PATHOGENESIS OF LEUKEMIAS

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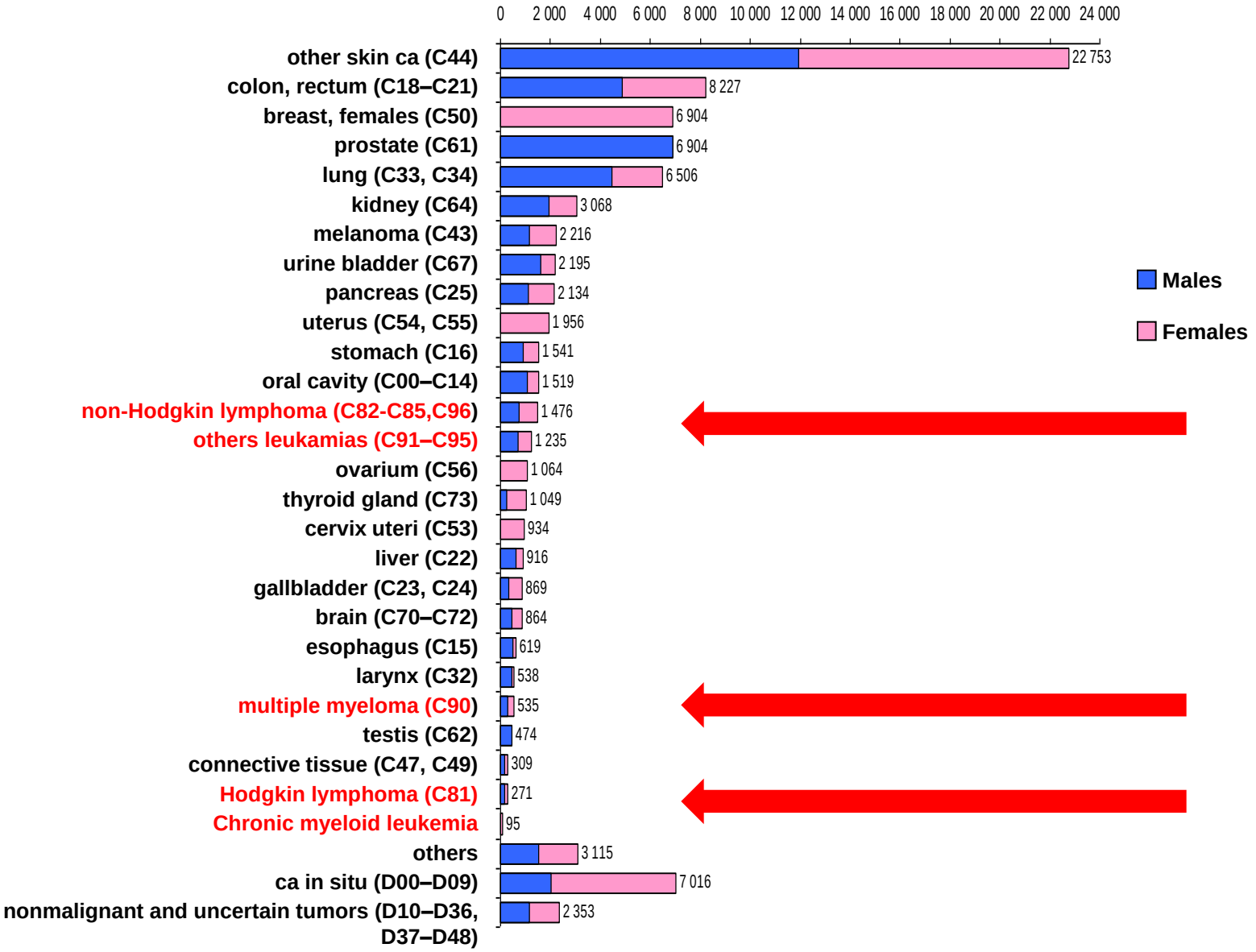
…what I am going to say…

- Epidemiology, frequency
- Model diseases, prototypes
- What leukemias are, clinical signs
- Key subtypes
- Elementary principles of pathogenesis
- Implications of these facts for diagnostics and therapy
- Emphasize to
  - time relationships of different discoveries
  - original data from the medical literature

Cancer incidence In Czechia, 2010-2014

Average number of yearly diagnosed cases



Source: National Cancer Registry, ÚZIS ČR

Archiv

für

pathologische Anatomie und Physiologie

und für

klinische Medicin.

Herausgegeben

von

R. Virchow und **B. Reinhardt.**

Erster Band.

Mit 4 Tafeln.

Berlin,
Druck und Verlag von G. Reimer.
1847.

heiten während ihres ganzen Verlaufes
gröfseren Theils desselben permanente,
derungen in der Blutmischung in Anspru
war geradezu ein Denkfehler, ganze F
heits-Entitäten im naturhistorischen Sinne
ein non-ens zurückzuführen. Wenn die
bute auf Faserstoffmangel beruhten, so hät
stens sagen sollen, ob der Faserstoff, de
Krankheit machte, oder der, welcher übrig
ob etwa jener die Typhen machte und
Diese Art von confusem Denken, dieses Z
schlecht untersuchten Thatsachen und unl
mufs einmal aufhören. Räumen wir auch
zusammengebrochenen Systeme weg, und
Platze auch noch nicht lange Strafsen vol
richten können, nun, so haben wir eine
freiere Aussicht. —

II. Weifses Blut (Leukämie).

Es giebt gewisse Wahrheiten, welche sich in der Wissenschaft nur sehr langsam schrittweise Geltung verschaffen. So scheint es meine ungen über weifses Blut (d. h. eine Vermehrung osen Blutkörperchen in dem Maafse, dafs die rothe es Blutes dadurch in eine röthlich-, gelblich- oder chweifse verwandelt wird) und dem Zusammenhang desselben mit chronischen Milzanschwellungen zu ergehen. Bei der ersten Veröffentlichung des von mir beobachteten Falls (Froriep's N. Notiz. 1845. No. 780.) hob ich schon diesen Zusammenhang hervor und zeigte den Unterschied dieser Blutveränderung von der sogenannten pyämischen. Trotzdem übergeht Bischoff (Müller's Archiv 1846. Jahresber. p. 135.) in seinem Referat den ersteren ganz und bemerkt nur, dafs eine chemische Untersuchung nicht angestellt sei und dafs der Fall mit anderen, unter dieser Bezeichnung aufbewahrten Fällen nur die Aehnlichkeit des äufseren Ansehens

WHO classification, 2016 upgrade

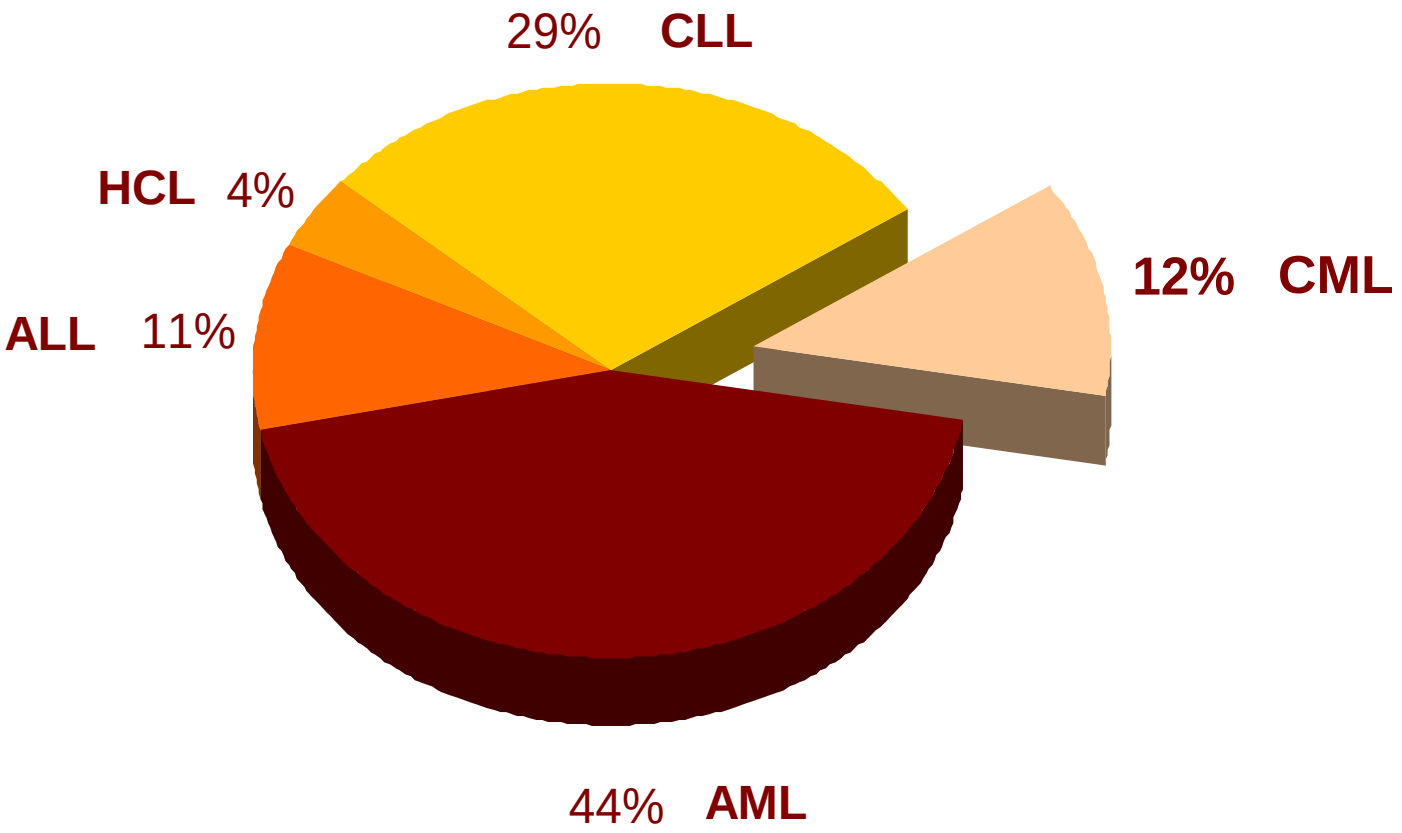
- More than 50 types and subtypes
- Leukemias – **simply, disturbances in the regulation of growth and differentiation of WBC, white blood cells**
- Key types:
- CML, chronic myeloid leukemia
- AML, acute myeloid leukemia
  - APL, acute promyelocytic leukemia
- ALL, acute lymphoblastic leukemia
- CLL, chronic lymphocytic leukemia
  - HCL, hairy cell leukemia

Key clinical signs, pathogenesis

- Leukocytes
  - leukocytosis, hyperviscosity
  - leukopenia, neutropenia
  - diminished cellular immunity, diminished humoral immunity (CLL)
  - **infections**
- Thrombocytes
  - thrombocytopenia
  - **bleeding**
  - thrombocytosis (CML)
- Erythrocytes
  - **anemia**
- Organ infiltration
  - **bone marrow**, spleen, liver, lymph nodes, brain, testis, skin, …
  - myelosarcoma

1000 leukemia cases

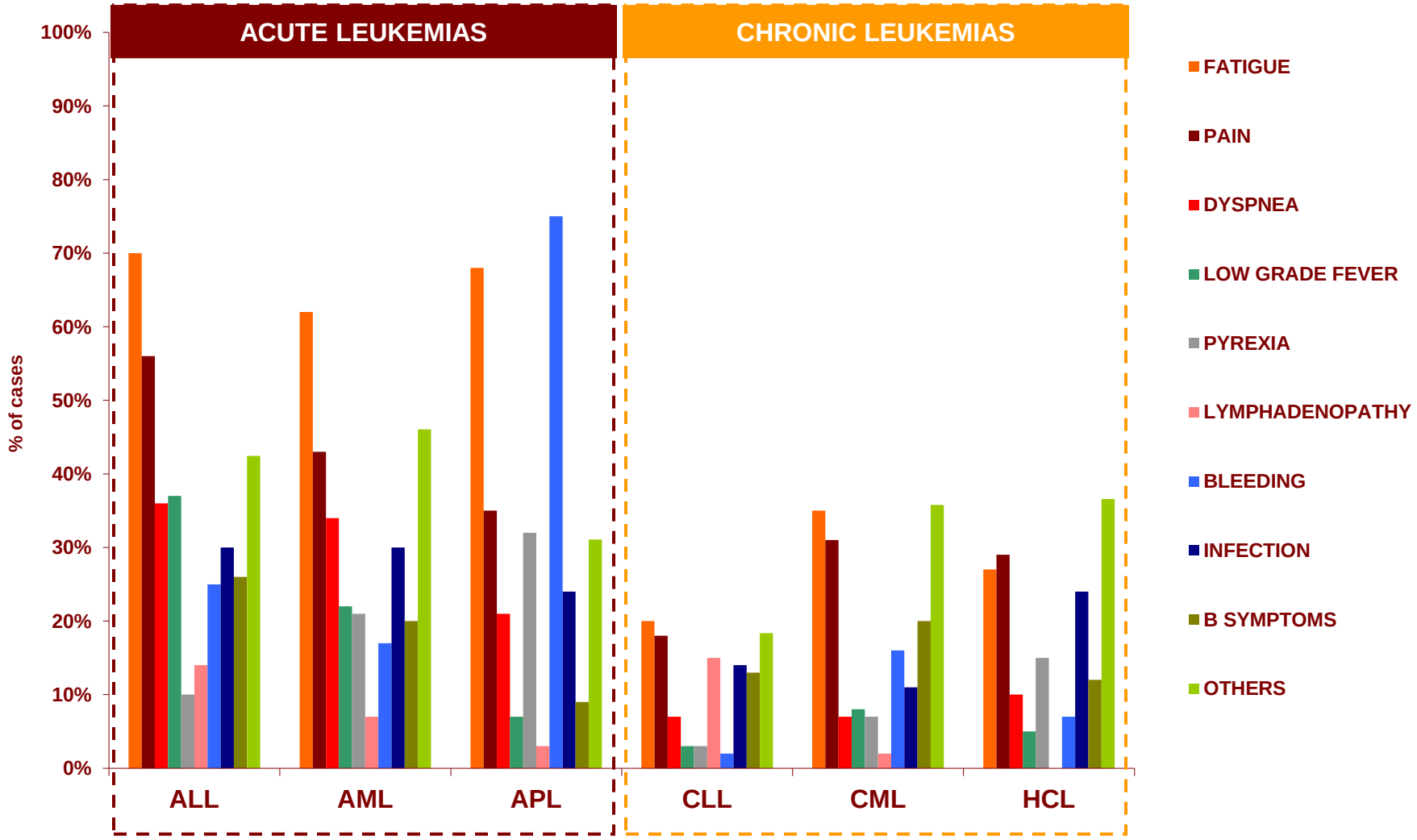
Number of analyzed cases (2004-2009) – 1007 patients



Ráčil et al., Am J Hematol 86, 2011, 800–803

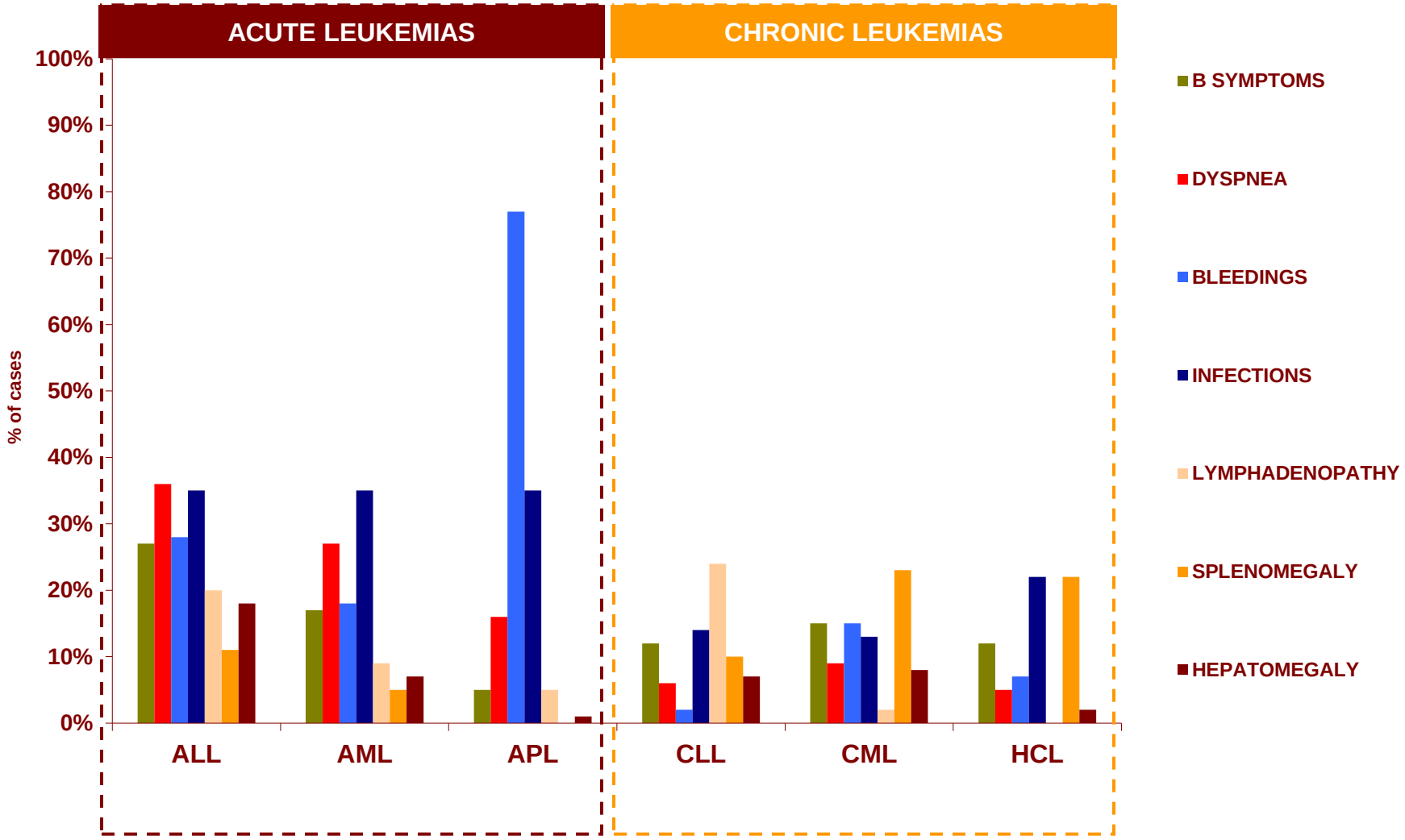
Clinical symptoms

Subjective complaints of patients according to the history at diagnosis



Clinical symptoms

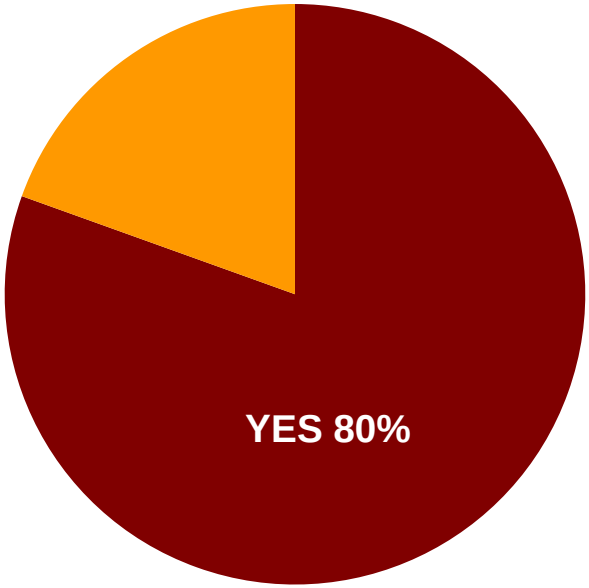
Objective findings by the first visited physician



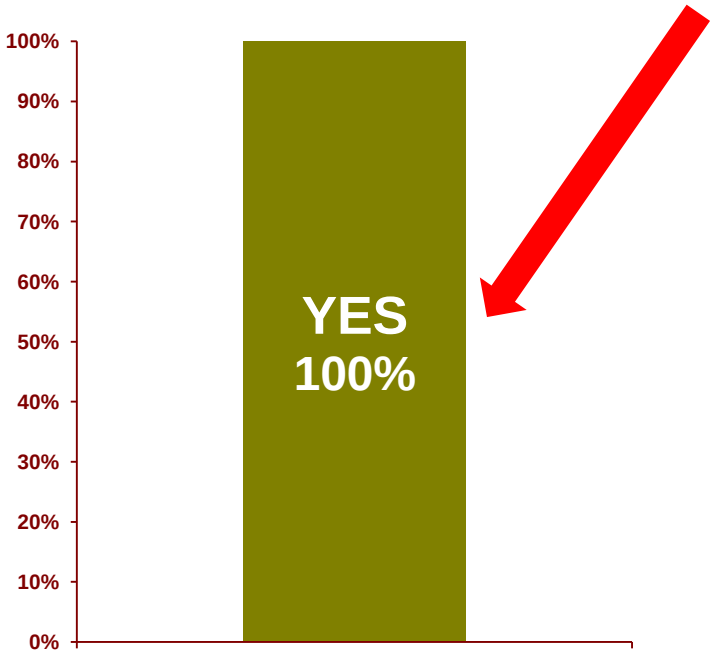
Blood count

Lab exam by the first visited physician

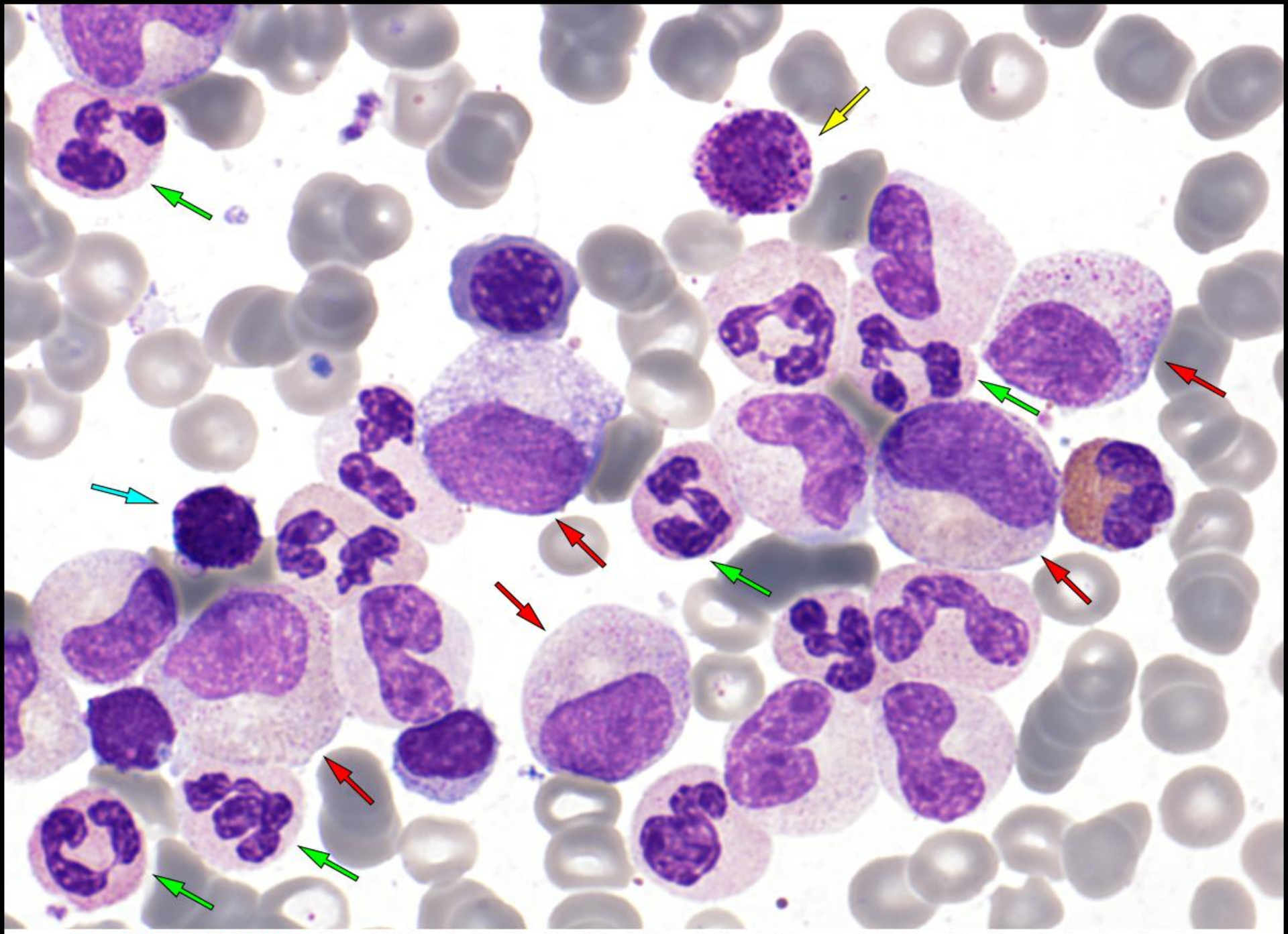
PERFORMED?

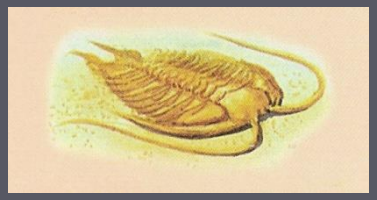


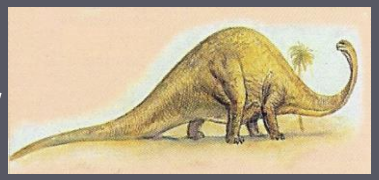
ABNORMALITY?

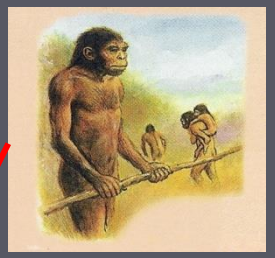


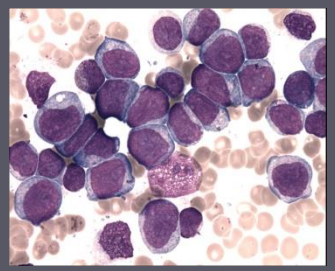
CML











time

„nothing"

a lot of

leukemia (1850)

chemotherapy (1950)

Ph^1 (1960)

BMT IFN

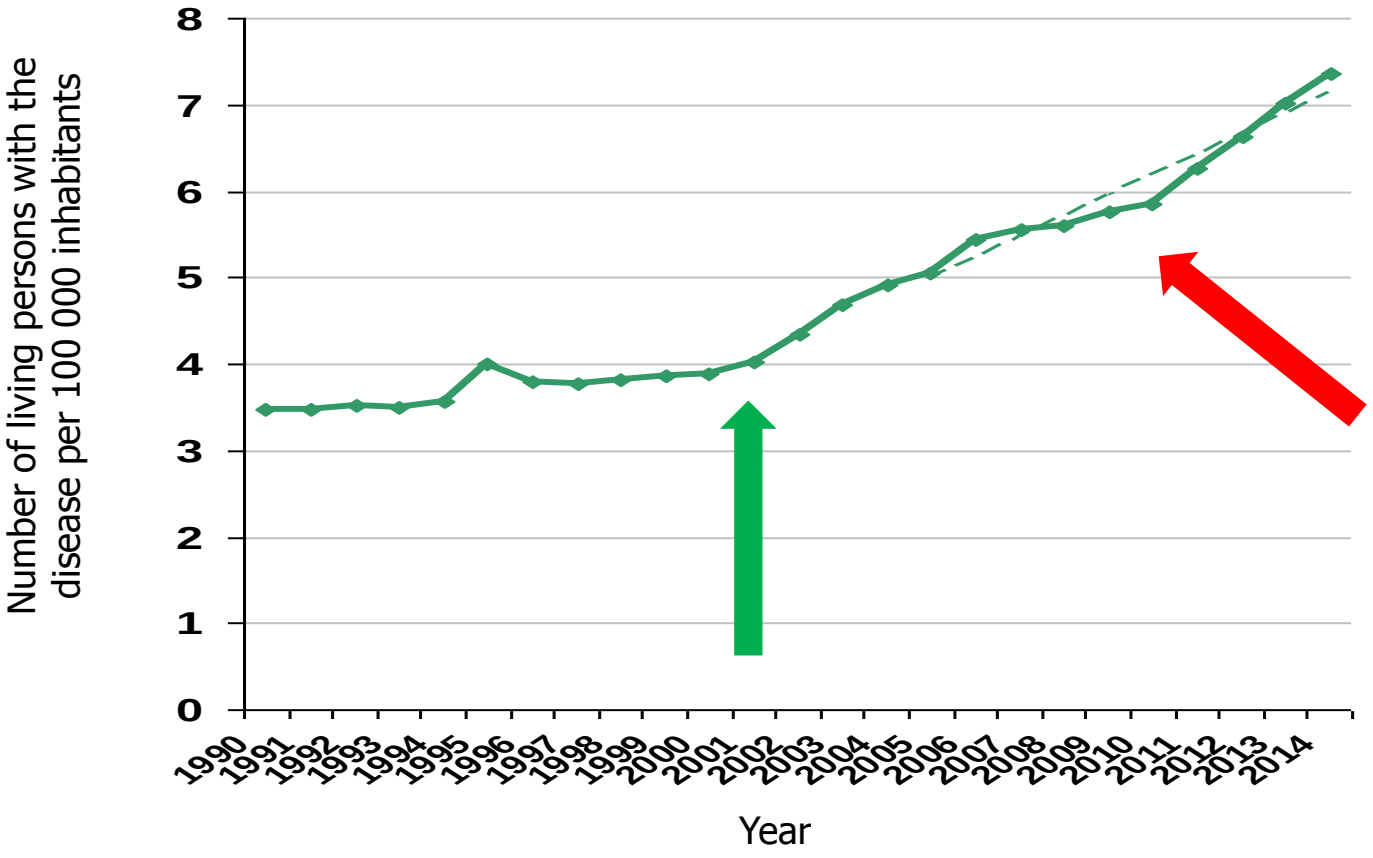
BCR/ABL PCR

imatinib, dasatinib, nilotinib

others

Epidemiology of CML in Czechia

Prevalence



Source: National Cancer Registry, ÚZIS ČR

BLOOD *The Journal of Hematology*

MARCH, 1960 VOL. XV, NO. 3

Leukemia in Hiroshima Atomic Bomb Survivors

By ROBERT HEYSSEL, A. BERTRAND BRILL, LOWELL A. WOODBURY, EDWIN T. NISHIMURA, TARUNENDU GHOSE, TAKASHI HOSHINO AND MITSURU YAMASAKI

Table 5.—*Incidence of Leukemia by Type*

Type of Leukemia	Japanese Exposed Survivors *			
	< 2,000 m		2,000-10,000 m	
	No.	Incidence	No.	Incidence
Acute Granulocytic	12	80	7	20
Chronic Granulocytic	15	100	1	3
Acute Lymphatic	3	20	0	-
Acute - Type Unspec.	2	13	1	3
Chronic Lymphatic	0	-	1	3

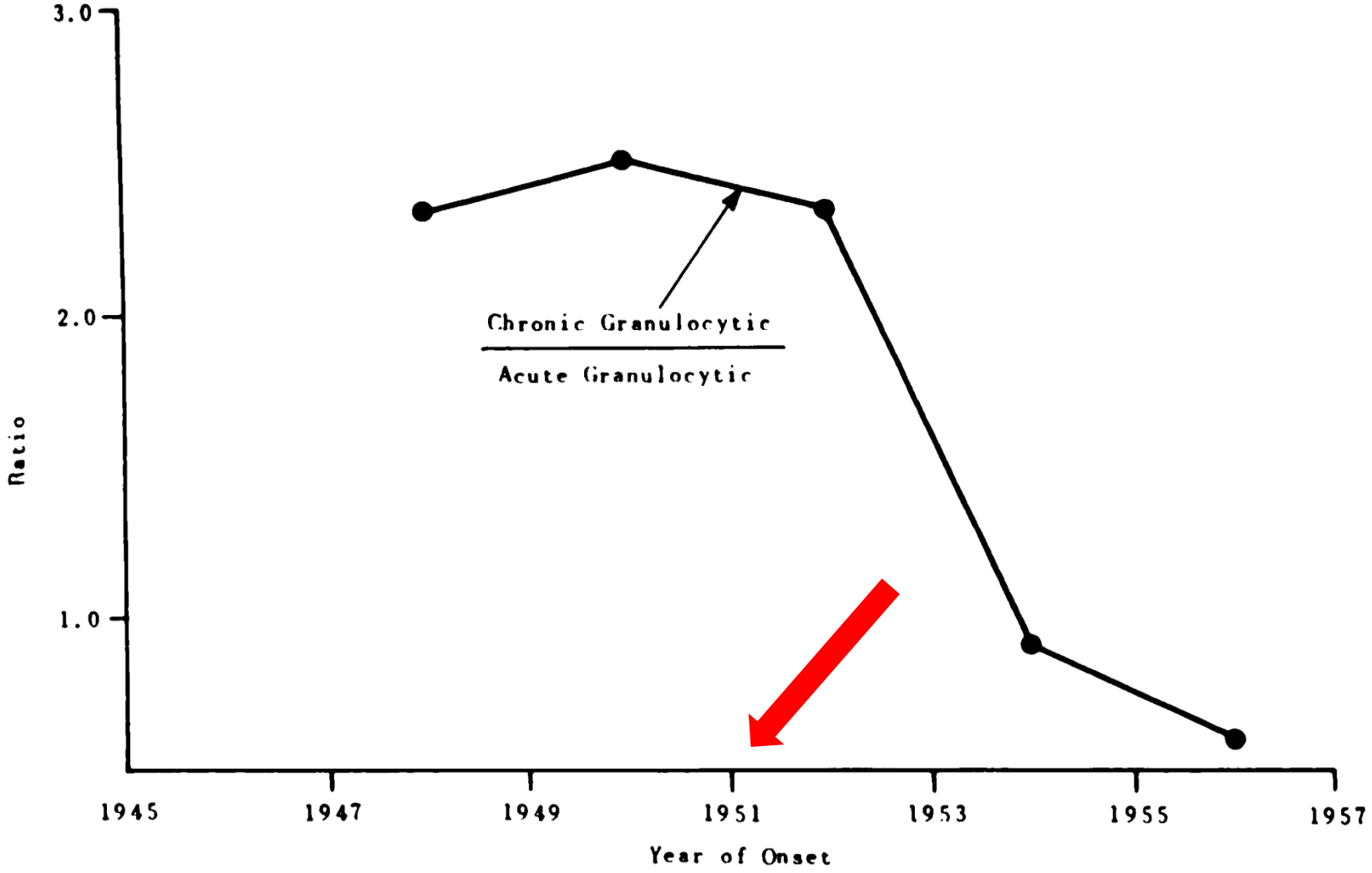


Fig. 6.—Annual changes in type distribution—exposed leukemia patients.

A Minute Chromosome in Human Chronic Granulocytic Leukemia

In seven cases thus far investigated (five males, two females), a minute chromosome has been observed replacing one of the four smallest autosomes in the chromosome complement of cells of chronic granulocytic leukemia cultured from peripheral blood. No abnormality was observed in the cells of four cases of *acute* granulocytic leukemia in adults or of six cases of acute leukemia in children. There have been several recent reports of chromosome abnormalities in a number of cases of human leukemia [including two of the seven cases reported here: Nowell and Hungerford, *J. Natl. Cancer Inst.* 25, 85 (1960)], but no series has appeared in which there was a consistent change typical of a particular type of leukemia.

Cells of the five new cases were obtained from peripheral blood (and bone marrow in one instance), grown in culture for 24–72 hours, and processed for cytological examination by a recently developed air-drying technique (Moorhead, *et al.*, *Exptl. Cell Research*, in press). The patients varied from asymptomatic untreated cases to extensively treated cases of several years duration in terminal myeloblastic crisis. All seven individuals showed a similar minute chromosome, and none showed any other frequent or regular chromosome change. In most of the cases, cells with normal chromosomes were also observed. Thus, the minute is not a part of the normal chromosome constitution of such individuals.

The findings suggest a causal relationship between the chromosome abnormality observed and chronic granulocytic leukemia.

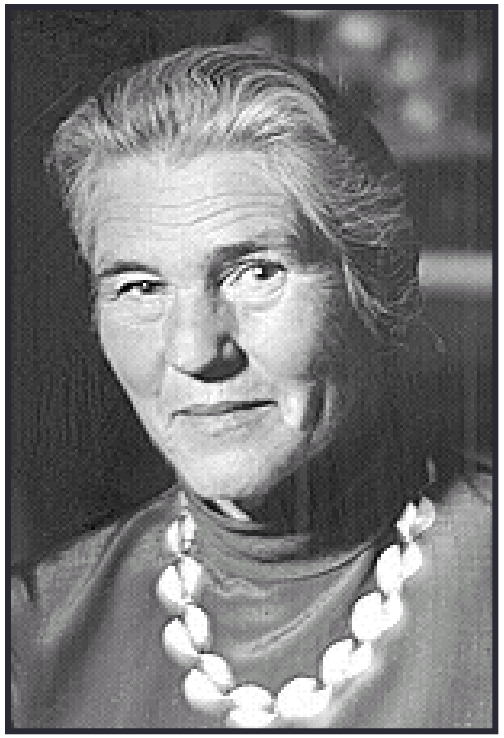
PETER C. NOWELL
School of Medicine, University of Pennsylvania
DAVID A. HUNGERFORD
Institute for Cancer Research

1960

A minute chromosome in human granulocytic leukemia. Science 132, 1960, 1497.

P.C. Nowell, D.A. Hungerford, University of Pennsylvania in **Ph**iladelphia

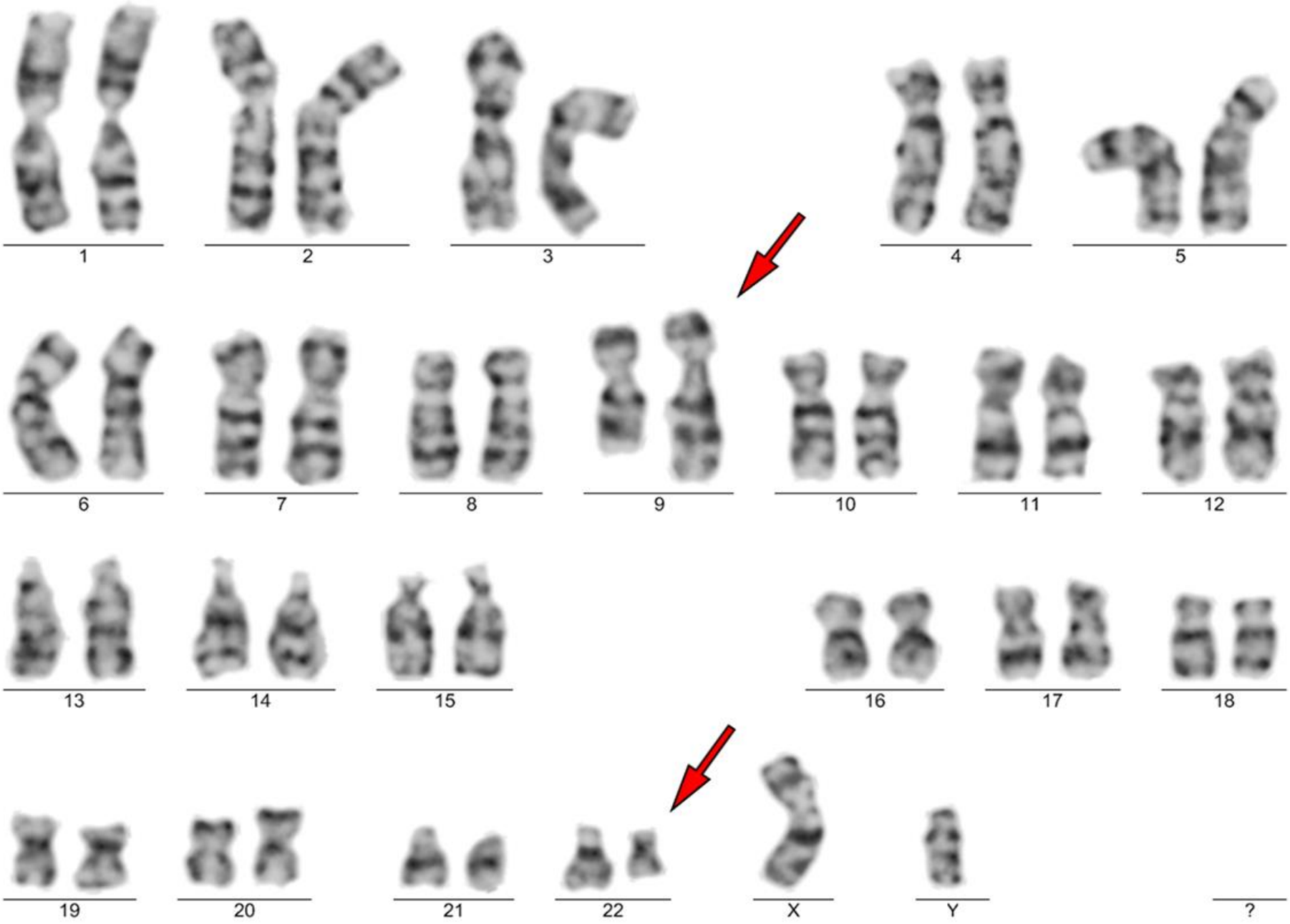
...the findings **suggest a causal relationship** between the chromosome abnormality observed and chronic granulocytic leukemia…

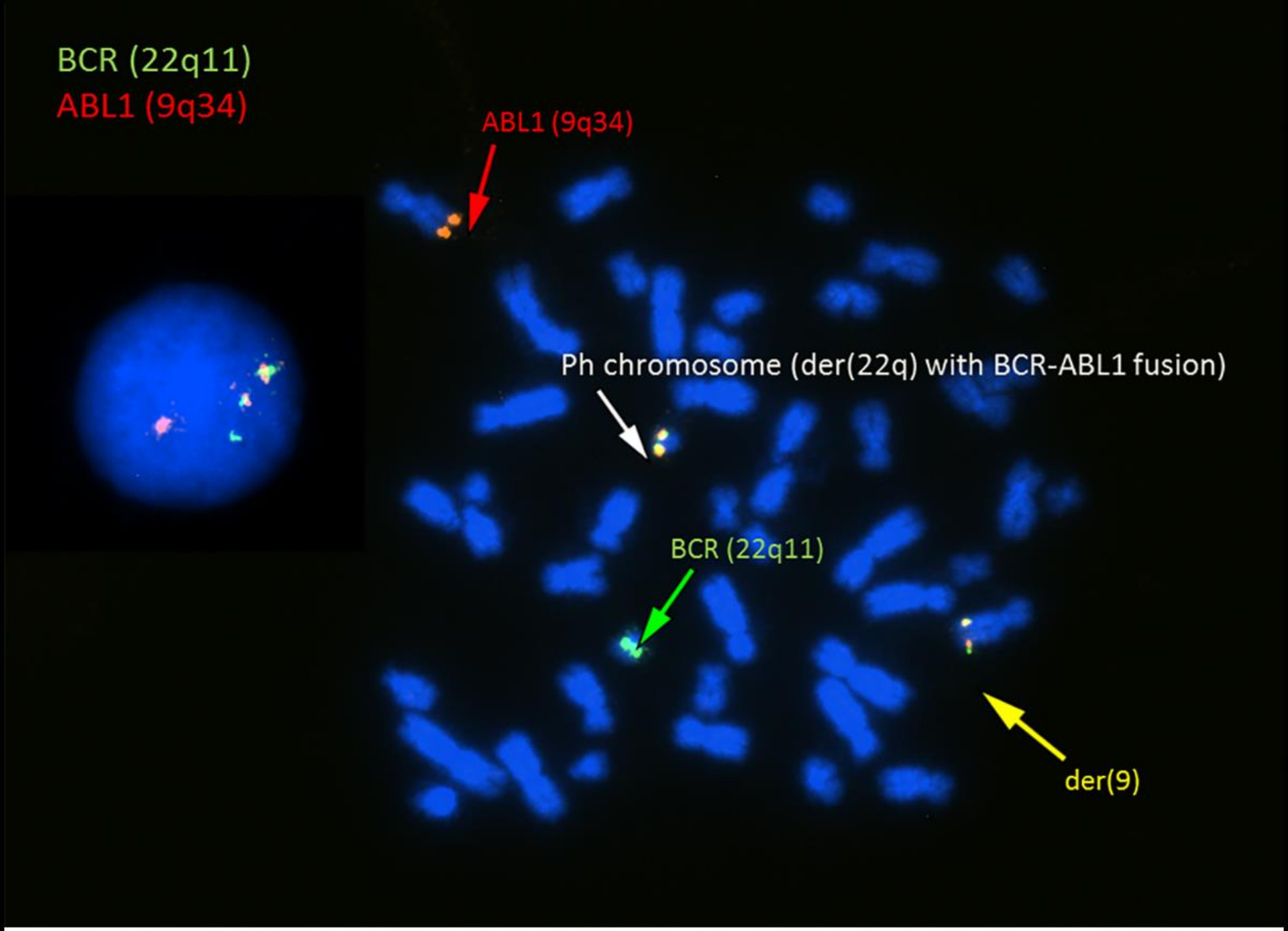


1973: translocation of chromosomal material

Rowley JD: A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature, 243, 290-293, 1973

…suggesting that there may be a hitherto undetected translocation between the long arm of 22 and the long arm of 9, producing the 9q+ chromosome…





1982: abl localized on chromosome 9

- *Heisterkamp N et al.: Chromosomal localization of human cellular homologues of two viral oncogenes. Nature 299, 1982, 747-749.*

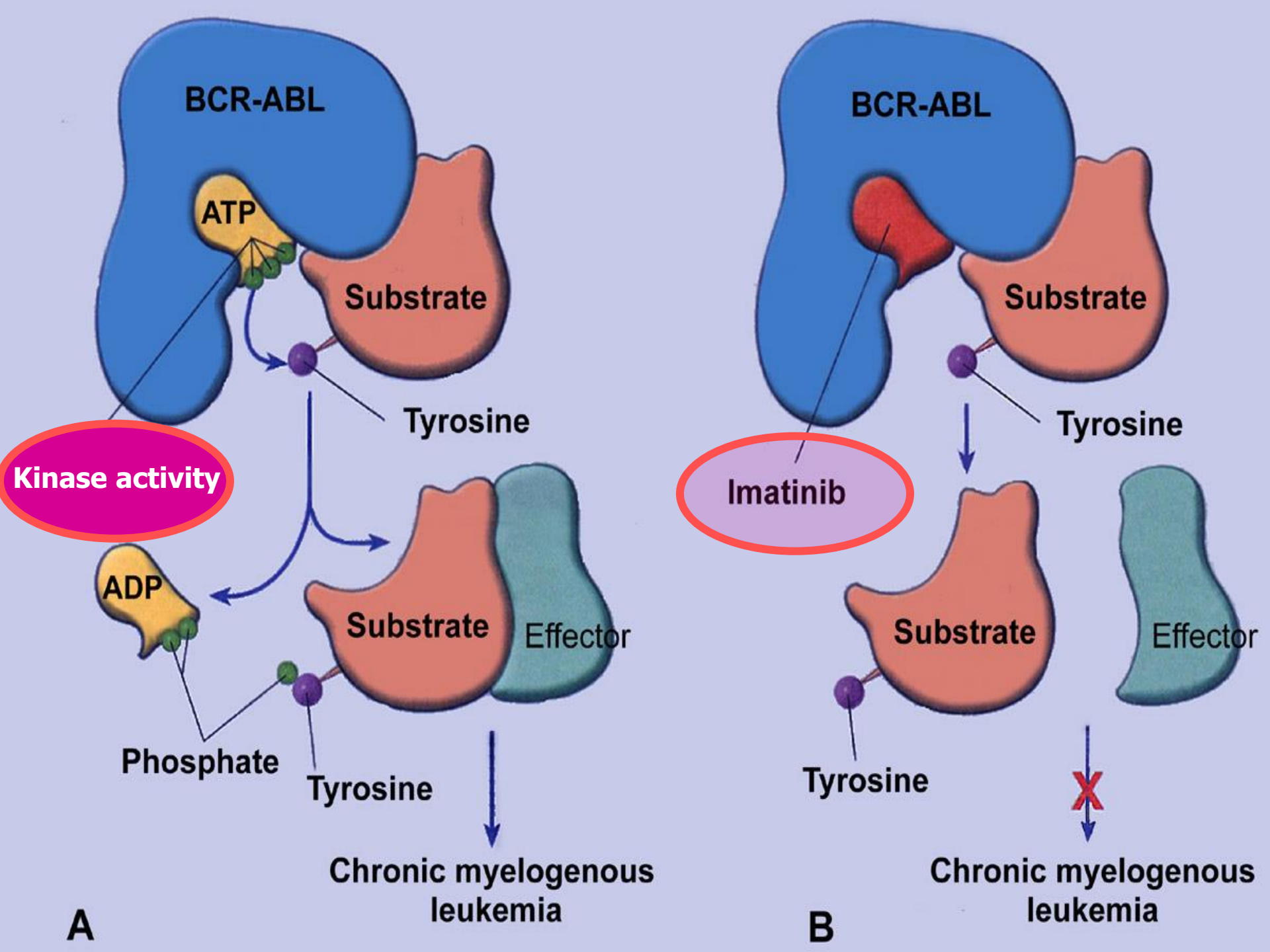
- …we now show that the human equivalents of c-fes and c-abl are localized on human chromosomes 15 and 9, respectively. It is of interest that both of these chromosomes are involved in specific rearrangements found in certain forms of human cancer…

- *ABL* gene = the human homologue of the *v-abl* oncogene of the Abelson murine leukemia virus. Abelson HT, Rabstein LS: Proc Am Assoc Cancer Res 10: 1, 1969

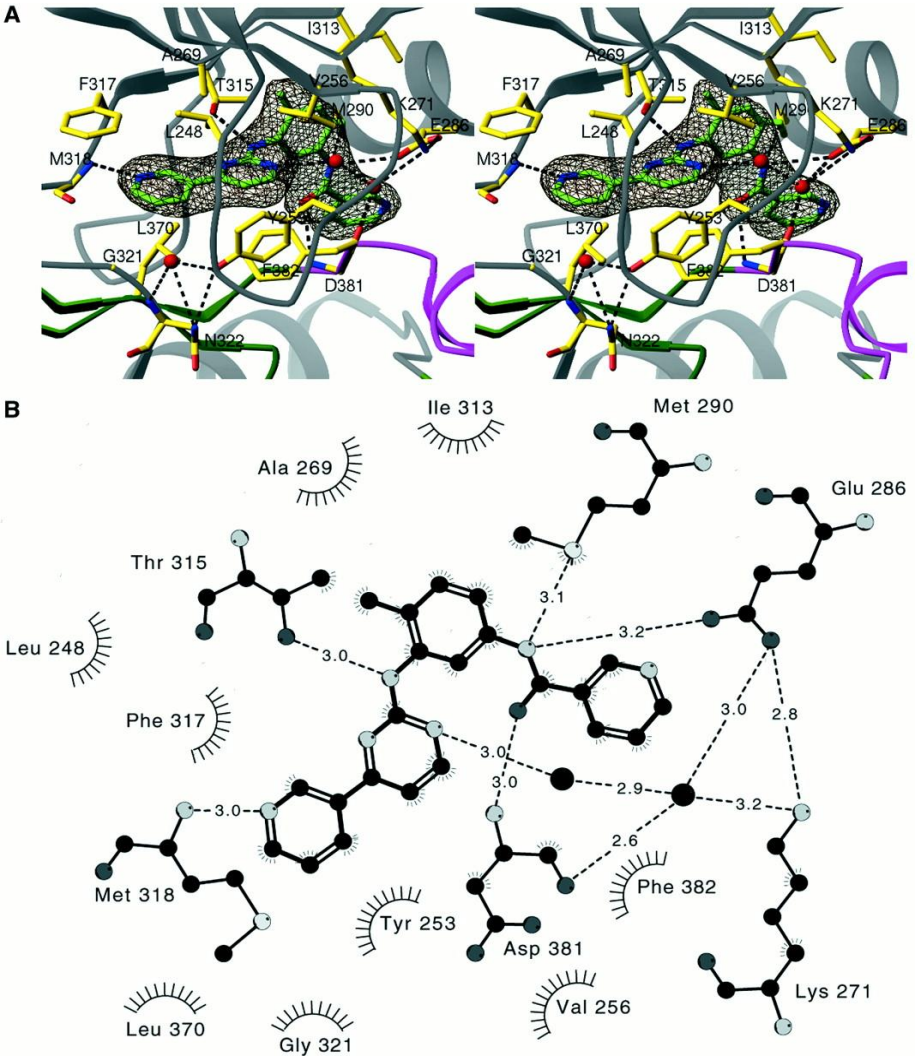
1985: fused protein BCR-ABL

- *Shtivelman E et al.: Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature 315, 1985, 550-554.*

- …characterization of an 8-kilobase RNA specific to chronic myelogenous leukaemia shows it to be a FUSED transcript of the two genes. The FUSED protein that would be produced is probably involved in the malignant process…

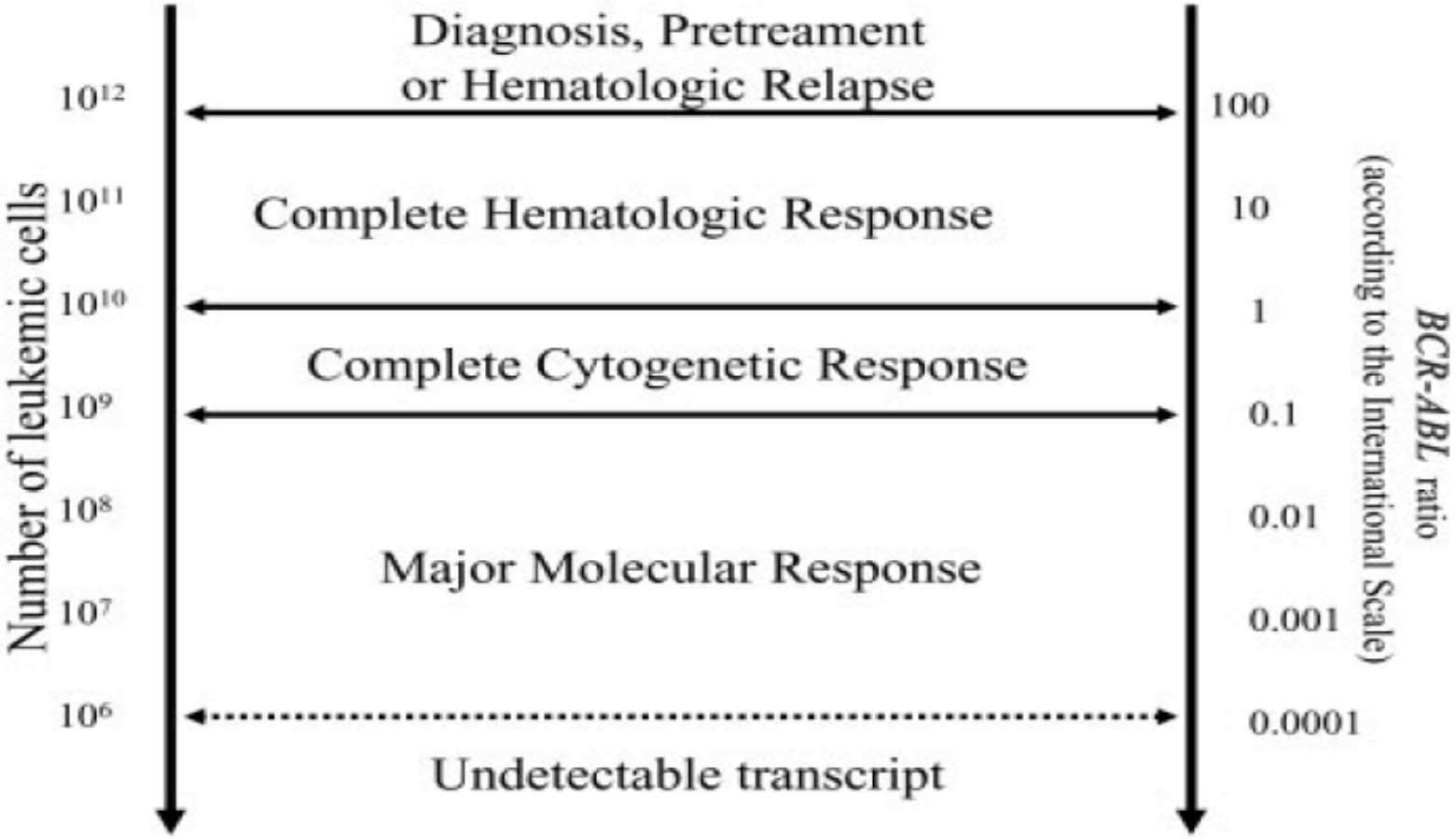

BCR-ABL
ATP
Substrate
Tyrosine
Kinase activity
ADP
Substrate
Effector
Phosphate
Tyrosine
Chronic myelogenous leukemia
A
BCR-ABL
Substrate
Tyrosine
Imatinib
Substrate
Effector
Tyrosine
X
Chronic myelogenous leukemia
B

Crystal structure solved, but resistance described



- Schindler T et al.: Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. Science 289, 2000, 1938-1942.
- Gorre ME et al.: Clinical resistance to STI-571 cancer therapy caused by BCR-ABL GENE MUTATION or amplification. Science 293, 2001, 876-880.

Relationship between the number of malignant cells, therapy response, and *BRC-ABL*

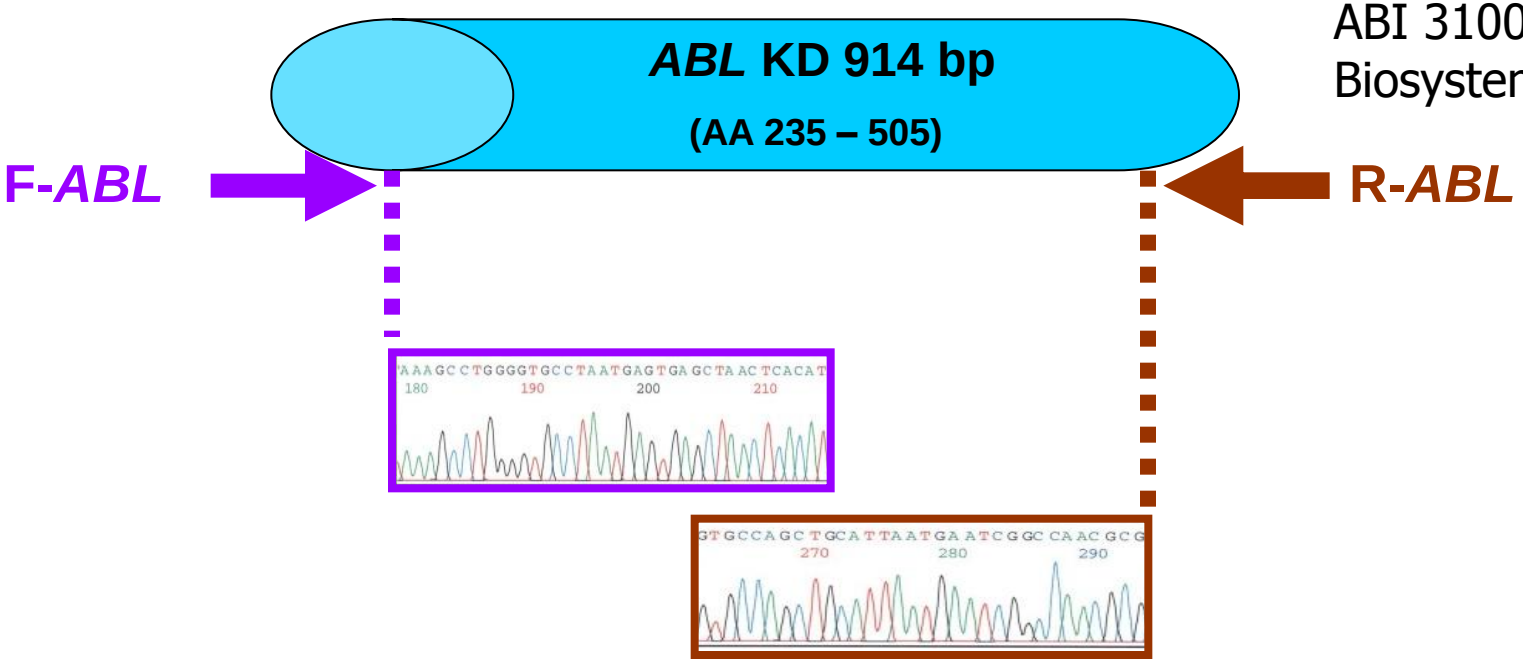


Baccarani et al.: Blood 108, 2006, 1809-1820

Direct sequencing

- BigDye v3.1 Termination kit (Applied Biosystems)

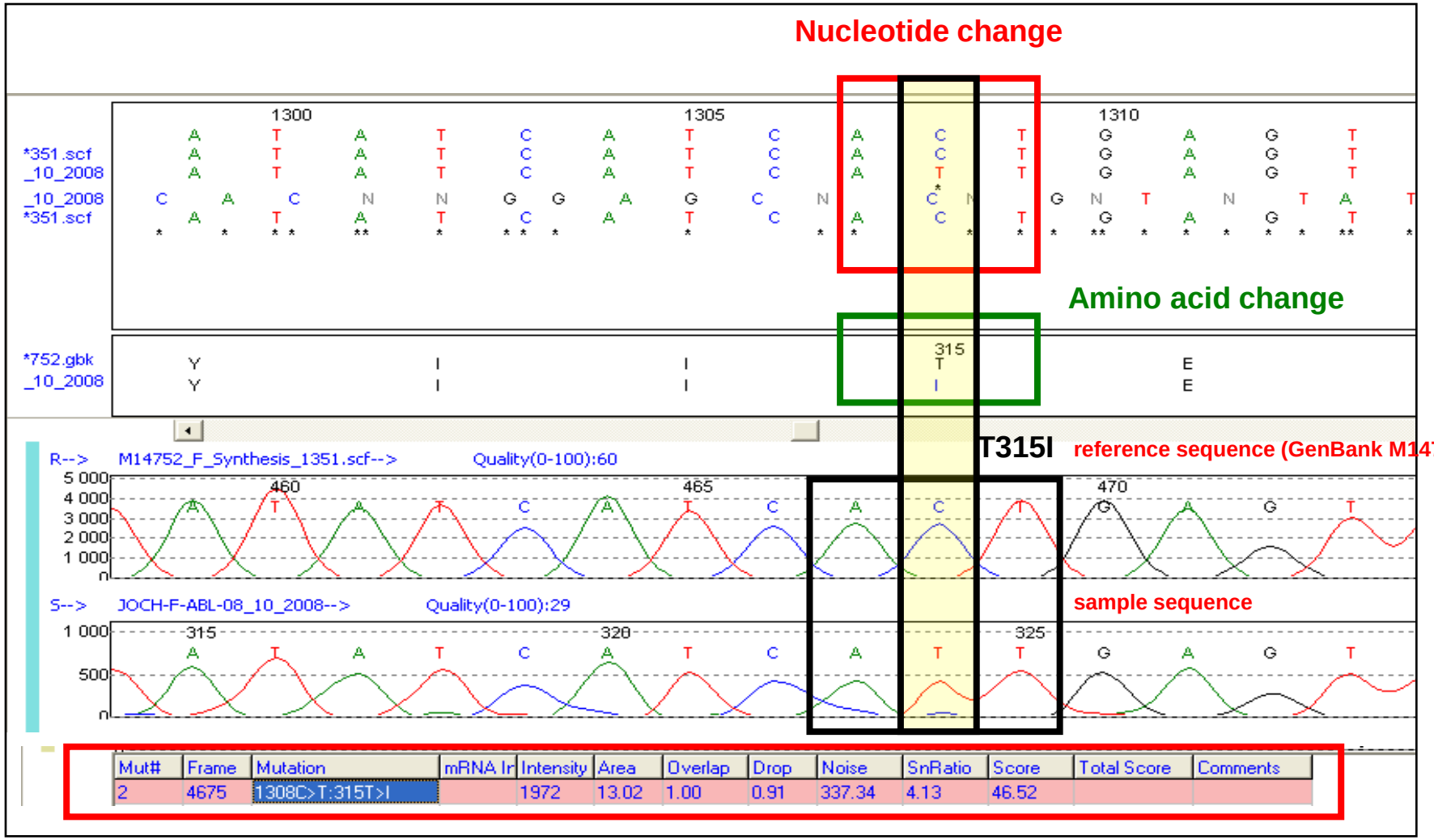
ABI 3100 (Applied Biosystems)



Department of Internal Medicine,
Hematology and Oncology,
University Hospital Brno
and Masaryk University, School of Medicine

Mutation detection - MutationSurveyor® software (Softgenetics)

Department of Internal Medicine,
Hematology and Oncology,
University Hospital Brno
and Masaryk University, School of Medicine



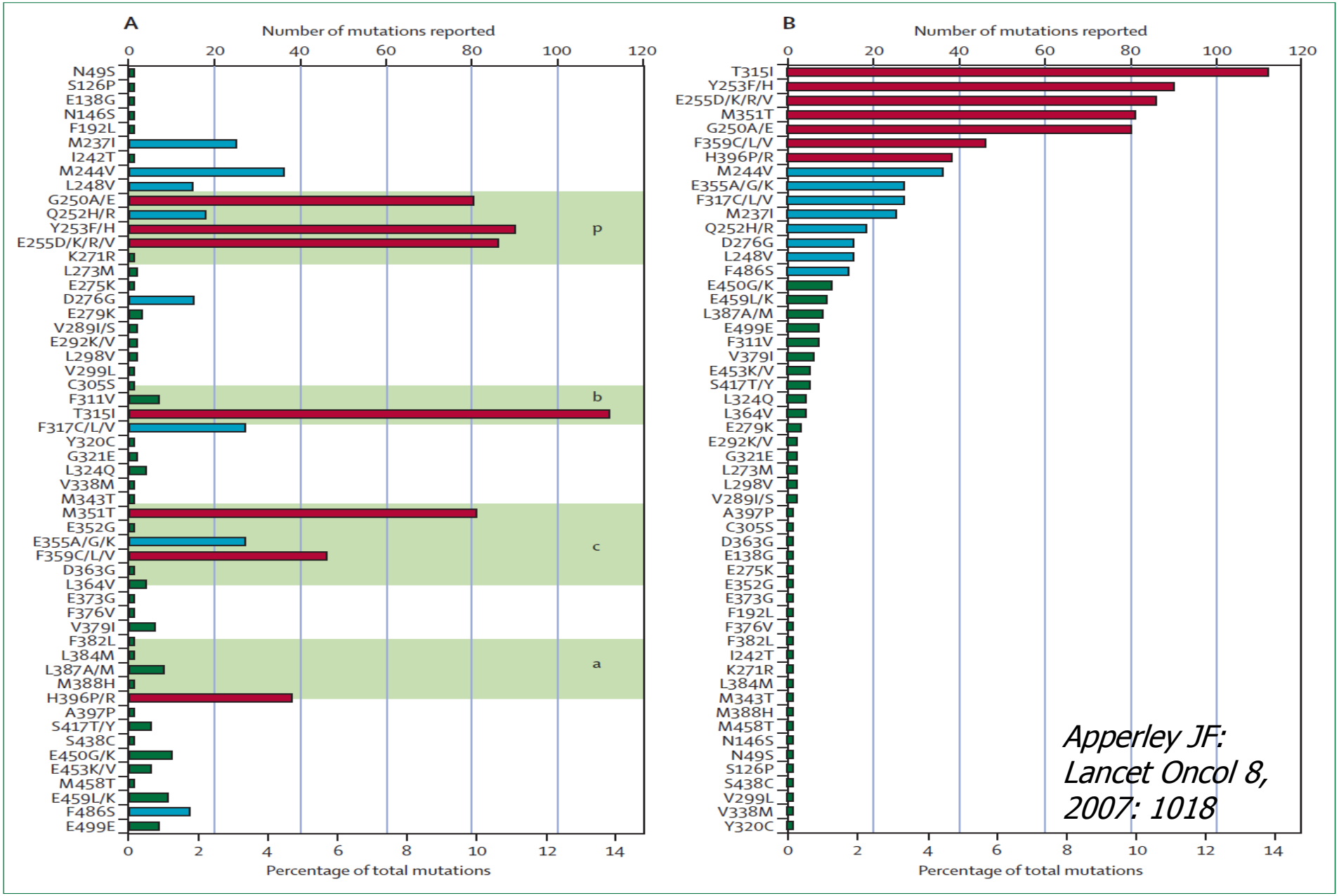
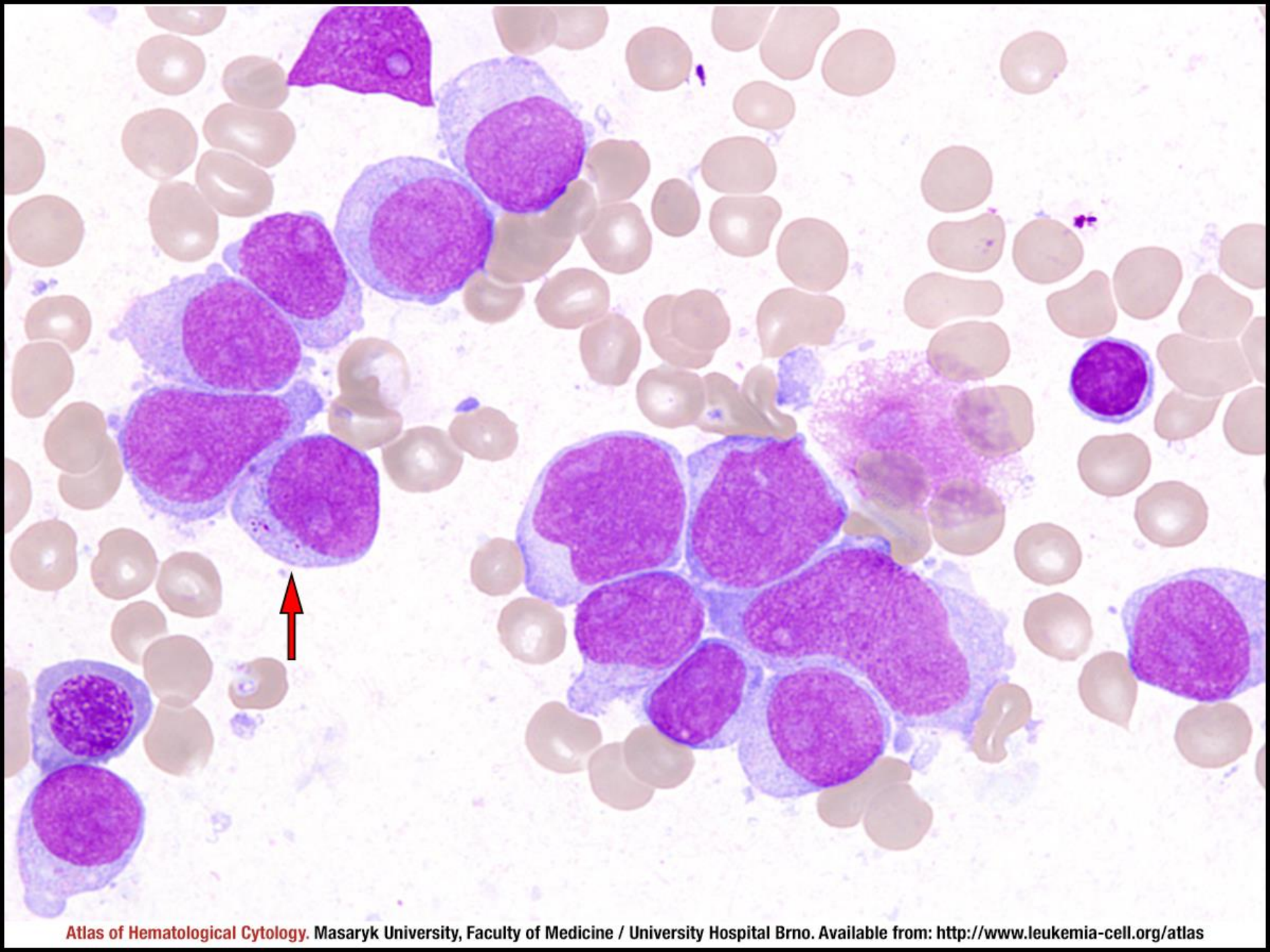


Figure 3: **Incidence of mutations in clinical practice**

(A) Incidence of mutations within the kinase domain by absolute number reported and by percentage of total. The seven most frequent mutations are depicted in red and the following eight most common mutations in blue. Specific regions of the kinase domain are indicated as P-loop or ATP binding site (p), imatinib binding site (b), catalytic domain (c) and activation loop (a). (B) Incidence of mutations in order of frequency; the seven most frequent mutations are depicted in red and the following eight most common mutations in blue.

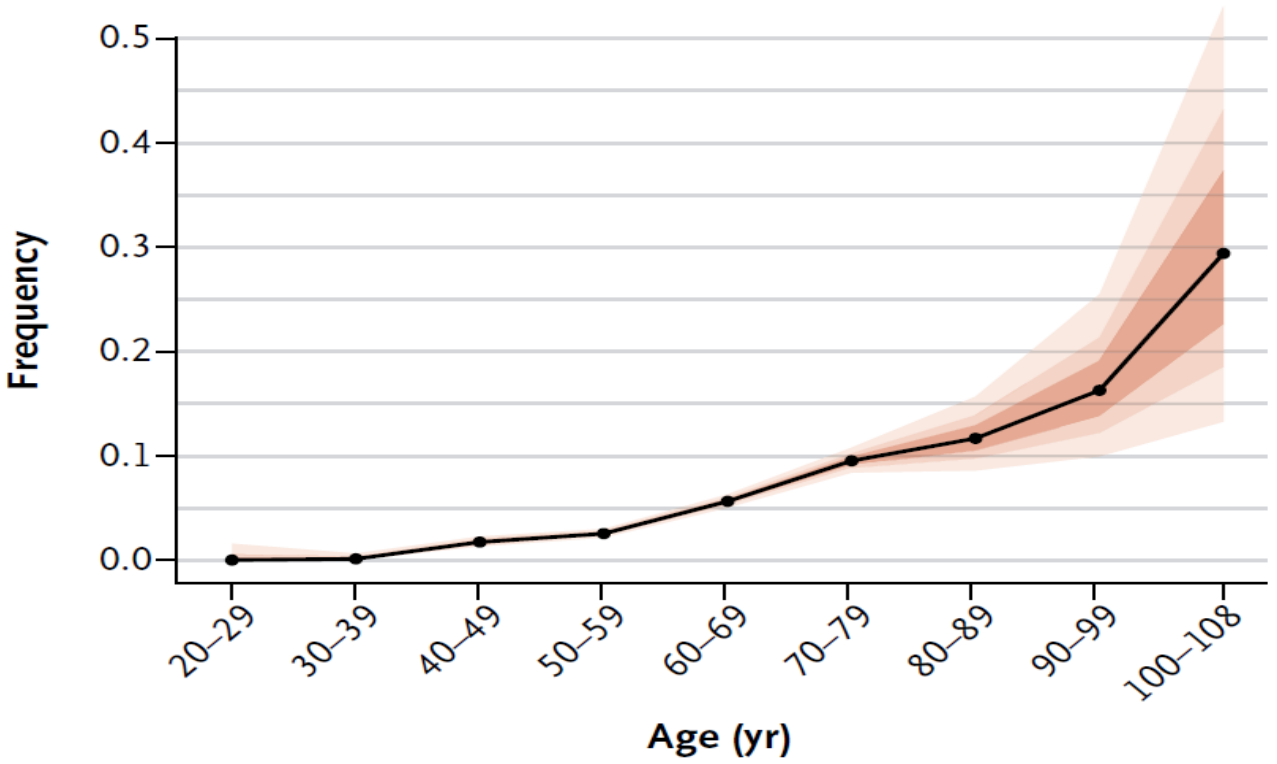
AML



AML: outline

- Myeloid pattern of the malignant cells
- Variable maturation grade
- Amazing genetic heterogeneity significantly influencing the prognosis
- Prognostic stratification used in clinical practice for selecting the best therapeutic strategy
- New molecular pathogenesis findings lead to the development of new, targeted therapies very recently

Clonal hematopoiesis

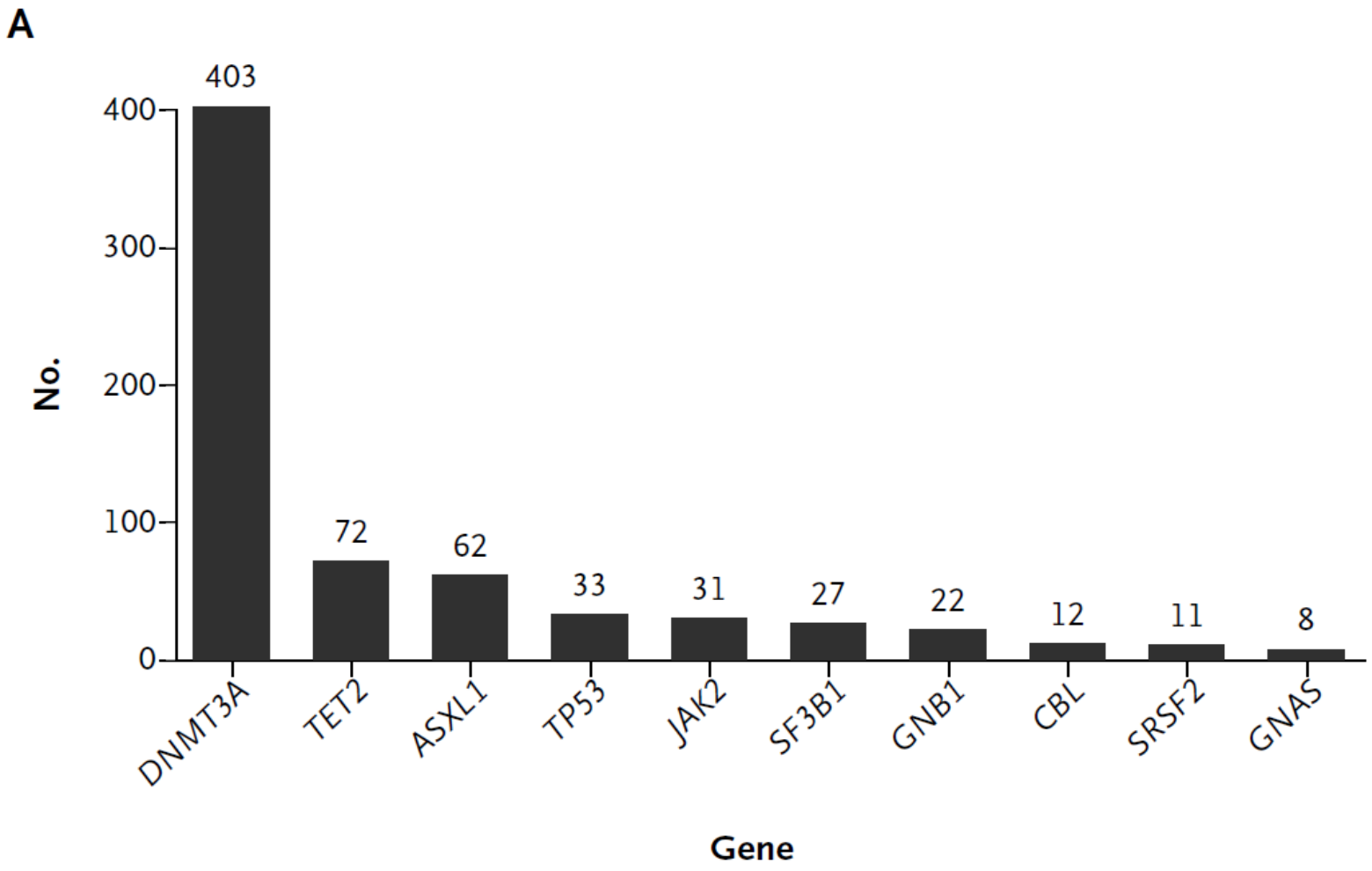


No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

Figure 1. Prevalence of Somatic Mutations, According to Age.
Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

Jaiswal S et al. N Engl J Med 2014;371:2488-2498

Clonal hematopoiesis



Jaiswal S et al. N Engl J Med 2014;371:2488-2498

DNMT3A

- DNA (cytosine-5)-methyltransferase 3A is an enzyme that catalyzes the transfer of methyl groups to specific CpG structures in DNA, a process called DNA methylation. The enzyme is encoded in humans by the DNMT3A gene.

- It is responsible for de novo DNA methylation. DNMT3A forms part of the family of DNA methyltransferase enzymes.

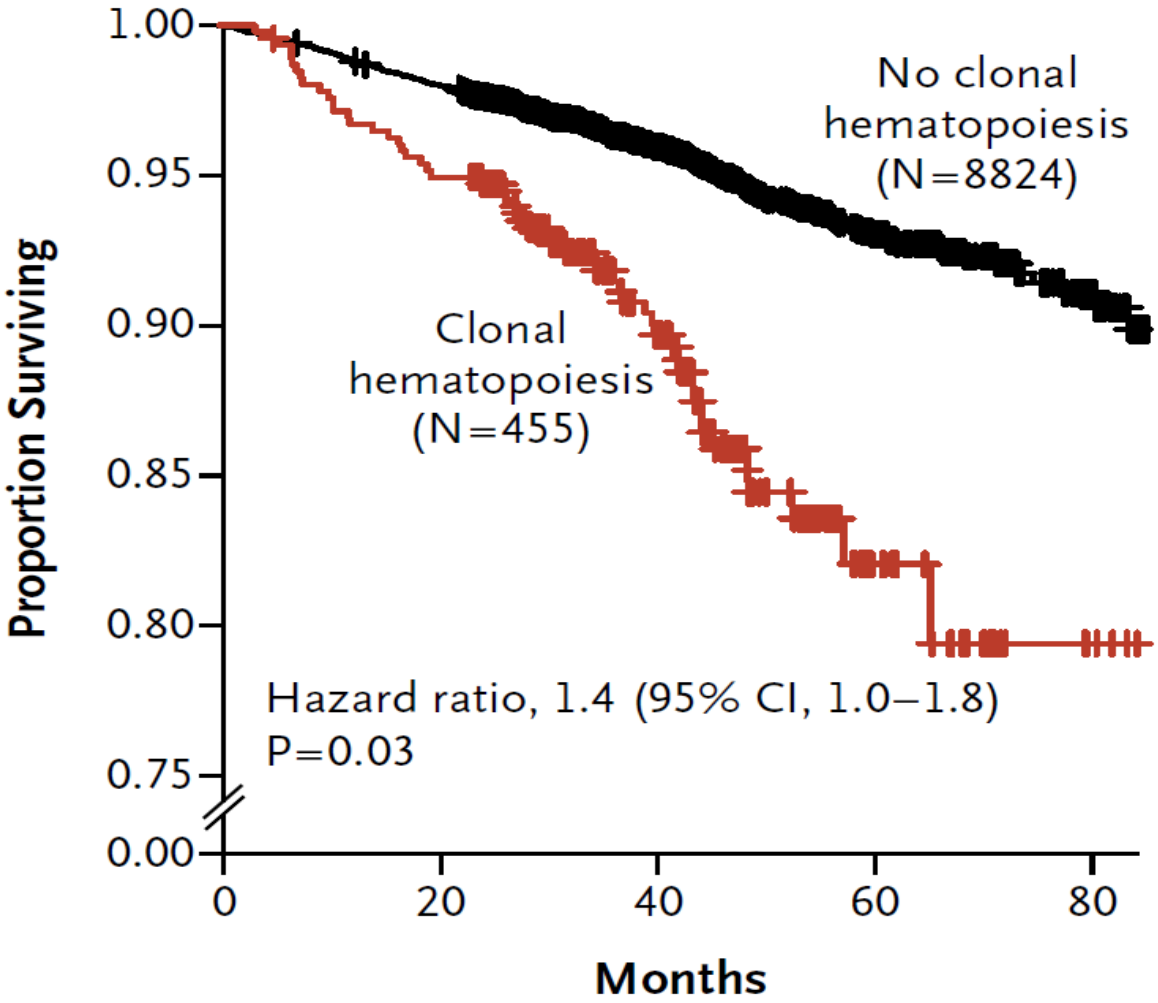
- While de novo DNA methylation modifies the information passed on by the parent to the progeny, it enables key epigenetic modifications essential for processes such as cellular differentiation and embryonic development, transcriptional regulation, heterochromatin formation, X-inactivation, imprinting and genome stability.

TET2

- TET2 tet methylcytosine dioxygenase 2
- The protein is a methylcytosine dioxygenase that catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine.
- The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders.

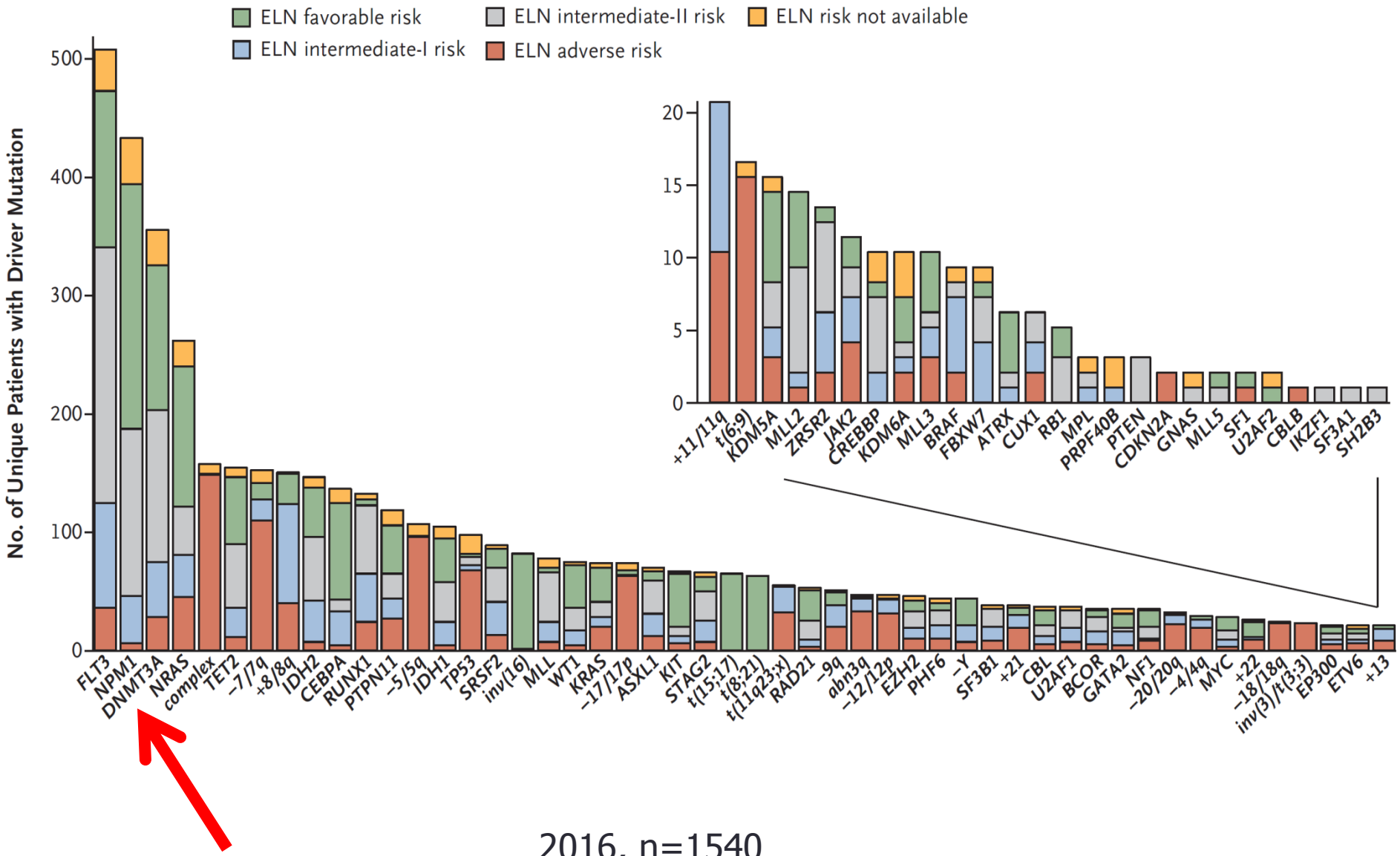
ASXL1

- The ASXL1 gene provides instructions for making a protein that is involved in a process known as chromatin remodeling.
- Through its role in chromatin remodeling, the ASXL1 protein regulates the expression of many genes, including a group of genes known as HOX genes.
- The ASXL1 protein may have an additional role in gene regulation by signaling to molecules to add a methyl group (a process called methylation) to an area near a gene called the promoter region, which controls gene activity. When a promoter region is methylated, gene activity is repressed, and when a promoter region is not methylated, the gene is active.



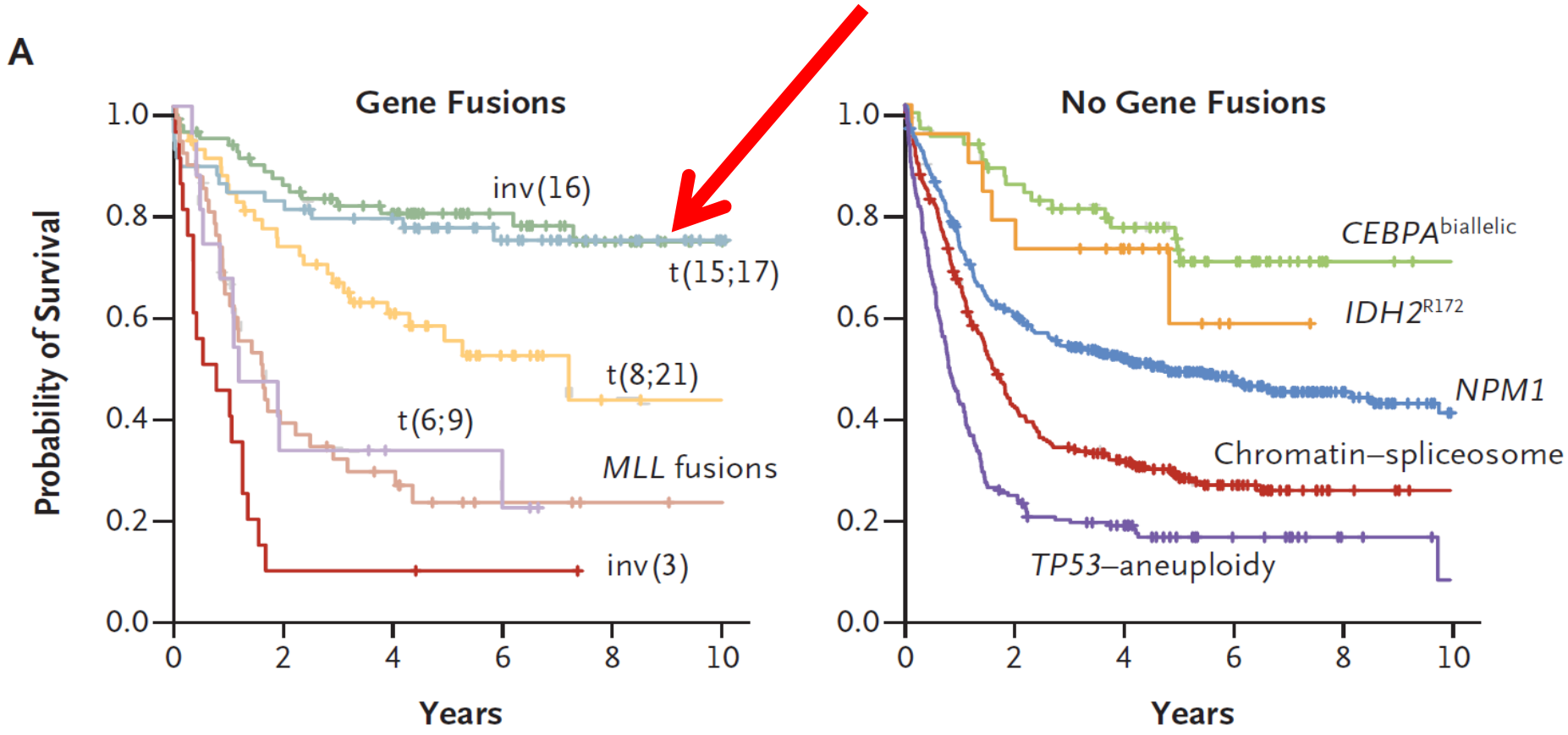
Clonal hematopoiesis: more frequent hematological malignancies, as well as cardiovascular diseases! Increase in total mortality.

Genovese G et al. N Engl J Med 2014;371:2477-2487

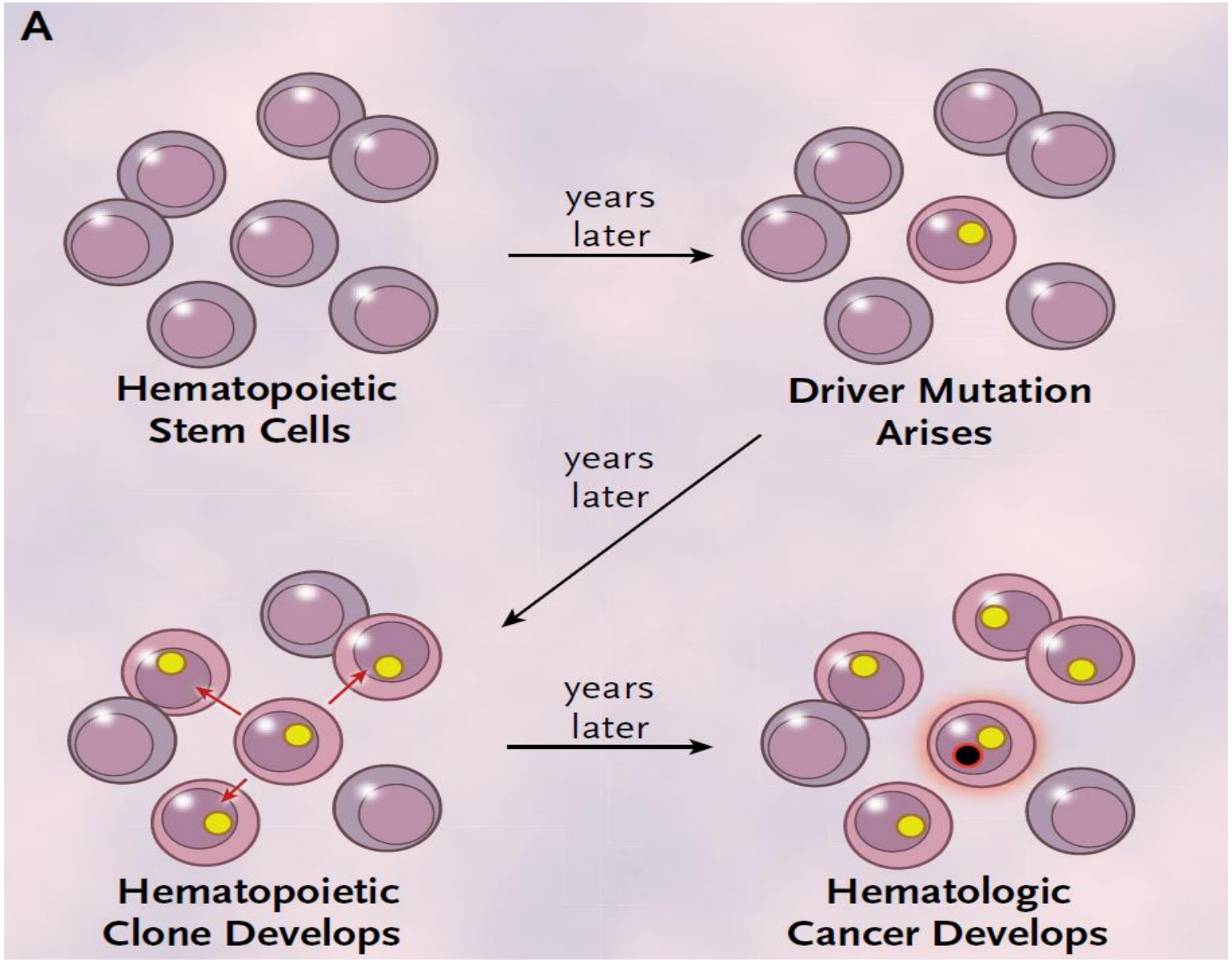


2016, n=1540

Papaemmanuil E et al. N Engl J Med 2016;374:2209-2221

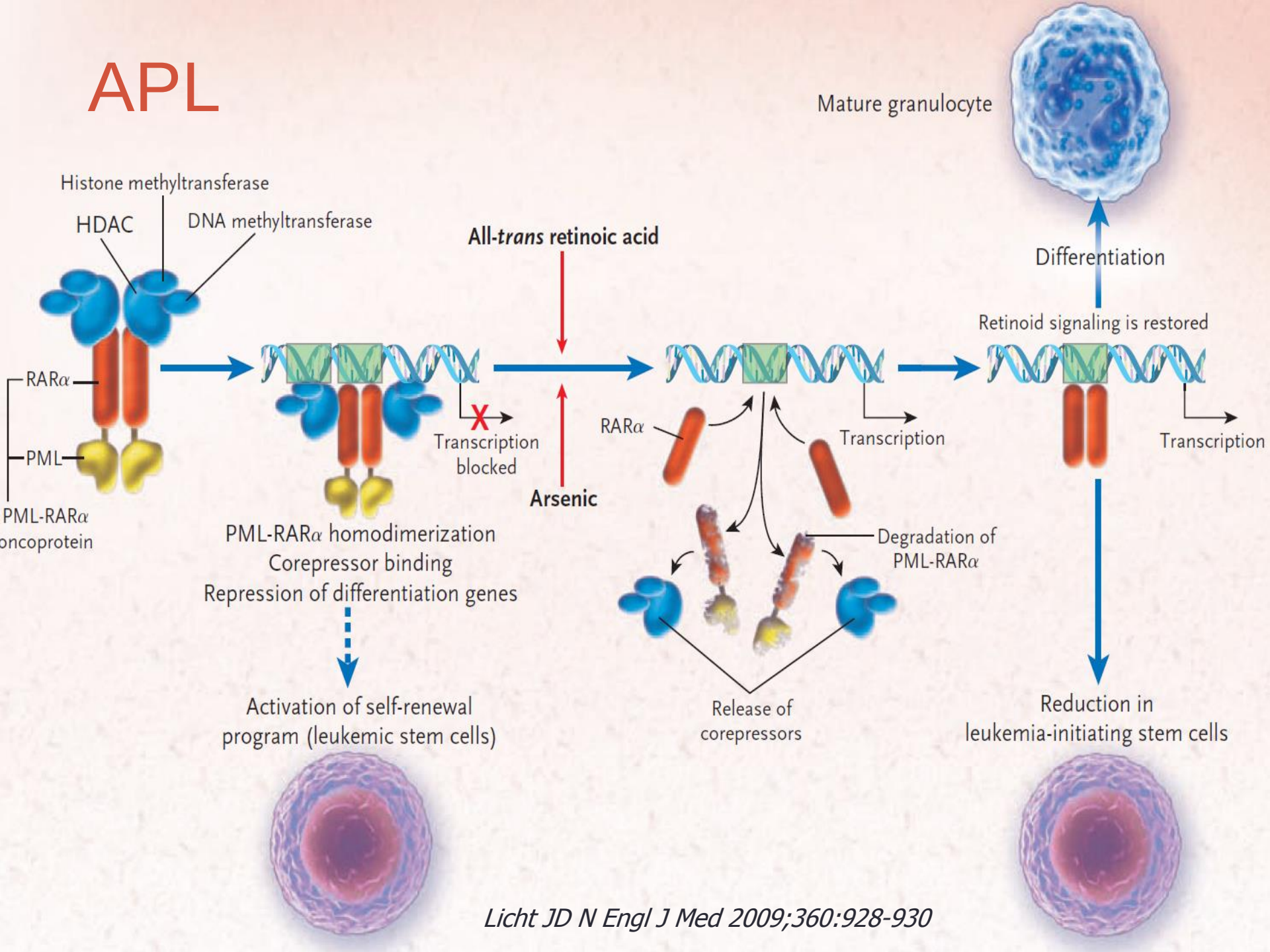


Papaemmanuil E et al. N Engl J Med 2016;374:2209-2221



Genovese G et al. N Engl J Med 2014;371:2477-2487

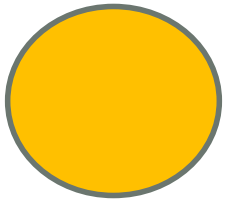
APL

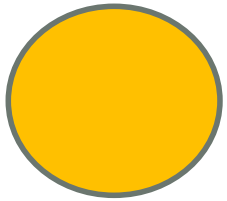


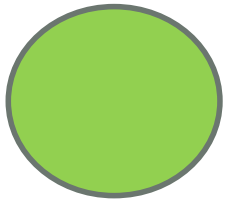
Licht JD N Engl J Med 2009;360:928-930

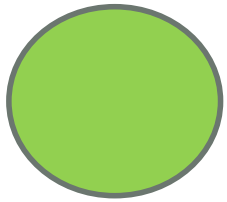
ALL

Monozygotic twins









ALL **3,8 y** **4,1 y** **5 y** **no ALL**

chemotherapy, BMT

therapy, relapse, death

is living

is living **relapse, death**

identical BCR ABL translocation

identical BCR ABL translocation from Guthri cards

hyperdiploidity **IKZF1 deletion**

slightly positive BCR ABL in blood

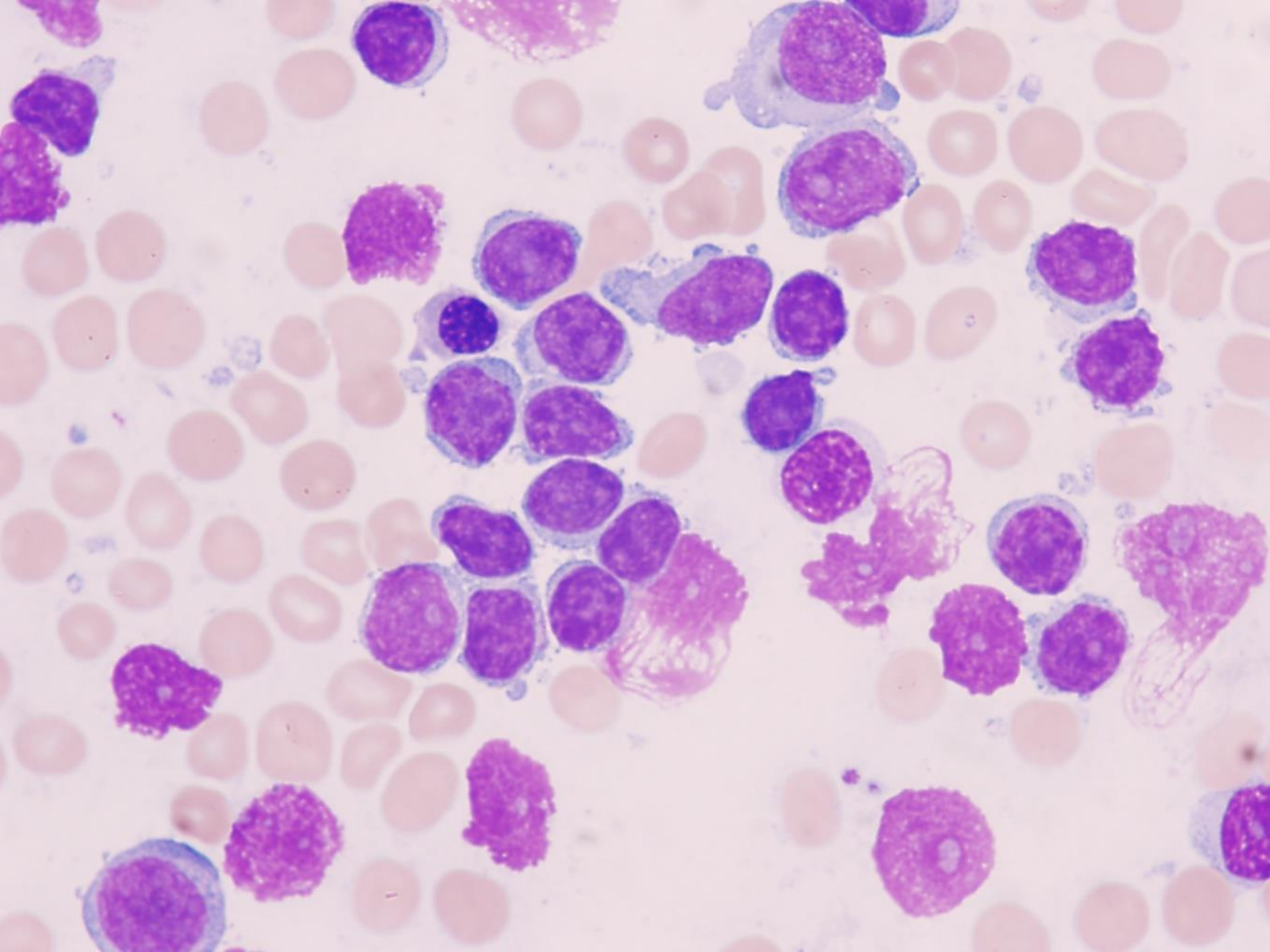
IKZF1 deletion **normal IKZF1**

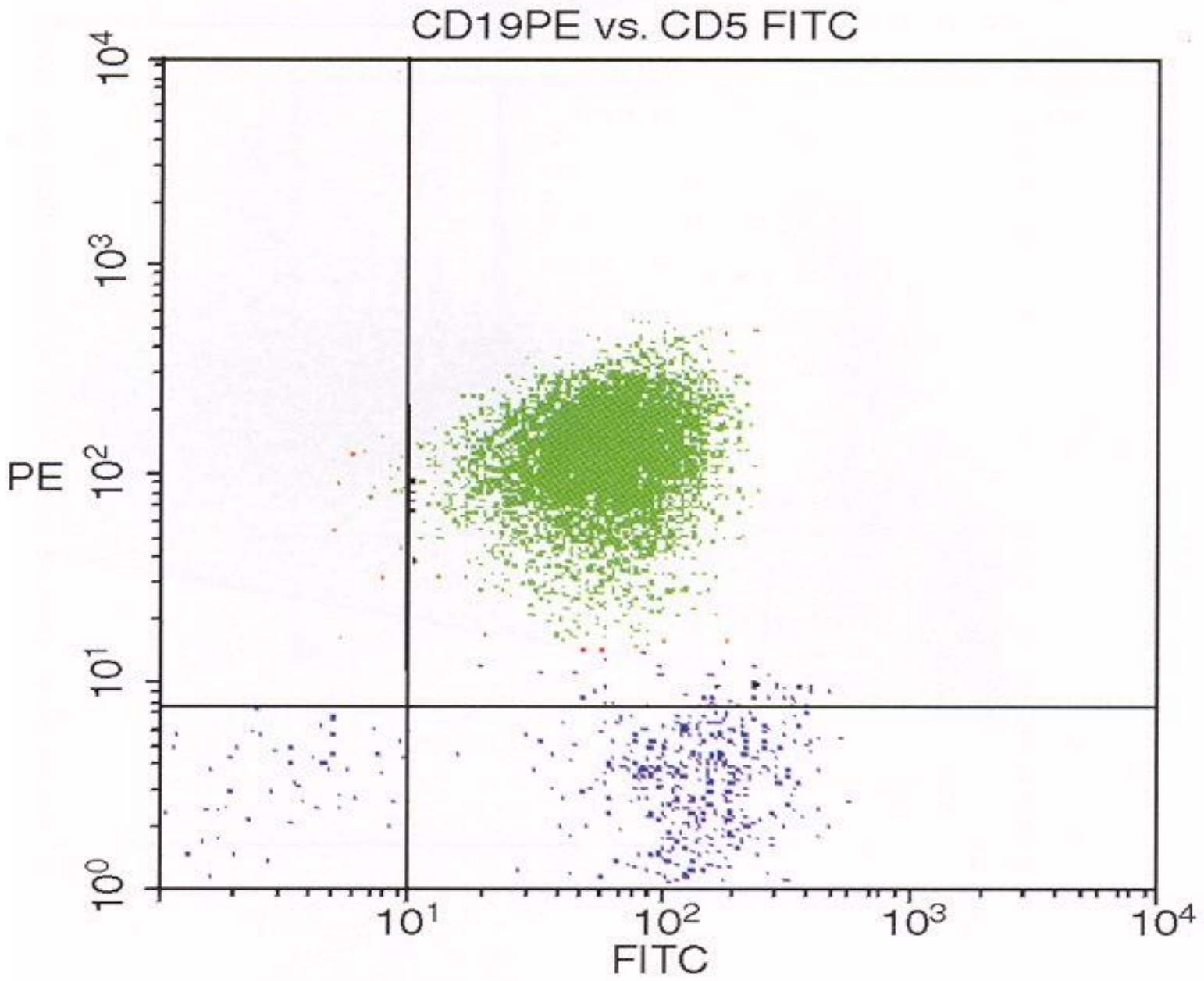
According to: Cazzaniga G et al. Blood 118, 2011, 5559-5564

IKZF1

- DNA-binding protein Ikaros also known as Ikaros family zinc finger protein 1 is a protein that in humans is encoded by the IKZF1 gene.
- This gene encodes a transcription factor that belongs to the family of zinc-finger DNA-binding proteins associated with chromatin remodeling.
- Ikaros displays crucial functions in the hematopoietic system and its loss of function has been linked to the development of lymphoid leukemia.
- Ikaros point mutant mice are embryonic lethal due to anemia; they have severe defects in terminal erythrocyte and granulocyte differentiation, and excessive macrophage formation.
- The expression of this protein is restricted to the fetal and adult hemo-lymphopoietic system, and it functions as a regulator of lymphocyte differentiation.

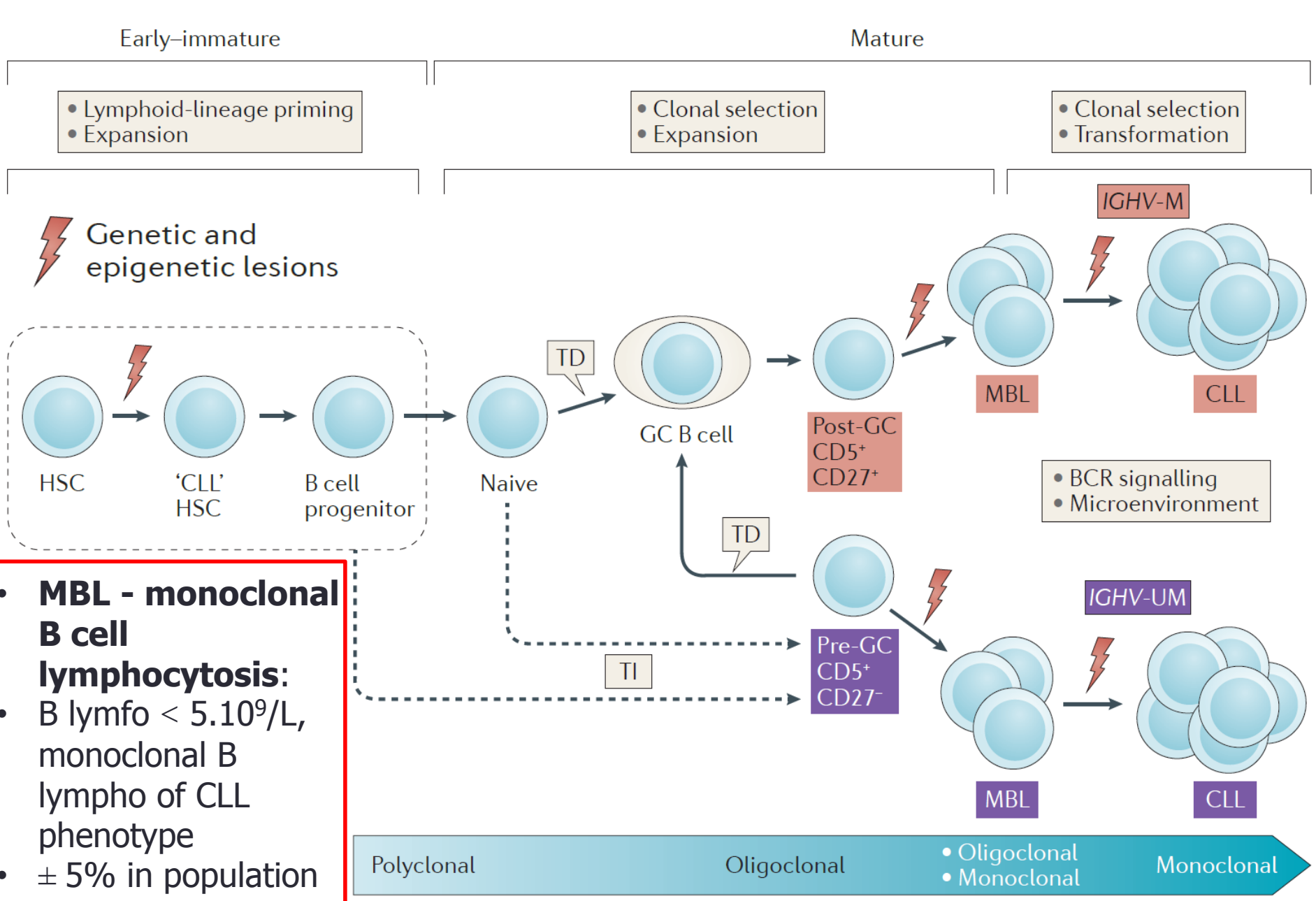
CLL




CD19PE vs. CD5 FITC
CD5 PE
10^0
10^1
10^2
10^3
10^4
10^1
10^2
10^3
10^4
FITC
CD19

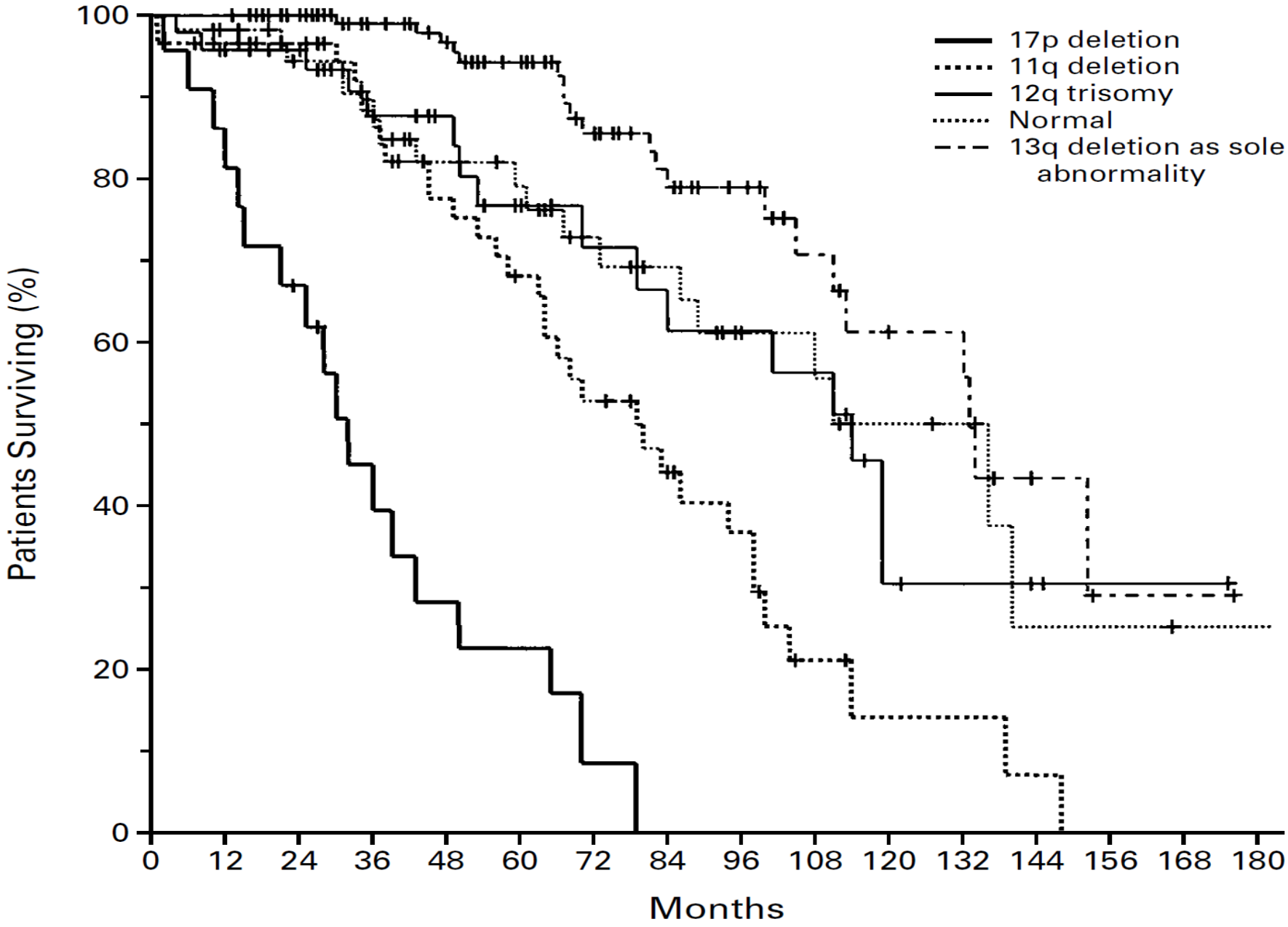
CLL - hallmarks

- Different behavior than AML, ALL, or CML
- Significant proportion of patients never require therapy (smoldering disease)
- According to our data, in 60% of patients just observation, watchful waiting
- The pathogenesis is extraordinarily complex, not yet fully understood, but big progress in recent years
- Not just the malignant cells are involved, also the interactions with microenvironment are crucial (vs AML)
- Due to recent progress, new therapies are emerging
- However, still incurable (but treatable) disease

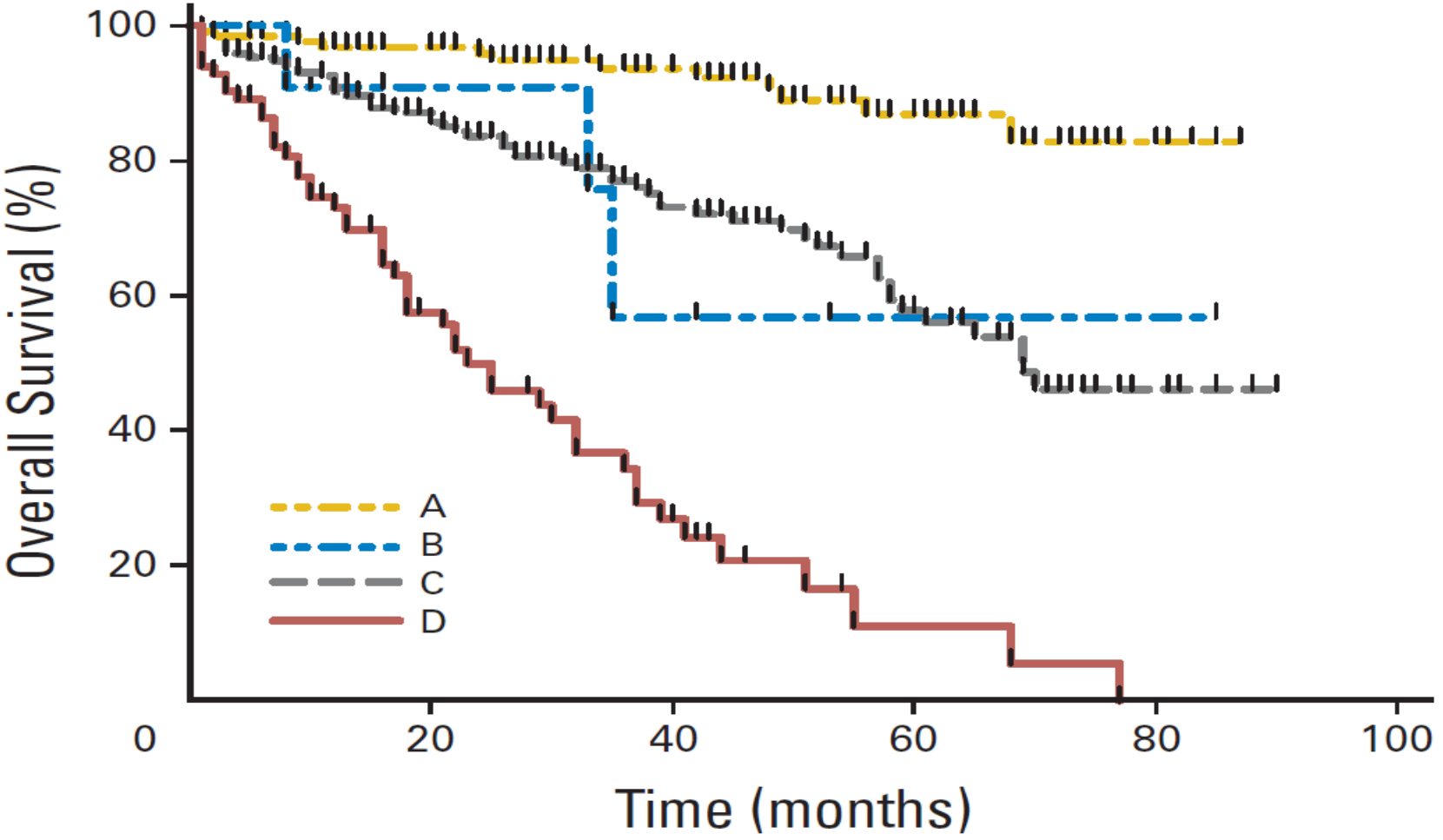


- **MBL - monoclonal B cell lymphocytosis**:
- B lymfo < 5.10^9/L, monoclonal B lympho of CLL phenotype
- ± 5% in population over 40 y, risk of progression into CLL

Fabbri G, Dalla-Favera R, Nat Rev Cancer 16, 2016, 145-162

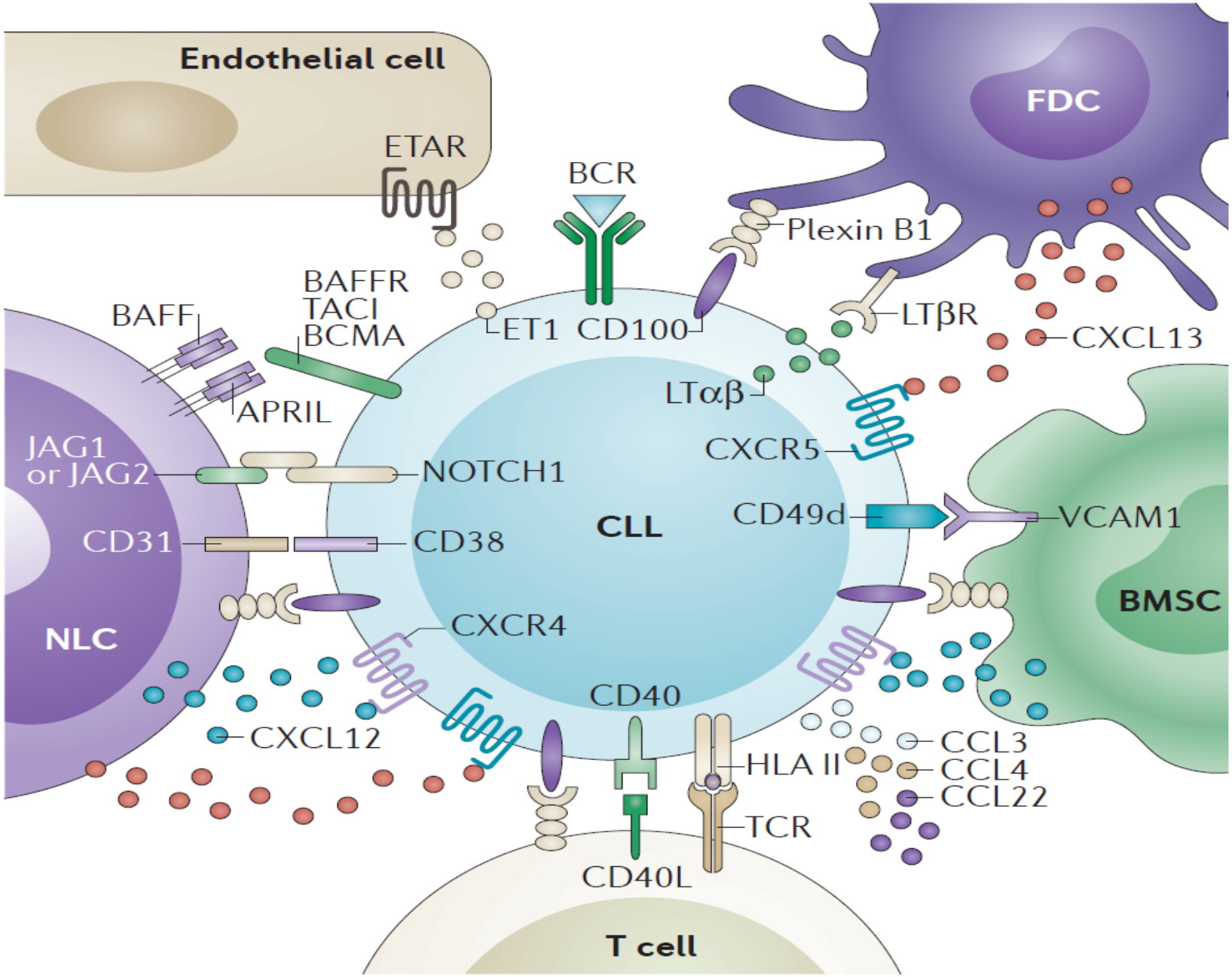


Döhner H et al.: N Engl J Med 2000; 343:1910-1916



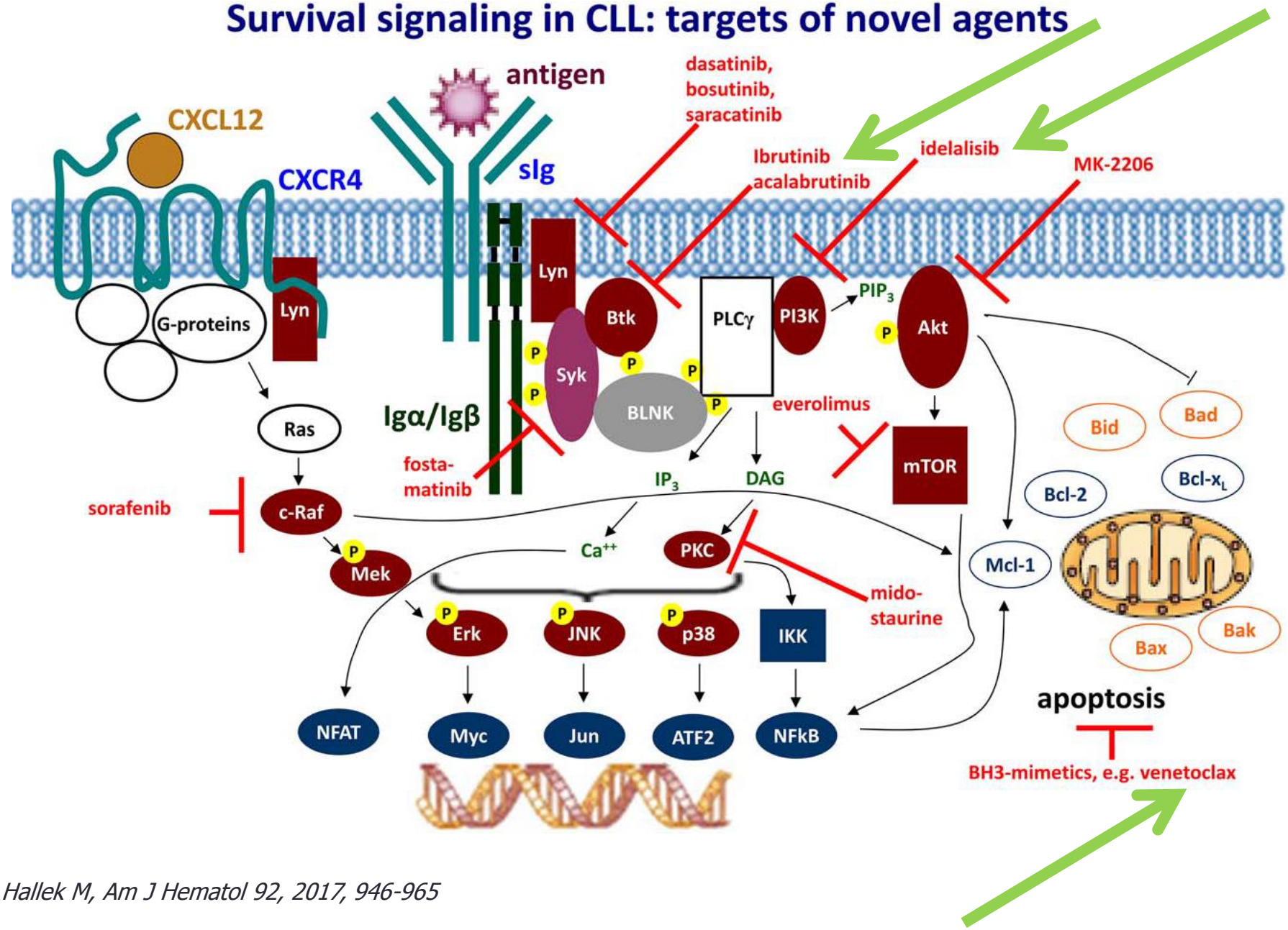
A, wild-type p53 and mutated IgVH
B, p53 defect and mutated IgVH
C, wild-type p53 and unmutated IgVH
D, p53 defect and unmutated IgVH

Trbusek M et al., J Clin Oncol 29, 2011, 2703-2708



Fabbri G, Dalla-Favera R, Nat Rev Cancer 16, 2016, 145-162

Survival signaling in CLL: targets of novel agents



Hallek M, Am J Hematol 92, 2017, 946-965

Summary

- After more than 150 years, the term leukemia still survive
- Leukemias have different clinical behavior, yet with some similar patterns
- Where the pathogenesis is relatively simple, just one targeted therapy may show miraculous effect (CML)
- Complex genetic changes in other types of leukemia, especially in advanced stages, preclude simple therapeutic strategy
- In CLL, disrupting the interactions with tumor microenvironment seems to be very important
- Classical non specific chemotherapy, in combination with monoclonal antibodies, or BMT, still serve as therapeutic option in many cases