009; 15: 151- 8

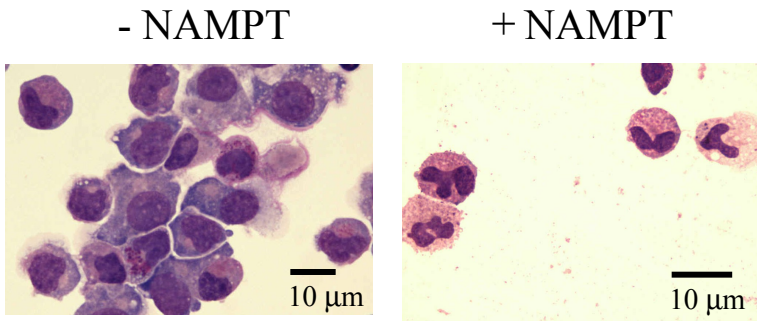
Nampt induces G-CSF and G-CSFR in CD34 cells via Sirt1/C/EBPs



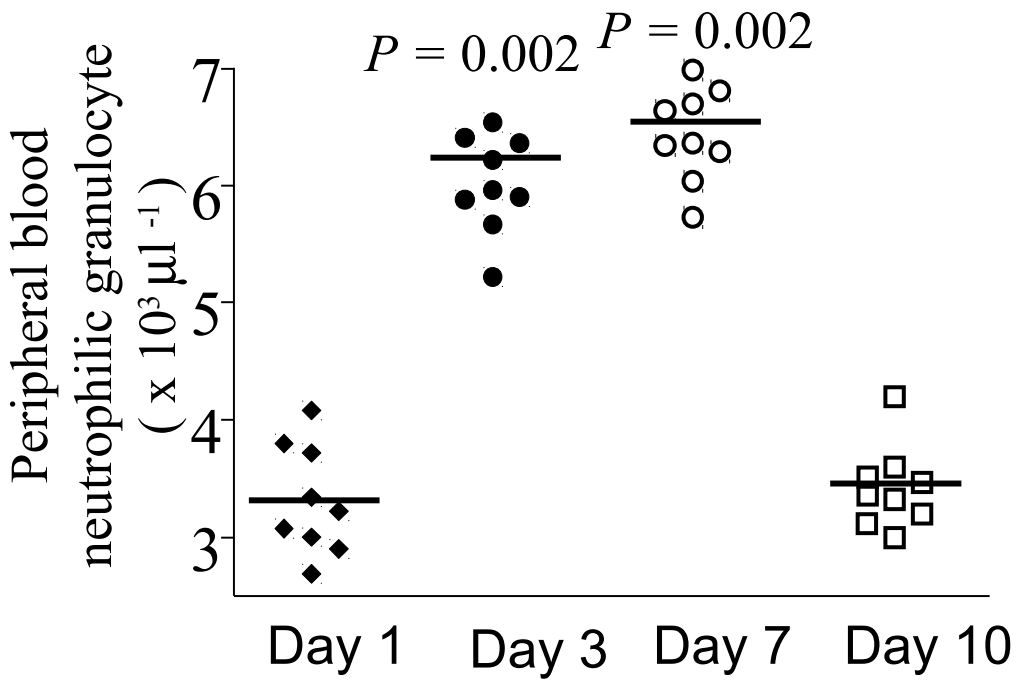
Nampt triggers myeloid differentiation of CD34⁺ cells







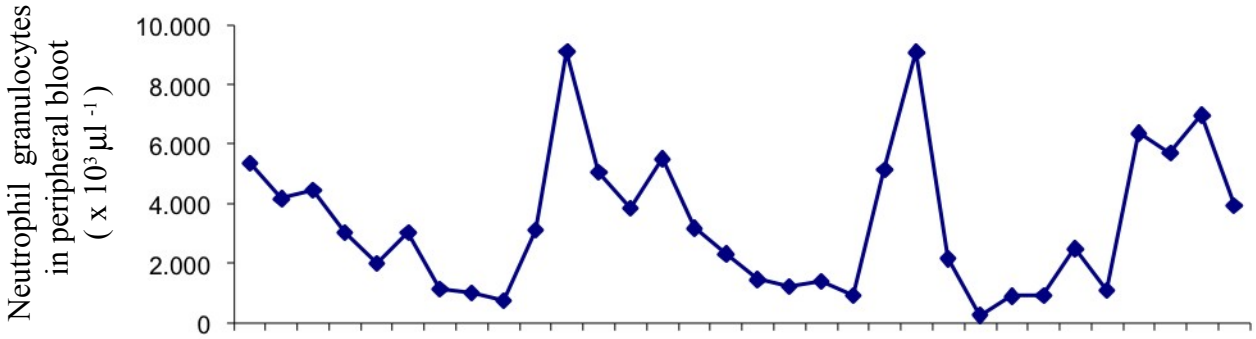
Vitamin B3 treatment increases number of neutrophilic granulocytes in healthy individuals



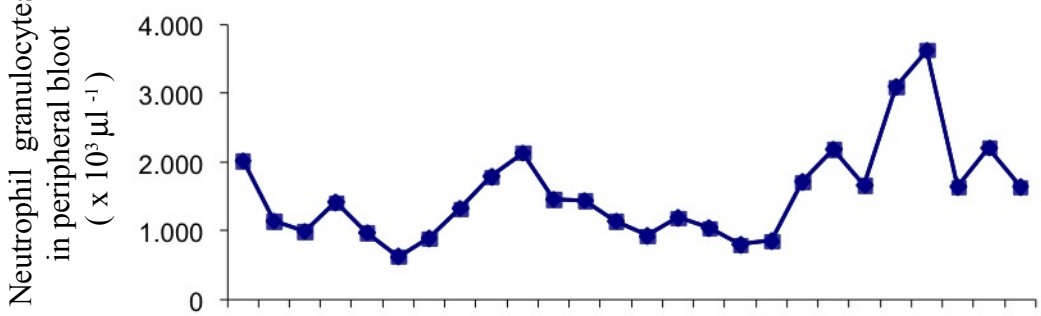
Vitamin B3 (200 mg/kg/d) treatment (day 1-7)

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

Vitamin B3 treatment of patient with cyclic neutropenia



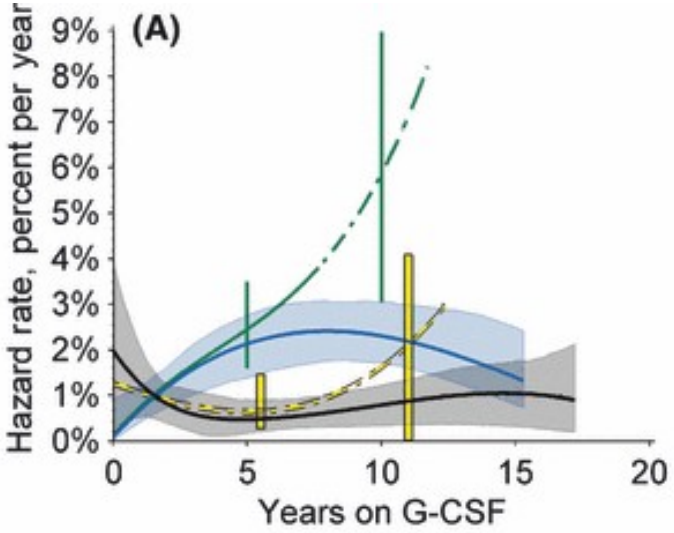
Treatment with G-CSF (3 ug/kg/d s.c.)



Treatment with Vitamin B3 (200 mg/kg/d p.o.)
without G-CSF

Hazard rates and cumulative incidence of MDS/AML and sepsis death in patients with SCN.

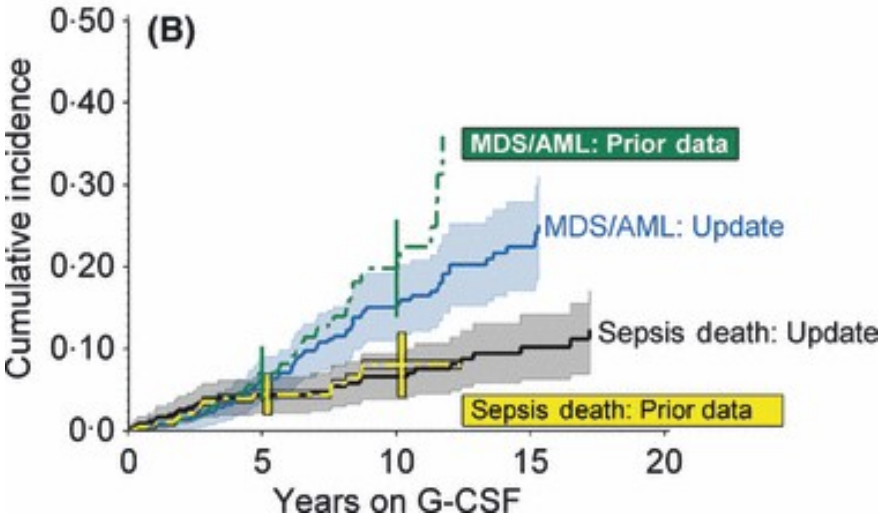
Cumulative incidence of MDS/AML (blue curve) and sepsis death (black curve) among patients who achieved an adequate mean absolute neutrophil count (ANC) by months 6–18 (≥2.188 cells × 109/l) at doses of G-CSF less than or equal to 8 μg/kg per d).



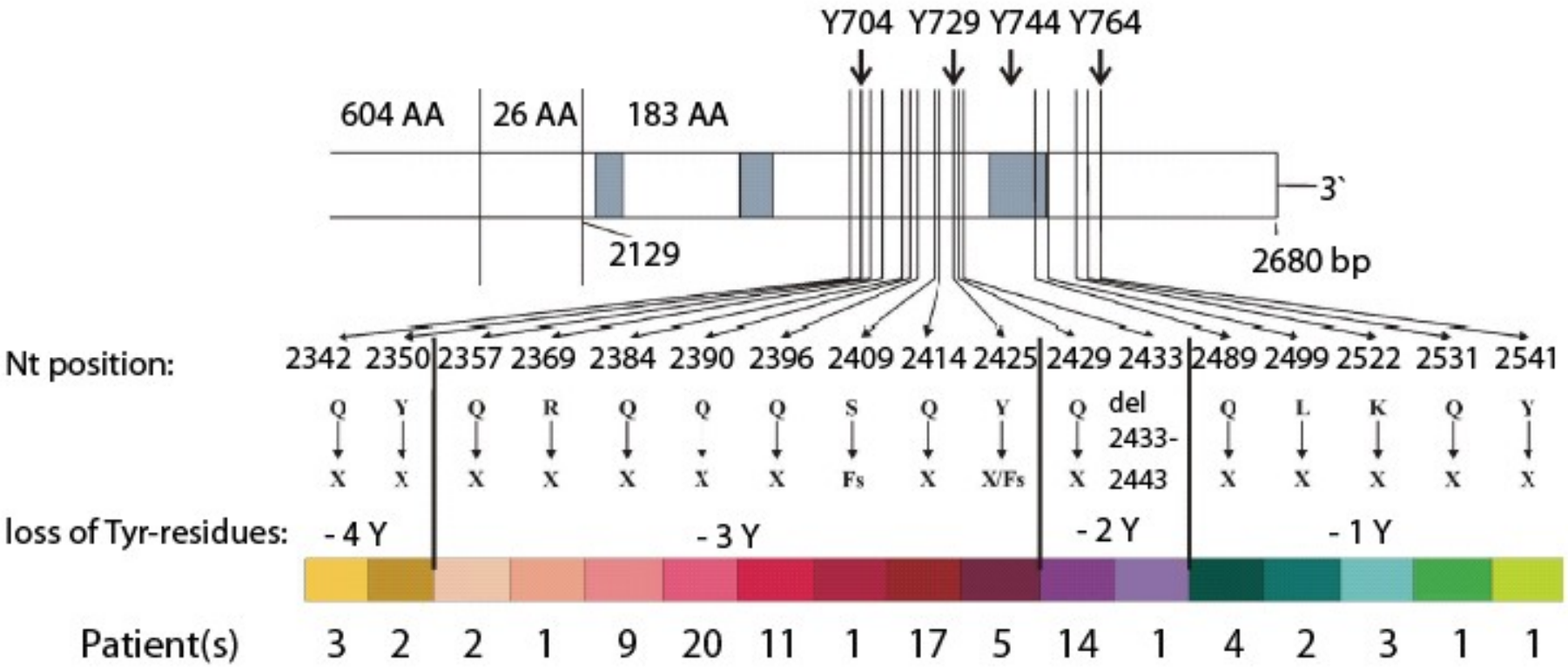
Corresponding cumulative incidence curves among patients who failed to achieve an adequate ANC despite higher doses of G-CSF.

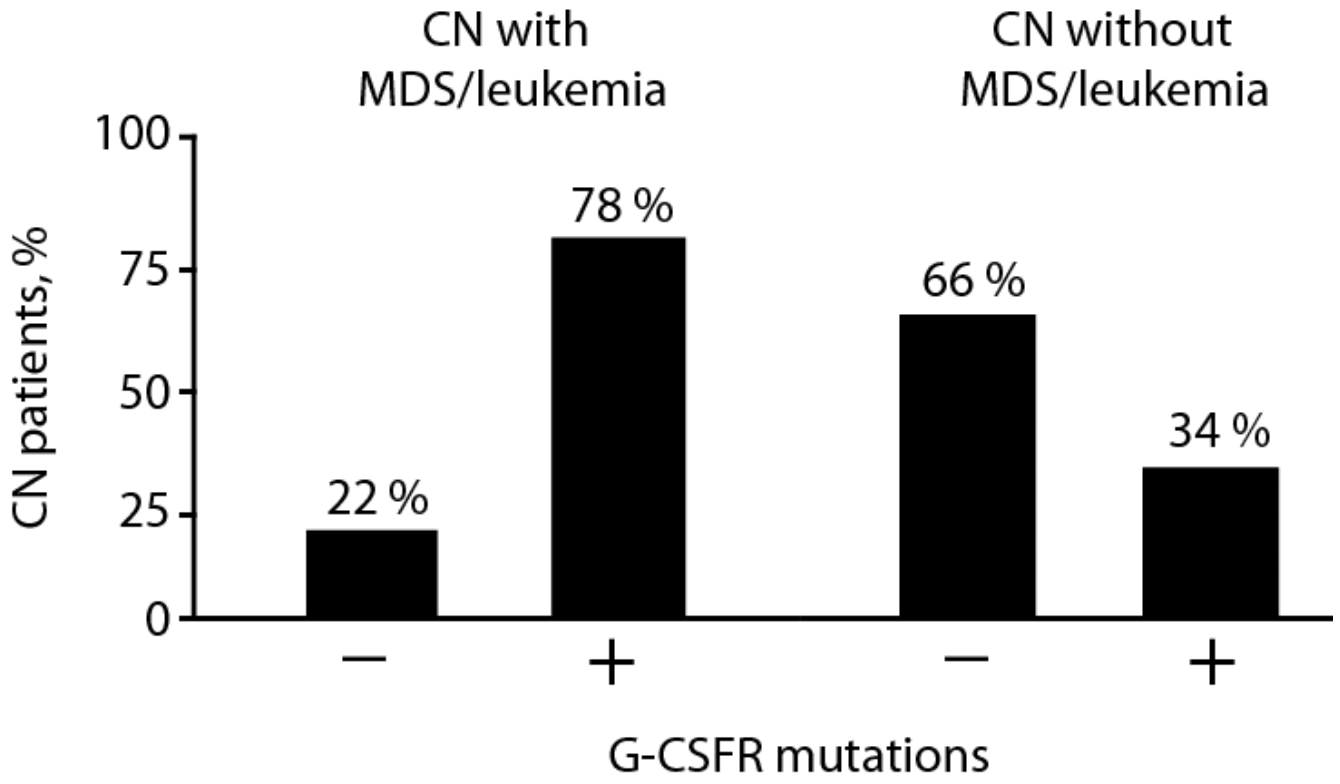
Rosenberg, Zeidler, et al., BJH 2010

Zeidler EHA 2010



G-CSFR mutations in CN patients

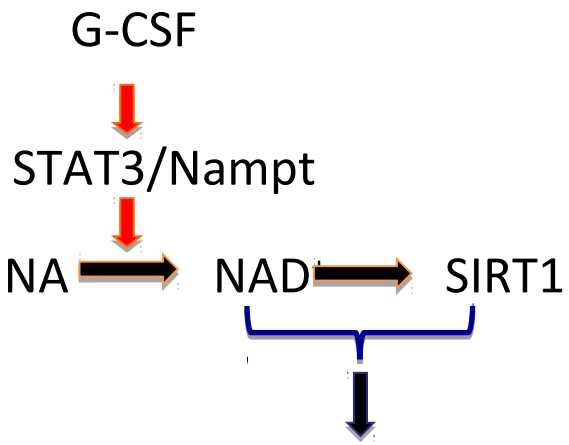




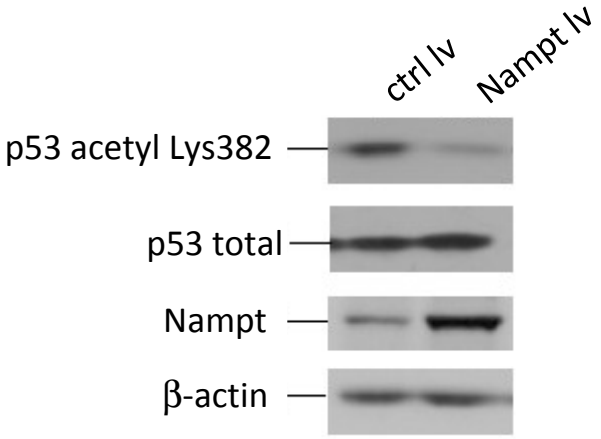
Adopted from Germeshausen et al., Blood 2006

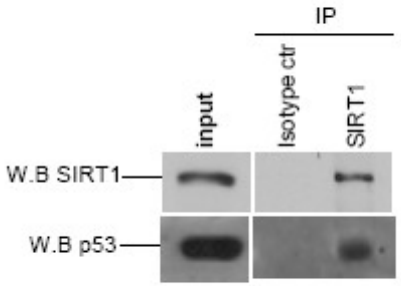
Leukemogenesis

Nampt deacetylates p53



Deactelylation of p53,
FOXO3A,
C/EBPs, etc.





Journal of Cell Science
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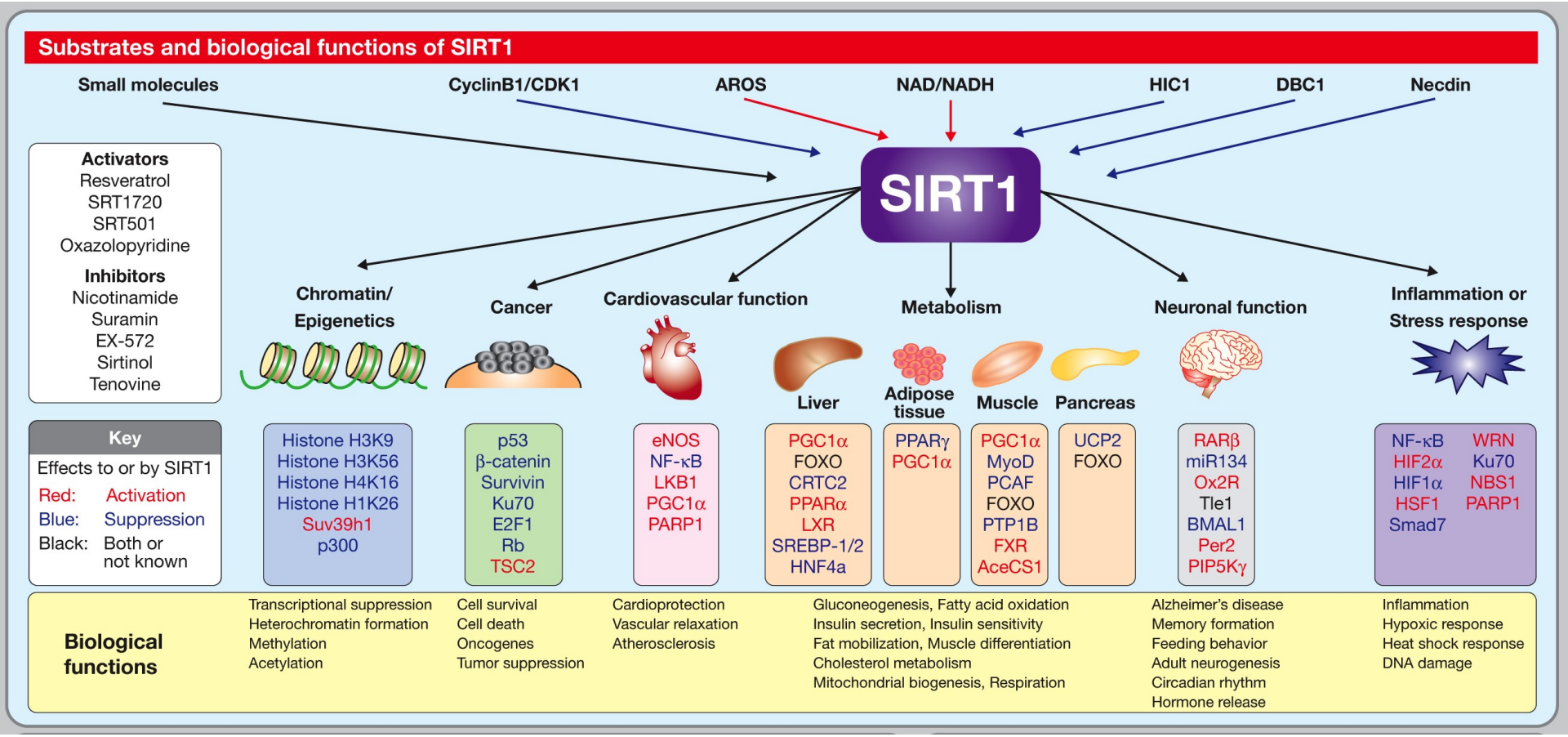
Sirtuins at a Glance

Takashi Nakagawa and Leonard Guarente



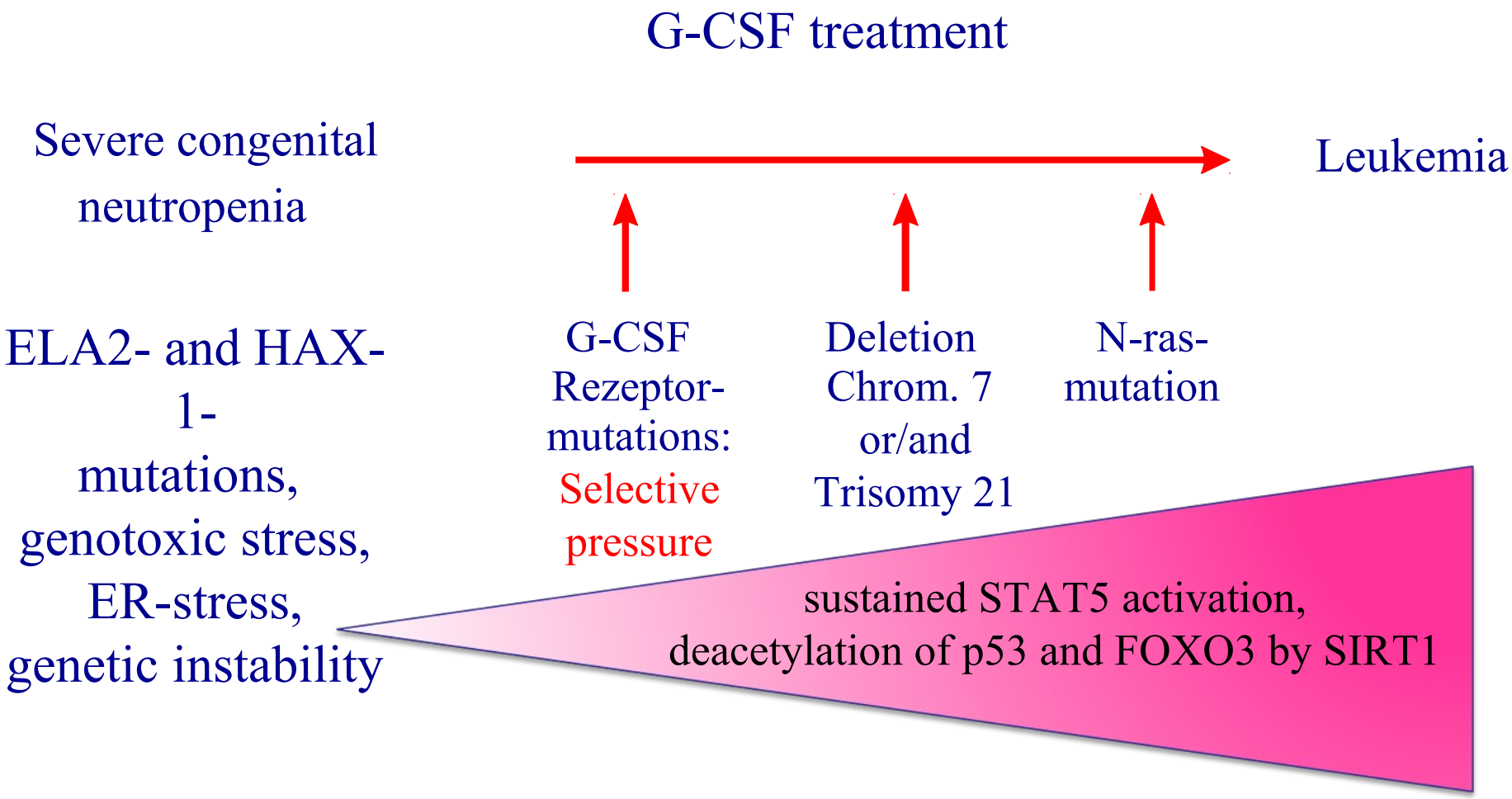
Leukemogenesis

SIRT1 deacetylates p53 and FOXO3a → functional

inactivation of p53 and FOXO3a

SIRT1 also deacetylates ß-catenin and by this may induce genomic instability

Model of the malignant transformation in CN



Donald Metcalf

Abstract | The four colony-stimulating factors (CSFs) are glycoproteins that regulate the generation and some functions of infection-protective granulocytes and macrophages. Recombinant granulocyte-CSF (G-CSF) and granulocyte–macrophage-CSF (GM-CSF) have now been used to increase dangerously low white blood cell levels in many millions of cancer patients following chemotherapy. These CSFs also release haematopoietic stem cells to the peripheral blood, and these cells have now largely replaced bone marrow as more effective populations for transplantation to cancer patients who have treatment-induced bone marrow damage.

Nature Review Cancer, 2010

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G-CSF signaling

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