Syndromes myéloprolifératifs : dépister et suivre l'évolution et la transformation.

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CNBH - Toulouse
27 septembre 2012

ACNBH

Agrément FMC
N° 100 168

41ème Colloque National
des Biologistes des Hôpitaux
Toulouse, 24-28 septembre 2012

DECLARATION D'INTERET DANS LE CADRE DE MISSIONS DE FORMATION REALISEES POUR L'ACNBH

Dr Eric Lippert
Exerçant au CHU de Bordeaux
déclare sur l'honneur

ne pas avoir d'intérêt, direct ou indirect (financier) avec les entreprises pharmaceutiques, du diagnostic ou d'éditions
en relation avec le DMDIV et/ou le sujet présenté.

Qu’est ce qu’un SMP ?

Quels sont les SMP ?

La LMC, comment ça marche ?

Quel bilan diagnostique ? quel suivi ?

Des nouveautés dans les SMP à éosinophiles ?

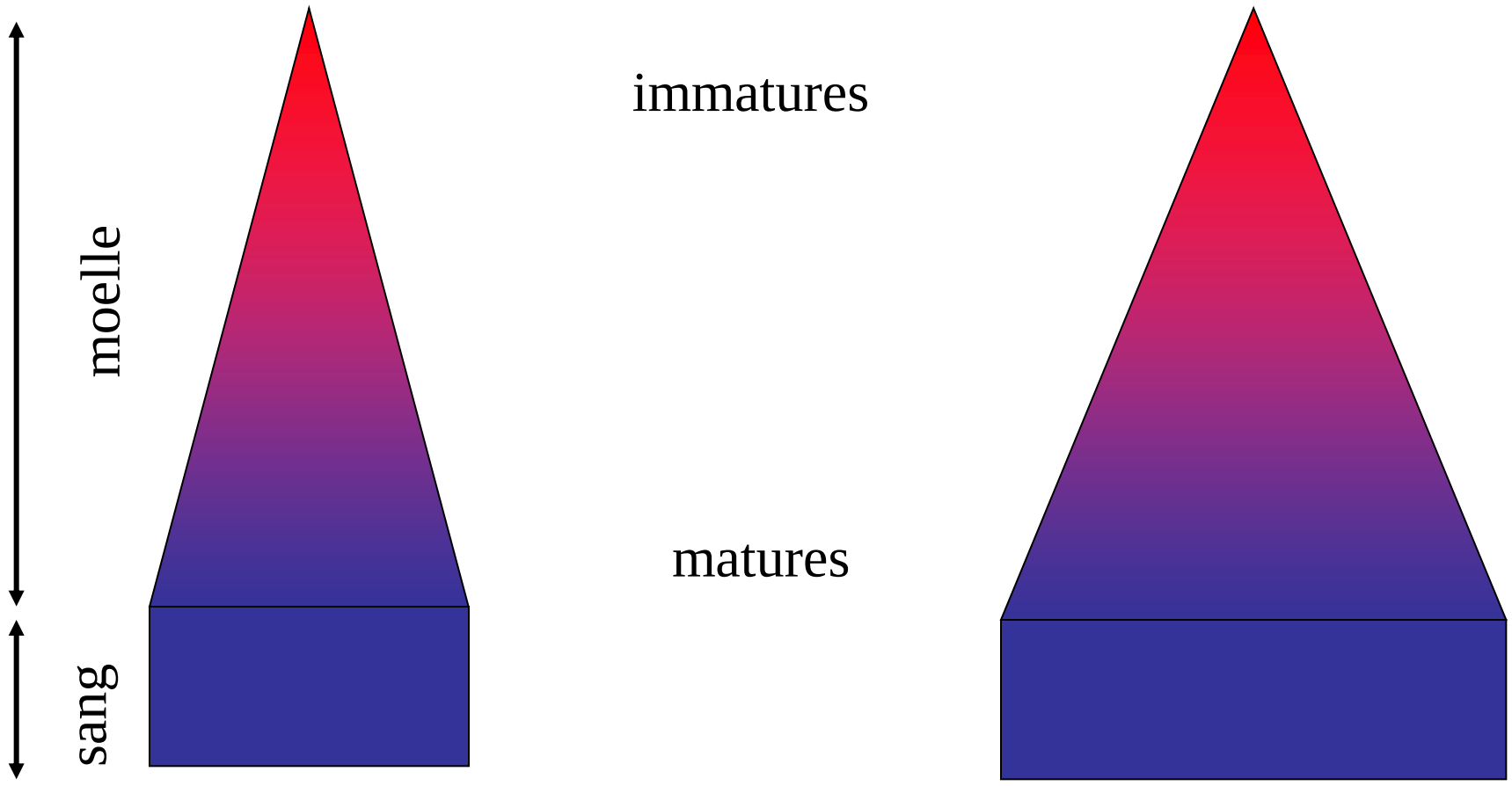
Et les autres alors ?

EDITORIAL

Some Speculations on the Myeloproliferative Syndromes

WITH ACCUMULATING EXPERIENCE, it becomes more and more evident that the bone marrow cells—erythroblasts, granulocytes, megakaryocytes—often proliferate *en masse* or as a unit rather than as single elements.

Thus, if we examine these various syndromes, all of them originating from bone marrow cells, as a group, we find it difficult to draw any clear-cut dividing lines; in fact, so many "transition forms" exist that one may with equal reasonableness call a single condition by at least two different terms. Polycythemia vera,



hématopoïèse normale

syndrome myéloprolifératif

"Classiques"

Leucémie Myéloïde Chronique

Polyglobulie de Vaquez

Thrombocytémie Essentielle

Splénomégalie Myéloïde
Myélofibrose Primitive

"Plus rares"

Leucémie Neutrophile Chronique

Leucémie Eosinophile Chronique
Syndrome HyperEosinophile

SMP chronique, inclassable

"Formes de chevauchement"

Leucémie MyéloMonocytaire Chronique

Leucémie Myéloïde Chronique Atypique

Leucémie MyéloMonocytaire Chronique Juvénile

SMP/SMD, inclassable

LEUCOCYTHEMIA,

OR

WHITE CELL BLOOD,

IN RELATION TO THE

PHYSIOLOGY AND PATHOLOGY OF THE LYMPHATIC GLANDULAR SYSTEM.

BY JOHN HUGHES BENNETT, M.D., F.R.S.E.,

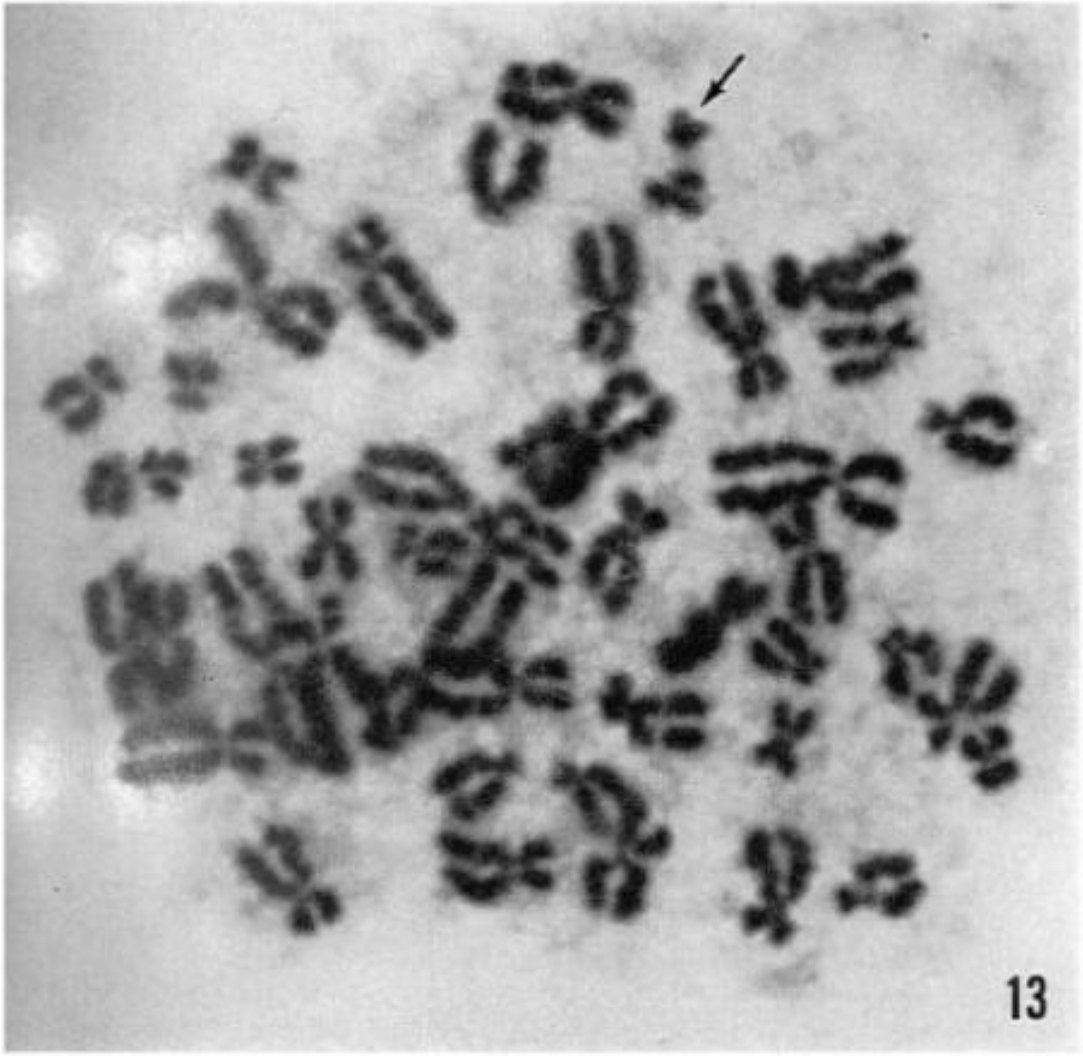
PROFESSOR OF INSTITUTES OF MEDICINE AND OF CLINICAL MEDICINE IN THE UNIVERSITY, AND PRESIDENT OF THE PHYSIOLOGICAL SOCIETY, EDINBURGH.
MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY; OF THE IMPERIAL SOCIETY OF PHYSICIANS OF VIENNA; OF THE MEDICAL ASSOCIATION OF PRUSSIA; OF THE ANATOMICAL AND BIOLOGICAL SOCIETIES OF PARIS; OF THE MEDICAL SOCIETIES OF SWEDEN, COPENHAGEN, ETC. ETC.

WITH TWO COLOURED LITHOGRAPHS, AND NUMEROUS WOODCUTS.

BIBLIOTH. COLL. REG. REG. EDIN.

EDINBURGH: SUTHERLAND AND KNOX.
LONDON: SIMPKIN, MARSHALL, & CO.

MDCCCLII.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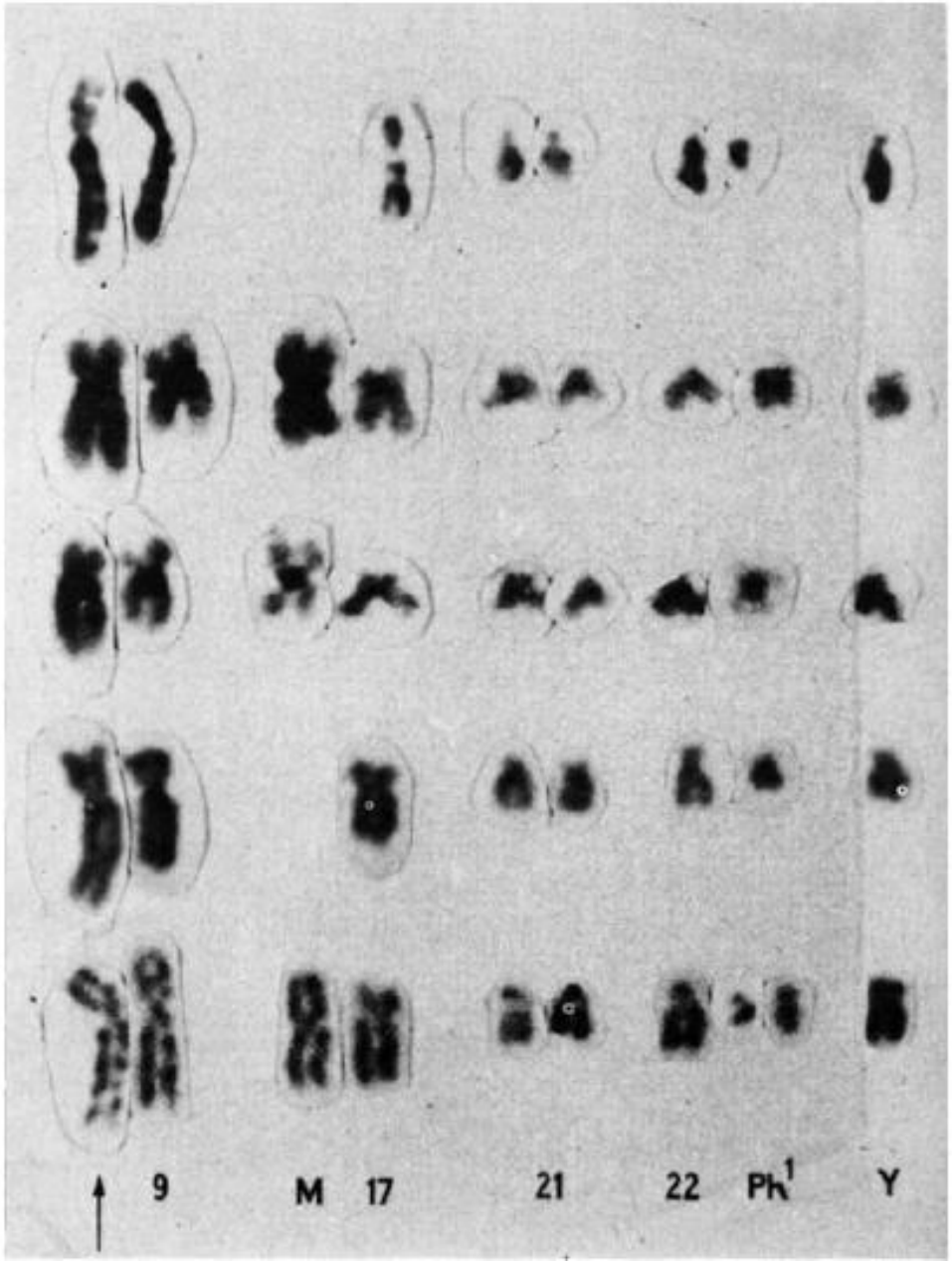
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Chromosomal localization of human cellular homologues of two viral oncogenes

Nora Heisterkamp*, John Groffen*, John R. Stephenson*, N. K. Spurr†, P. N. Goodfellow†, E. Solomon†, B. Carritt‡ & W. F. Bodmer†

* Laboratory of Viral Carcinogenesis, National Cancer Institute—FCRF, Frederick, Maryland 21701, USA
† Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK
‡ MRC Human Biochemical Genetics Unit, Galton Laboratory, University College, London WC2, UK

Nature Vol. 299 21 October 1982

A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia

Annelies de Klein*, Ad Geurts van Kessel*, Gerard Grosveld*, Claus R. Bartram*, Anne Hagemeijer*, Dirk Bootsma*, Nigel K. Spurr†, Nora Heisterkamp‡, John Groffen‡ & John R. Stephenson‡

* Department of Cell Biology and Genetics, Erasmus University, PO Box 1738 – 3000 DR Rotterdam, The Netherlands
† Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK
‡ Laboratory of Viral Carcinogenesis, National Cancer Institute-FCRF, Frederick, Maryland 21701, USA

Nature Vol. 300 23/30 December 1982

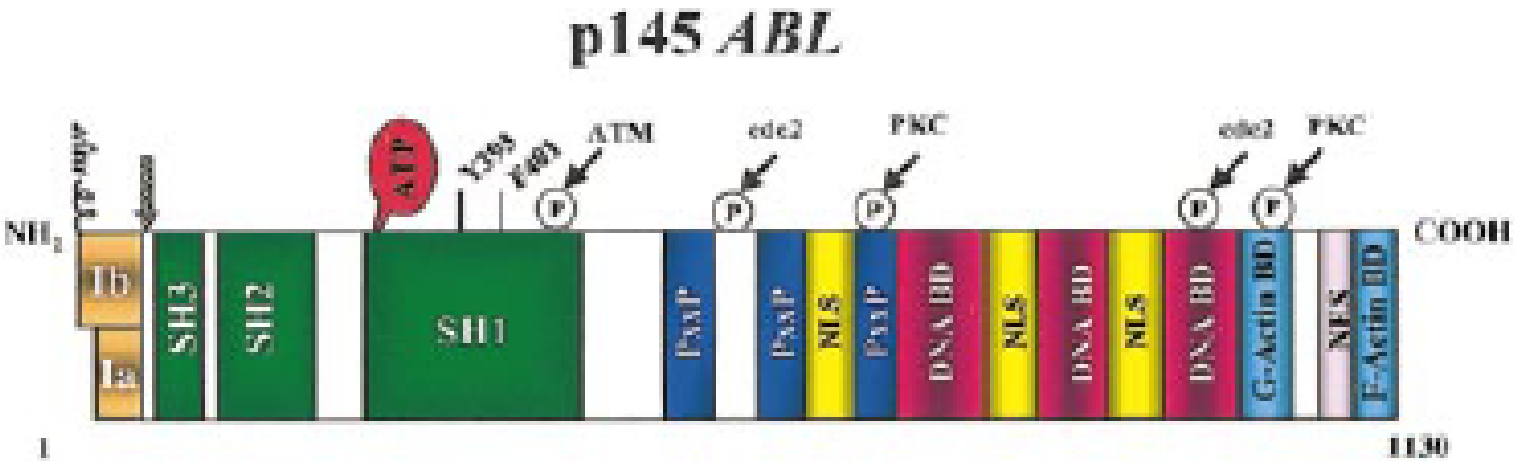
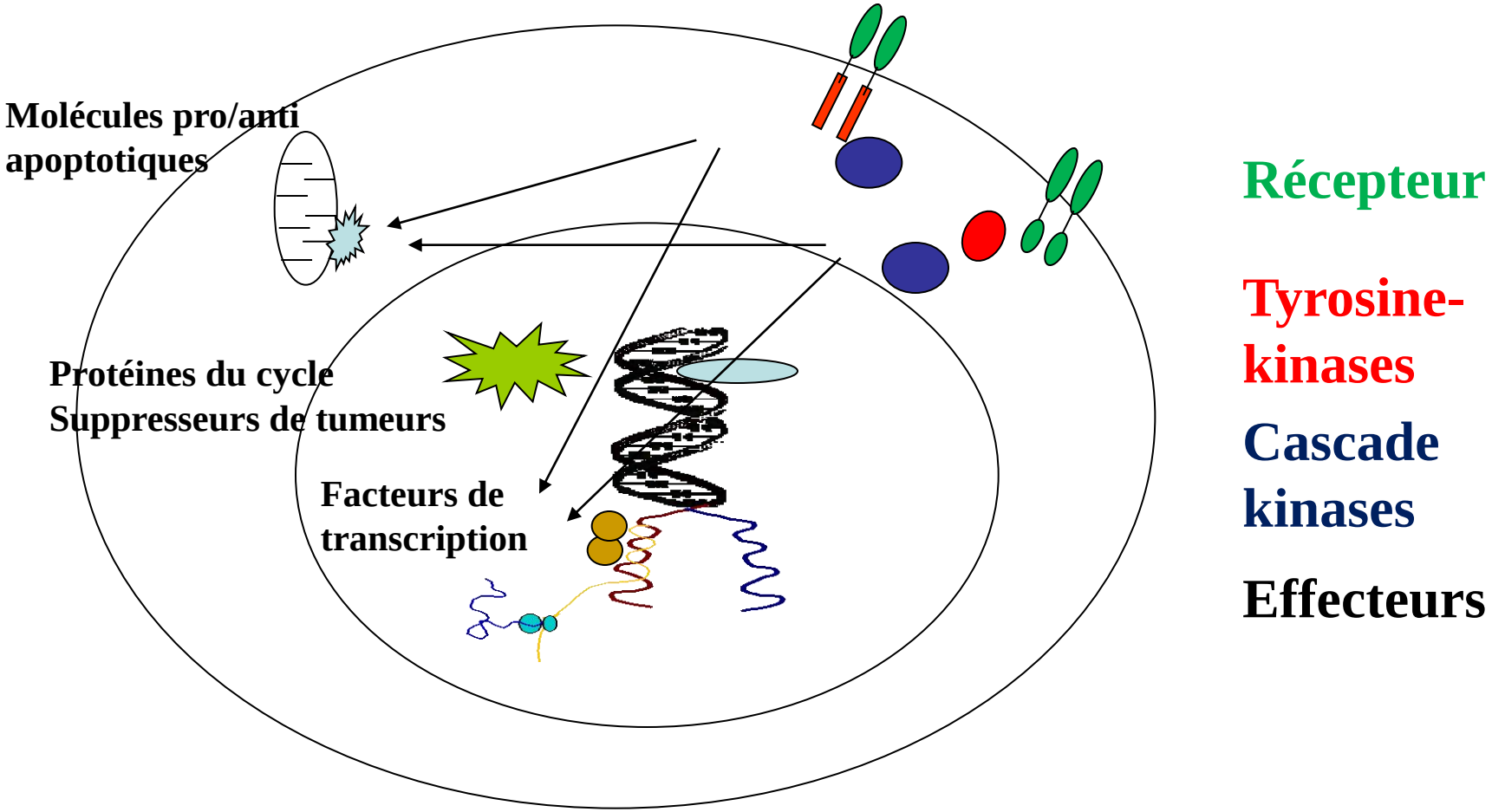


Figure 1. Structure of the Abl protein. Type Ia isoform is slightly shorter than type Ib, which contains a myristoylation (myr) site for attachment to the plasma membrane. Note the 3 SRC-homology (SH) domains situated toward the NH_2 terminus. Y393 is the major site of autophosphorylation within the kinase domain, and phenylalanine 401 (F401) is highly conserved in PTKs containing SH3 domains. The middle of each protein is dominated by proline-rich regions (PxxP) capable of binding to SH3 domains, and it harbors 1 of 3 nuclear localization signals (NLS). The carboxy terminus contains DNA as well as G- and F-actin–binding domains. Phosphorylation sites by Atm, cdc2, and PKC are shown. The arrowhead indicates the position of the breakpoint in the Bcr-Abl fusion protein.

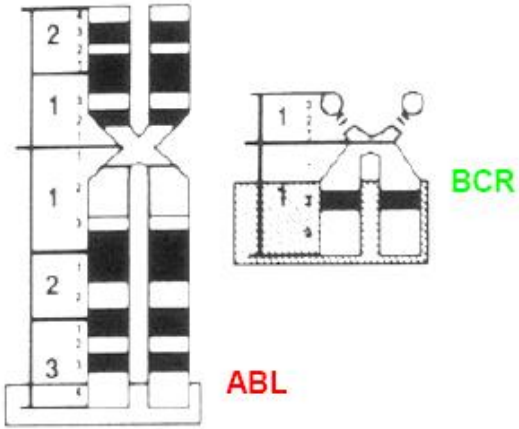
Les Tyrosine Kinases

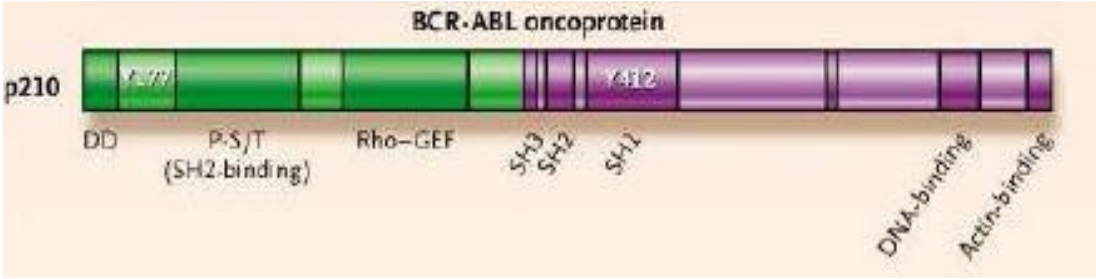
- Relais de signaux de prolifération
- Activées par dimérisation



p160 *BCR*

Figure 2. Structure of the Bcr protein. Note the dimerization domain (DD) and the 2 cyclic adenosine monophosphate kinase homologous domains at the N terminus.





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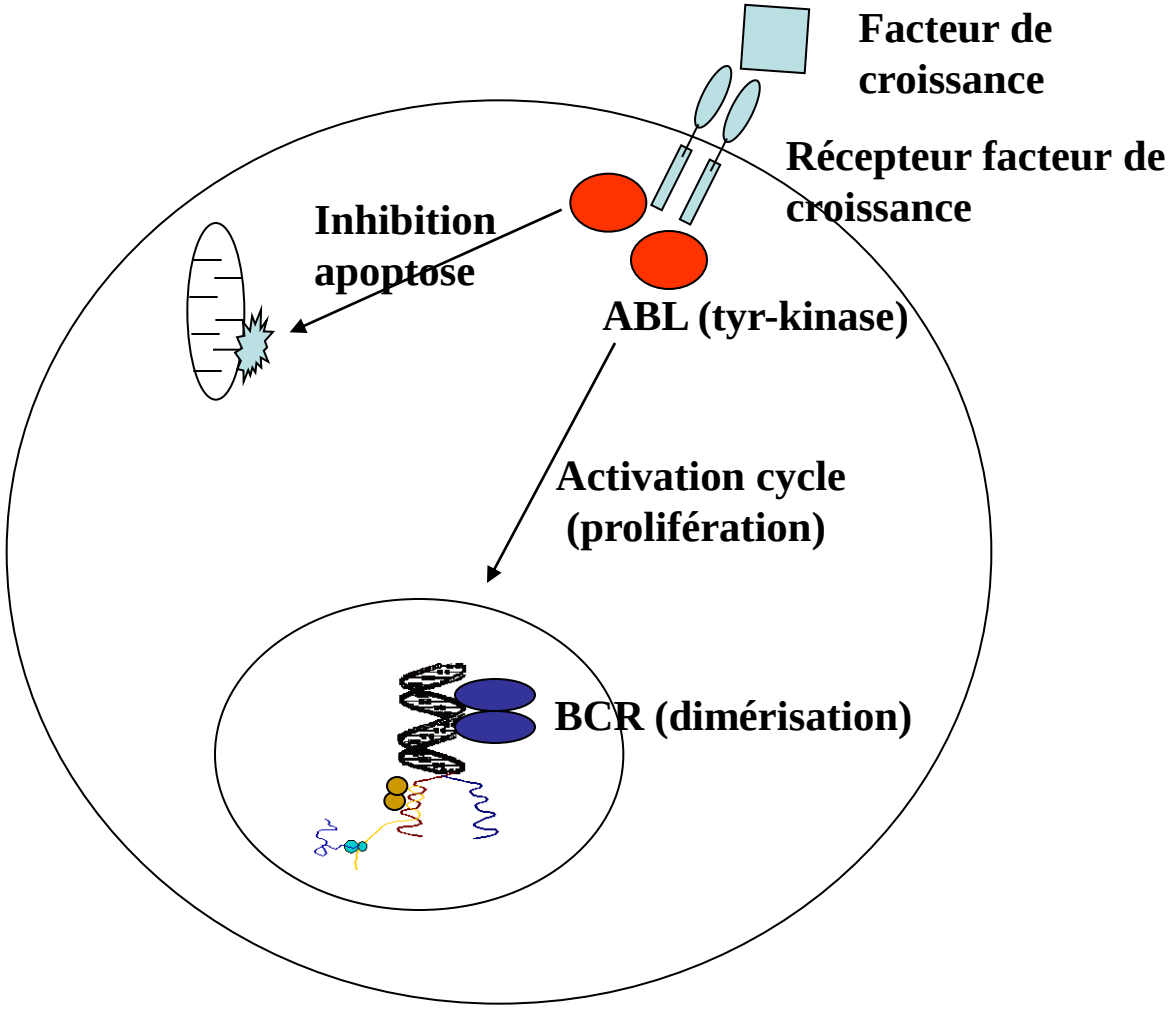
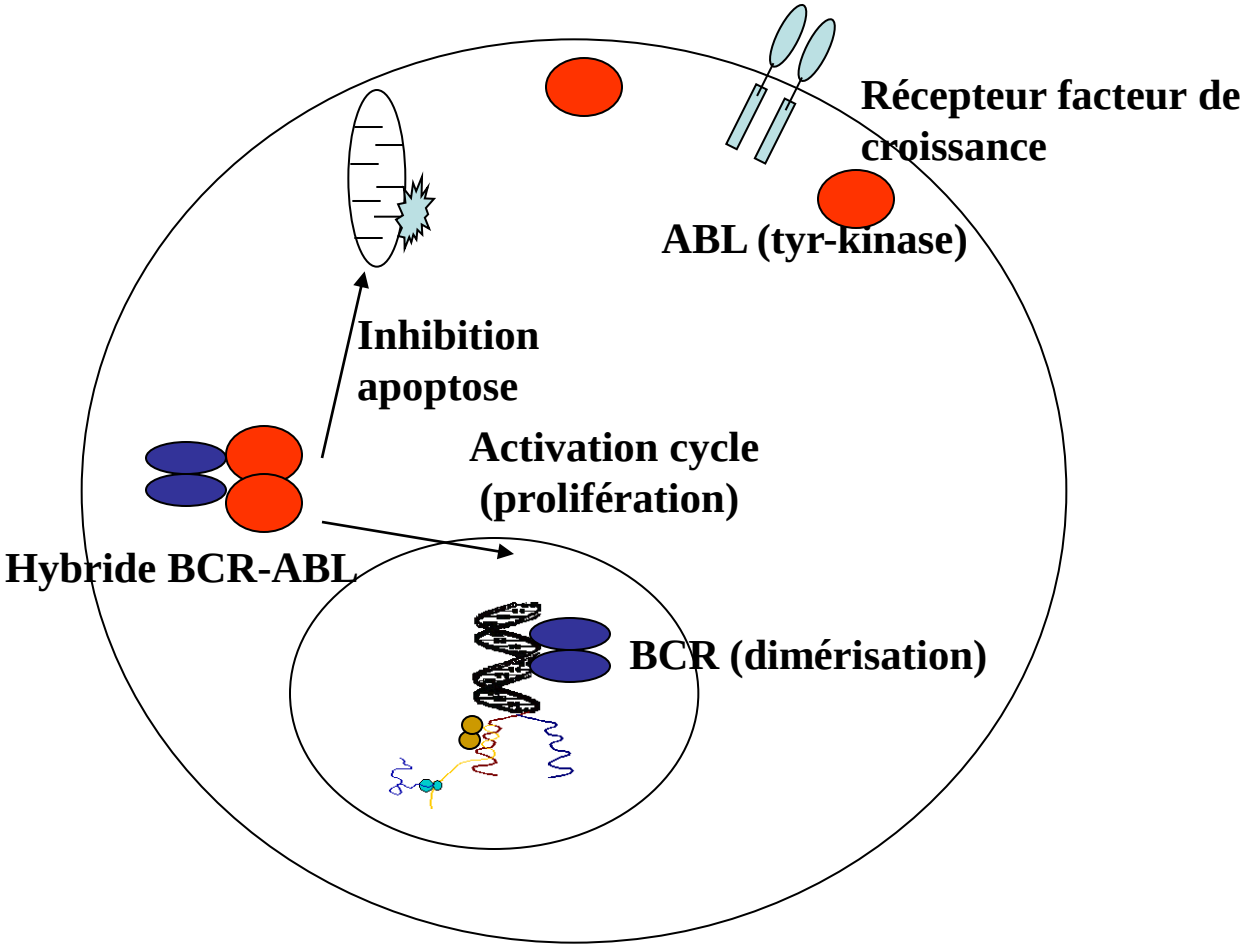
(Goldman and Melo, NEJM, 2003)

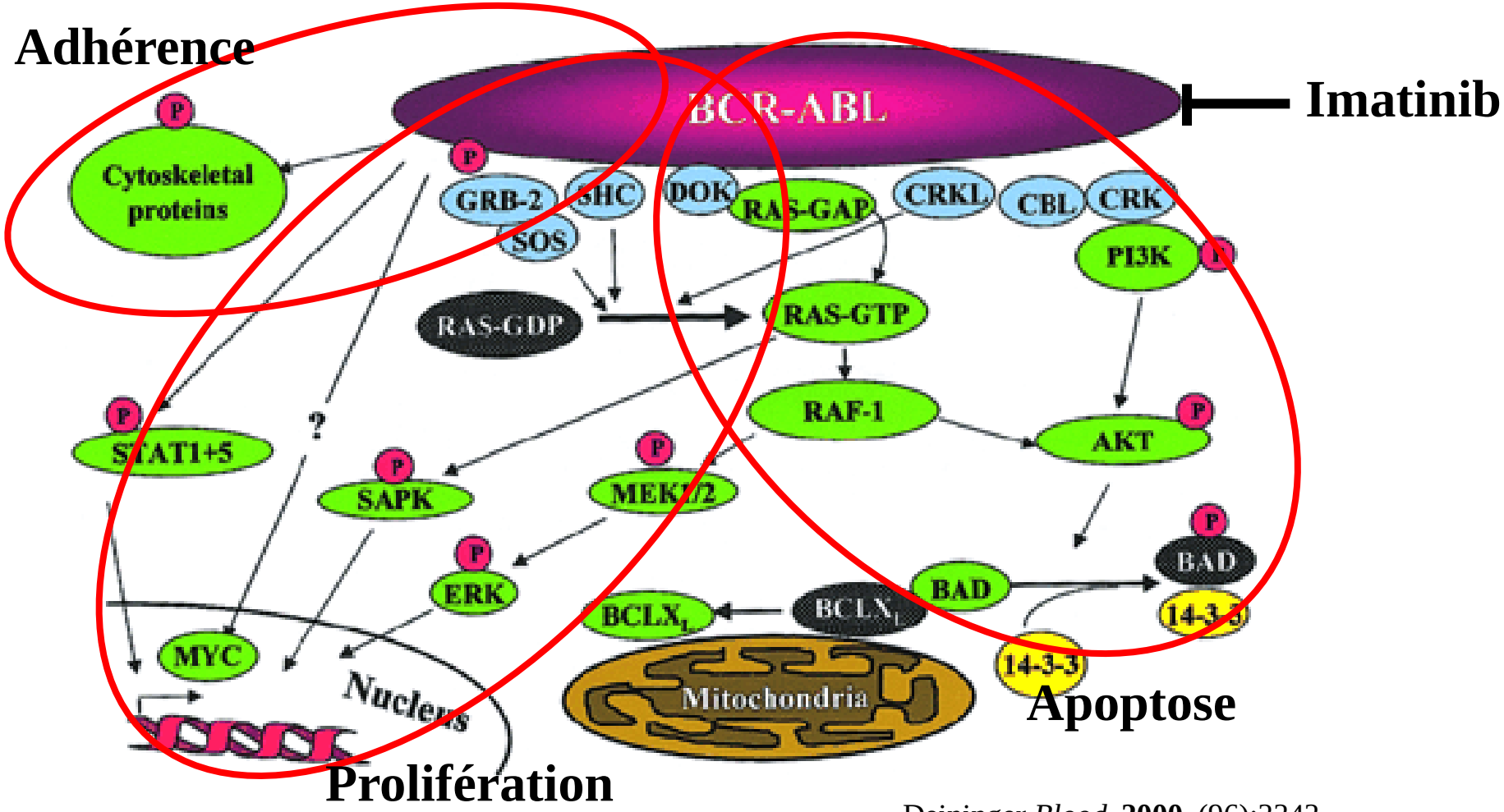
Signalisation ABL



Signalisation BCR-ABL

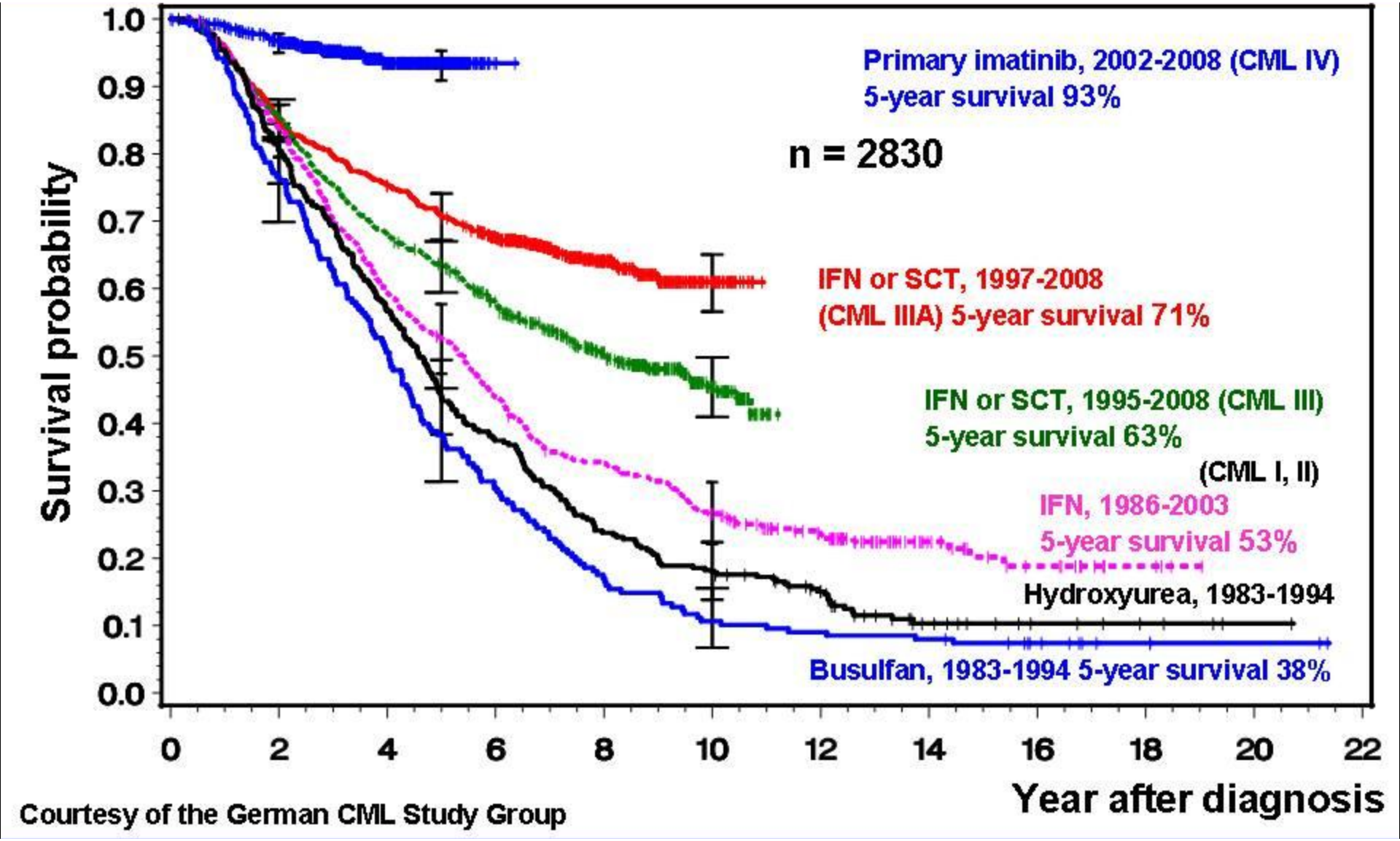


LMC : Activation d'ABL

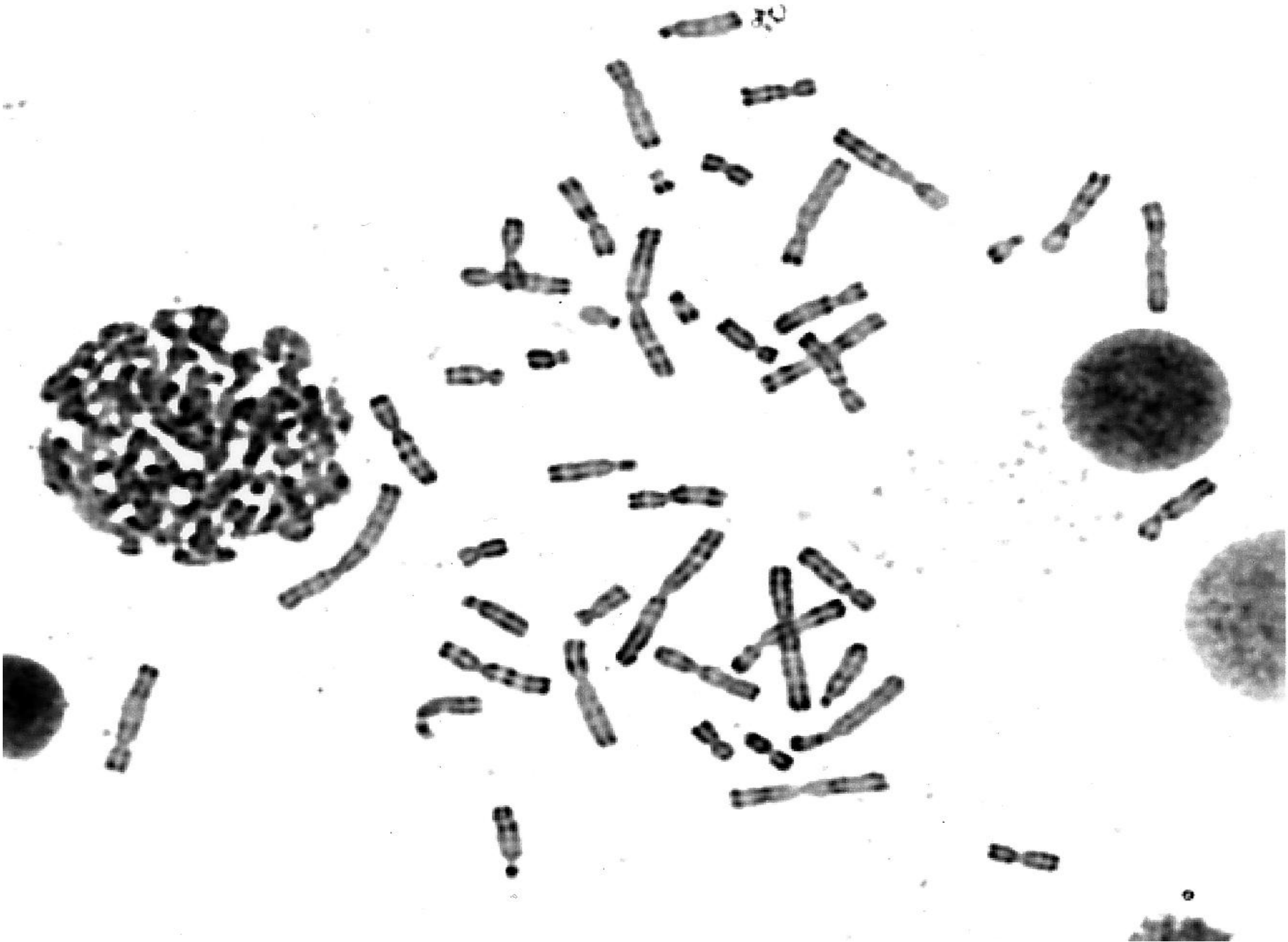


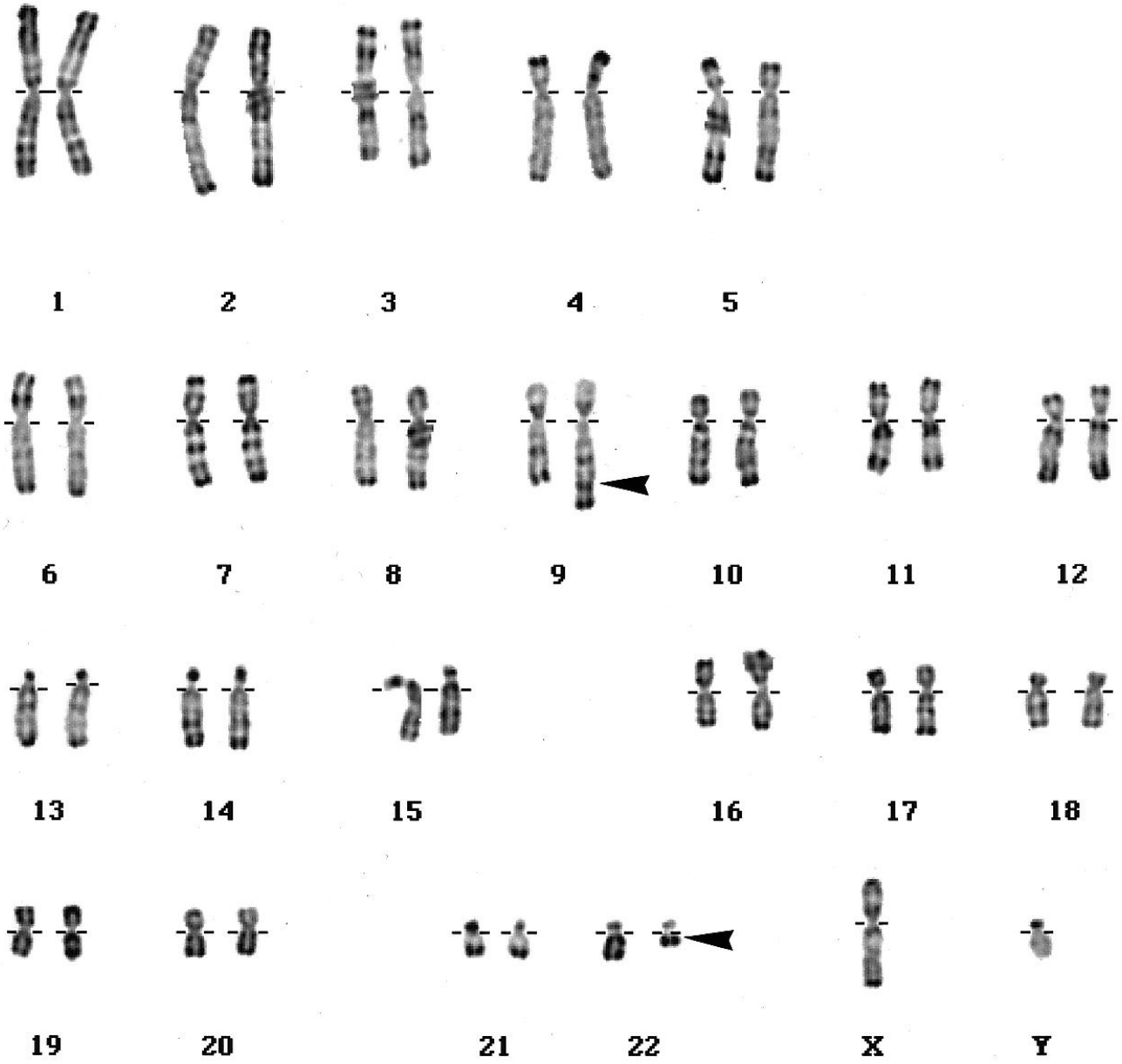
Deininger,*Blood*, **2000**, (96):3343

CML and survival



Courtesy of the German CML Study Group

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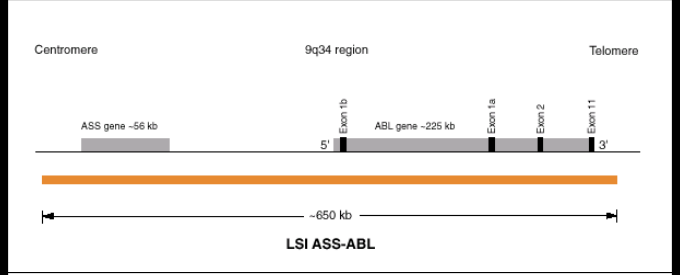
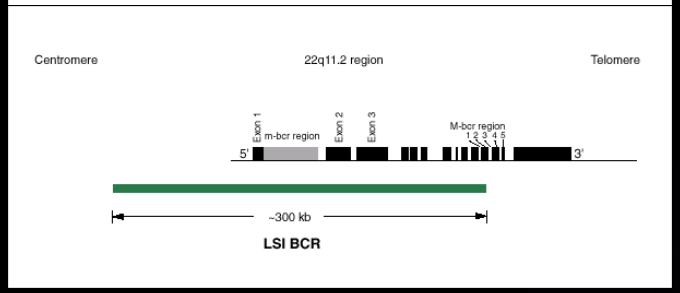

Centromere
9q34 region
Telomere
ASS gene ~56 kb
Exon 1b
ABL gene ~225 kb
Exon 1a
Exon 2
Exon 11
5'
3'
~650 kb
LSI ASS-ABL


Centromere
22q11.2 region
Telomere
Exon 1
m-bcr region
Exon 2
Exon 3
M-bcr region
1 2 3 4 5
5'
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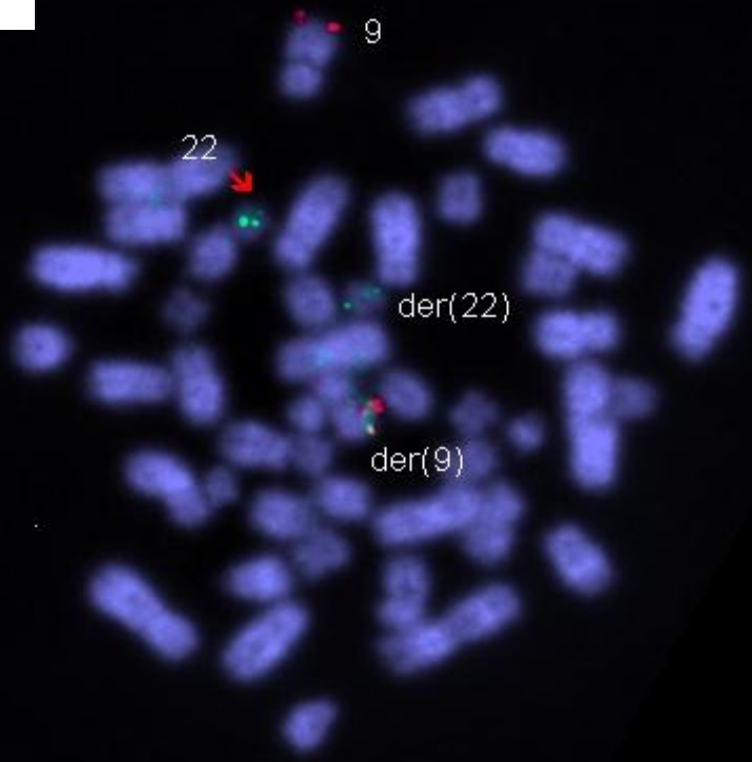
~300 kb
LSI BCR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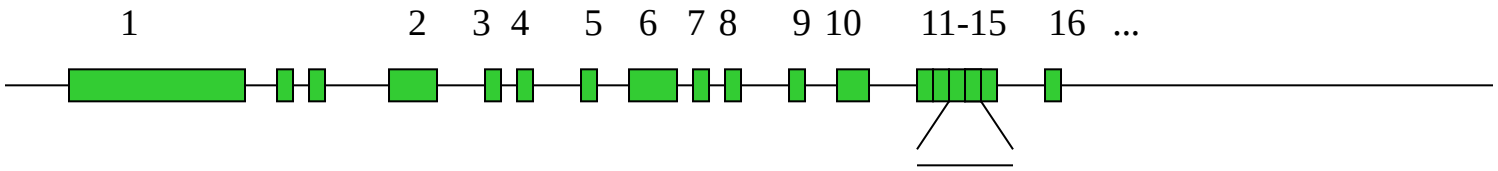
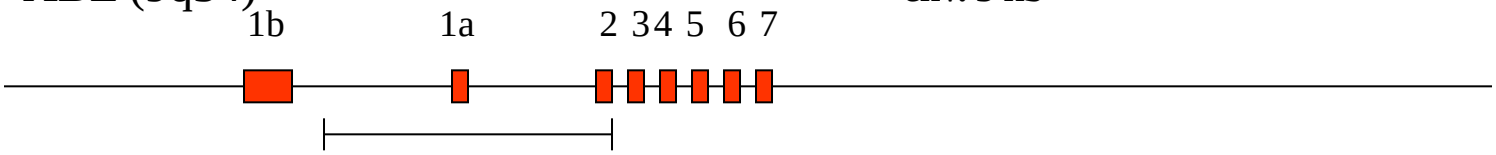
Recherche de transcrits de fusion : RT-PCR

BCR (22q11)



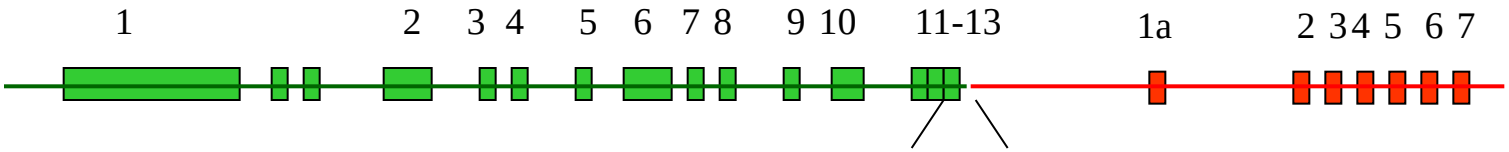
M-BCR
env. 3 kb

ABL (9q34)



Région de cassure
env. 200 kb

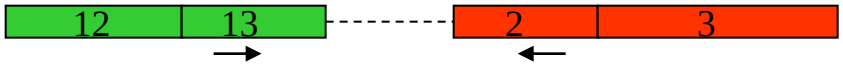
gène BCR-ABL

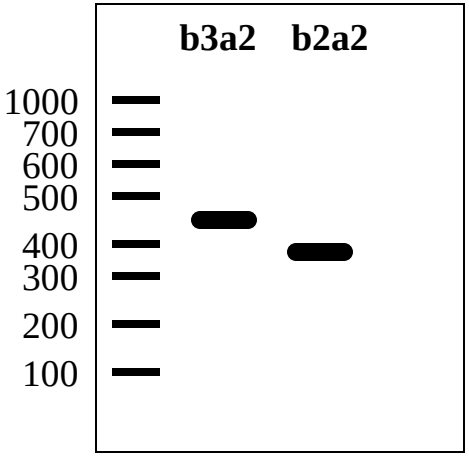


Transcrits de fusion

b3a2

b2a2





Proposition de stratégie

LMC très probable : hyperleucocytose PN+myélémie, baso...

- moelle pour cyto + caryo +/- FISH
- sang pour BM

LMC très peu probable, diagnostic simple (thrombocytose isolée, hyperleuco isolée...)

- BM seule sur sang

LMC peu probable, hémopathie probable, diagnostic difficile

- moelle : cyto, phéno, caryo

EVOLUTION NATURELLE DE LA LMC :

Classiquement : 3 phases
- chronique (SMP typique : prolifération+++/ pas d'anomalie maturation)
- Acutisation : Blastes (>20%), anomalies moléculaires additionnelles +++
- Accélération : entre les deux

Critères d'accélération discutables
OMS 2002 :
Persistance ou augmentation de la leucocytose > 10 G/L ou persistance SMG sous traitement
Thrombocytose persistante > 1000 ou thrombopénie non attribuable au traitement
Evolution clonale après le diagnostic
≥ 20% baso en périphérie
10-19% blastes dans la MO

OMS 2008 : ne reconnaît plus clairement ces critères car prise en charge non différente et suivi évolutif à prendre en compte

Table 2. Recommendations for monitoring individual patients.

At diagnosis
- Full blood count and 100 cell differential
- Calculation of Sokal score*
- Bone marrow aspirate with cytogenetic analysis of 30 metaphases
- [FISH for BCR-ABL1 in the absence of a Philadelphia chromosome]
- RQ-PCR for BCR-ABL1 transcripts to characterize the BCR-ABL1 junction [Optional]

Thereafter:
- Blood counts at intervals of two or more weeks
- Liver chemistry

At 3 months
- Blood count
- Bone marrow cytogenetics
- RQ-PCR for BCR-ABL1 transcripts

At 6 months
- Blood count
- Bone marrow cytogenetics
- RQ-PCR for BCR-ABL1 transcripts
- [Thereafter bone marrow aspirates are only required if CCyR has not been achieved]

At 3 month intervals thereafter:
- RQ-PCR for BCR-ABL1 transcripts indefinitely

Goldman *Hematology 2009*

Table 6. Evaluation of Overall Response to Imatinib First-Line in Early Chronic Phase

Evaluation Time, Months	Response			Warnings
	Optimal	Suboptimal	Failure	
Baseline	NA	NA	NA	High risk; CCA/Ph+*
3	CHR and at least minor CgR (Ph+ ≤ 65%)	No CgR (Ph+ > 95%)	Less than CHR	NA
6	At least PCgR (Ph+ ≤ 35%)	Less than PCgR (Ph+ > 35%)	No CgR (Ph+ > 95%)	NA
12	CCgR	PCgR (Ph+ 1% to 35%)	Less than PCgR (Ph+ > 35%)	Less than MMolR†
18	MMolR†	Less than MMolR†	Less than CCgR	NA
Any time during treatment	Stable or improving MMolR†	Loss of MMolR†; mutations‡	Loss of CHR; loss of CCgR; mutations§; CCA/Ph+	Increase in transcript levels‖; CCA/Ph−

Table 9. Provisional Definition of the Response to Second-Generation TKIs, Dasatinib and Nilotinib, As Second-Line Therapy of Patients With Imatinib-Resistant Disease in Chronic Phase

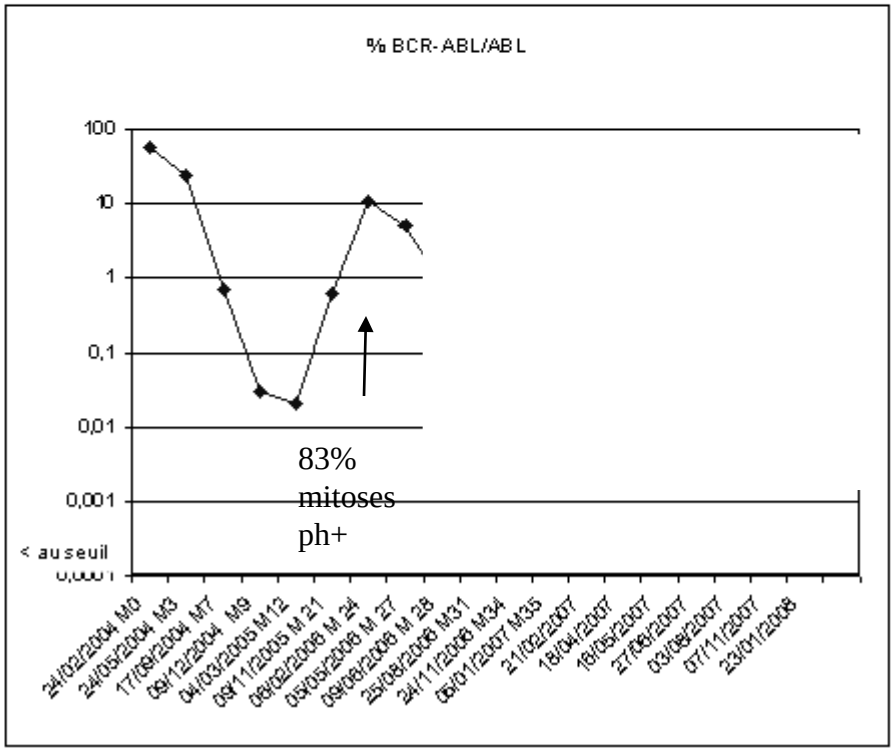
Evaluation Time, Months	Response		Warnings
	Suboptimal	Failure	
Baseline	NA	NA	Hematologic resistance to imatinib; CCA/Ph+ (ie, clonal progression); mutations*
3	Minor CgR (Ph+ 36% to 65%)	No CgR (Ph+ > 95%); new mutations*	Minimal CgR (Ph+ 66% to 95%)
6	PCgR (Ph+ 1% to 35%)	Minimal CgR (Ph+ 66% to 95%); new mutations*	Minor CgR (Ph+ 36% to 65%)
12	Less than MMolR†	Less than PCgR (Ph+ > 35%); new mutations*	

Baccarani, JCO, 2009

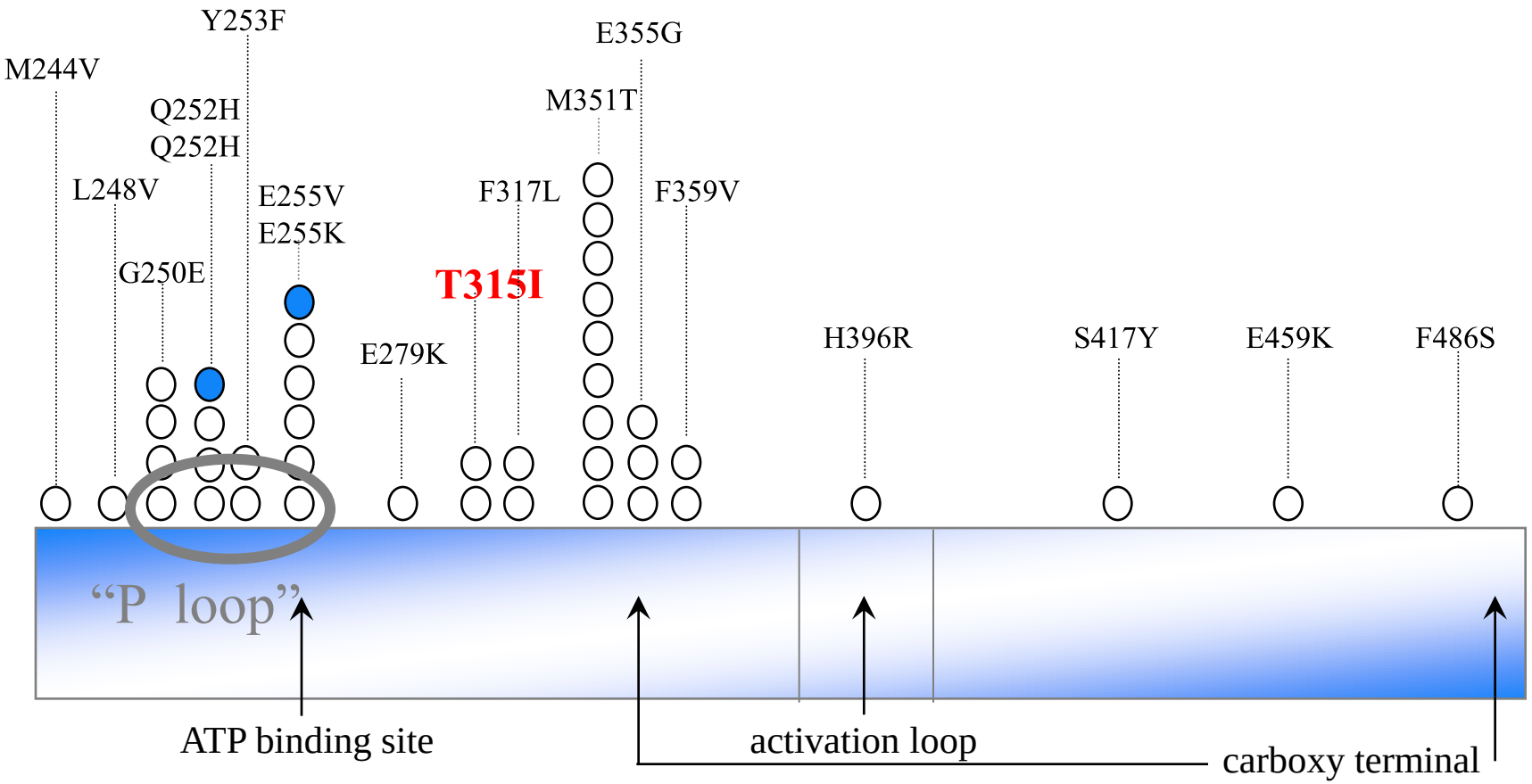
2/ RT-PCR :		
	BCR-ABL	**ABL**
Sensibilité RT-PCR sur Gamme d'ARN	10-5	
Sensibilité RT-PCR sur Gamme plasmidique	10 copies	10 copies
Efficacité PCR	**86,96**	**96,30**
Taux d'erreur PCR	**0,076**	**0,078**

3/ VALIDATION DU TEST : CC	
	Pour l'échantillon testé
Echec Technique (ET)	
PCR BCR-ABL négative et PCR-ABL positive faible	
PCR BCR-ABL négative et PCR-ABL positive	
PCR BCR-ABL et PCR-ABL positives	X

4/ QUANTIFICATION	
DATE	**% BCR-ABL/ABL**
24/02/2004 M0	56,20
24/05/2004 M3	24,41
17/09/2004 M7	0,72
09/12/2004 M9	0,03
04/03/2005 M12	0,02
09/11/2005 M 21	0,64
06/02/2006 M 24	10,83



Location of mutations within the kinase domain

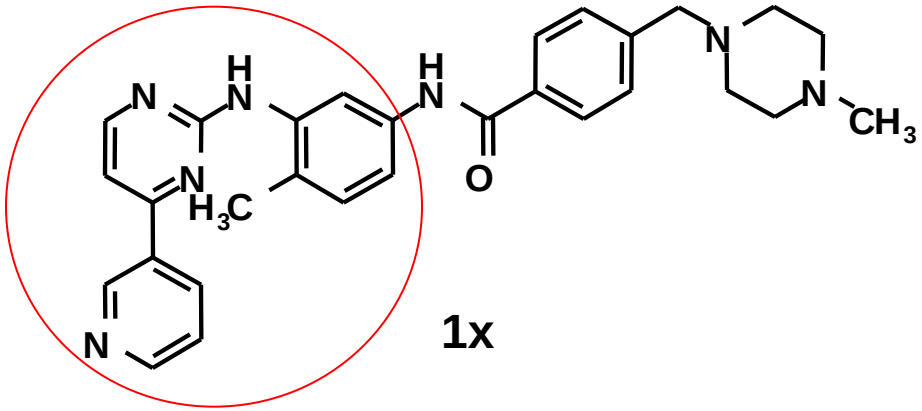


○ Represents the number of patient with each mutation

● Indicates a different mutation at the same amino acid

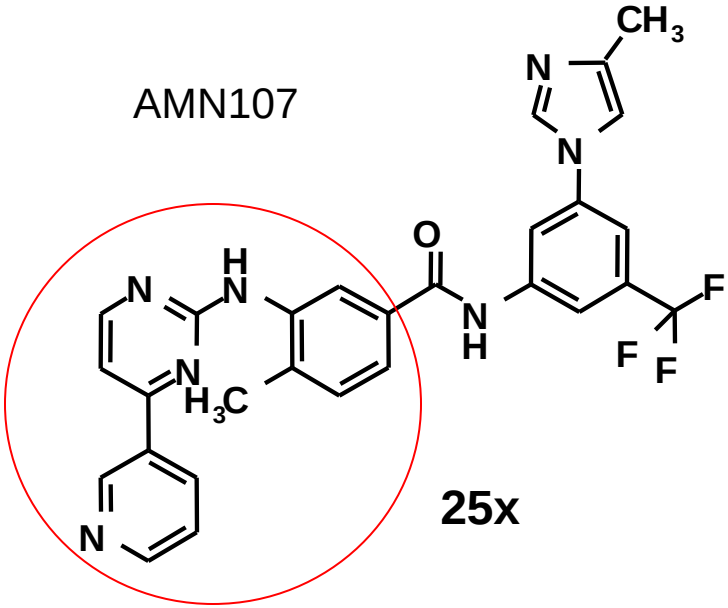
Novel ABL inhibitors in clinical trials

Imatinib (STI571)

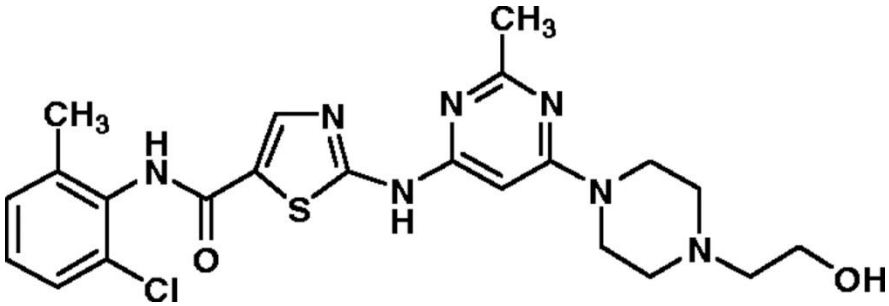


Binds inactive conformation

AMN107



Binds inactive conformation



Dasatinib

Binds inactive <u>and</u> active conformations, multitargeted (SRC)

2/ RT-PCR :		
	BCR-ABL	**ABL**
Sensibilité RT-PCR sur Gamme d'ARN	10-5	
Sensibilité RT-PCR sur Gamme plasmidique	10 copies	10 copies
Efficacité PCR	**86,96**	**96,30**
Taux d'erreur PCR	**0,076**	**0,078**

3/ VALIDATION DU TEST : CC	
	Pour l'échantillon testé
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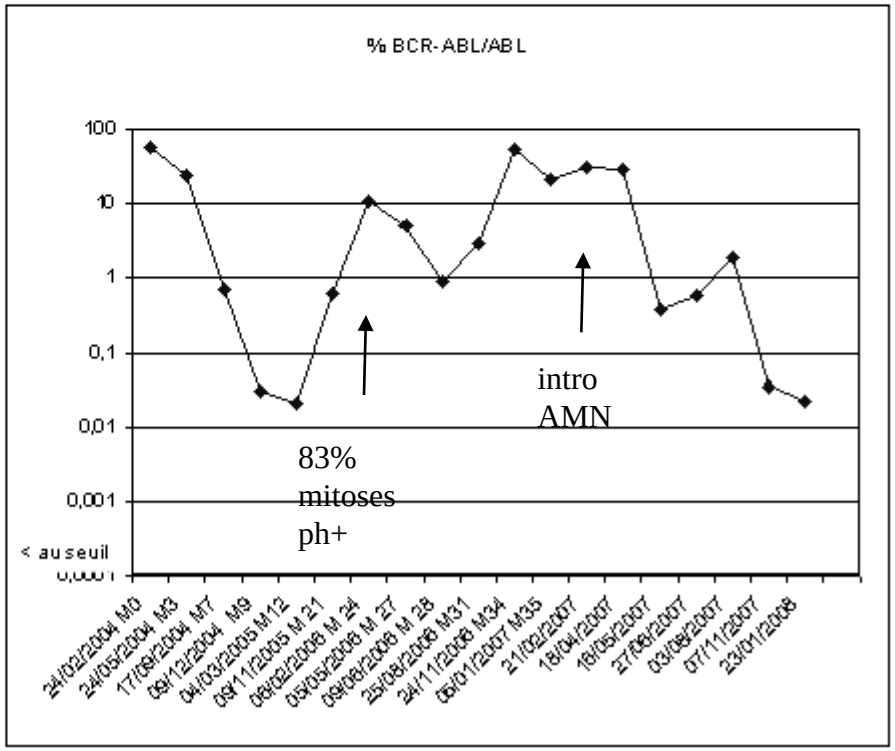
04/03/2005 M12	0,02
09/11/2005 M 21	0,64
06/02/2006 M 24	10,83



"Classiques"

Leucémie Myéloïde Chronique

Polyglobulie de Vaquez

Thrombocytémie Essentielle

Splénomégalie Myéloïde
Myélofibrose Primitive

"Plus rares"

Leucémie Neutrophile Chronique

Leucémie Eosinophile Chronique
Syndrome HyperEosinophile

SMP chronique, inclassable

"Formes de chevauchement"

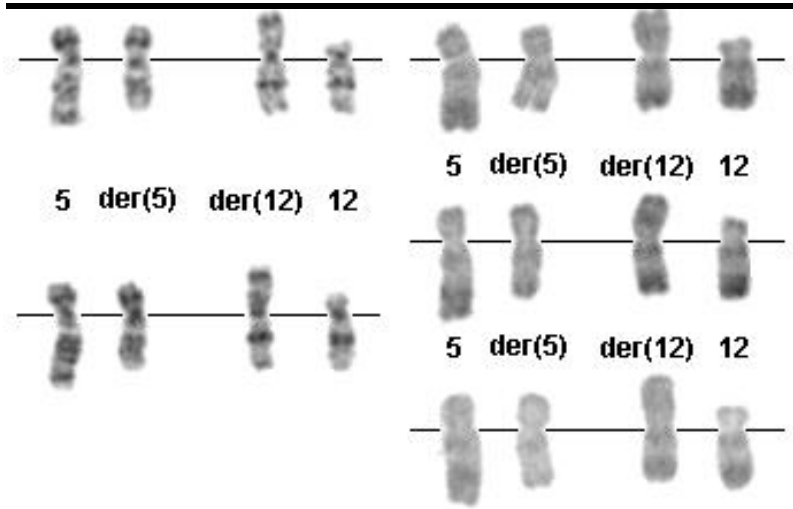
Leucémie MyéloMonocytaire Chronique

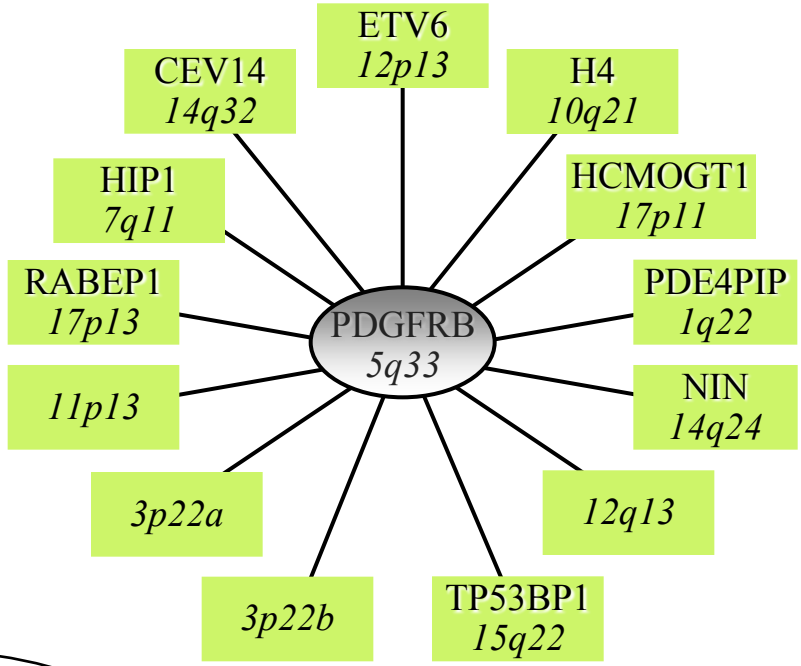
Leucémie Myéloïde Chronique Atypique

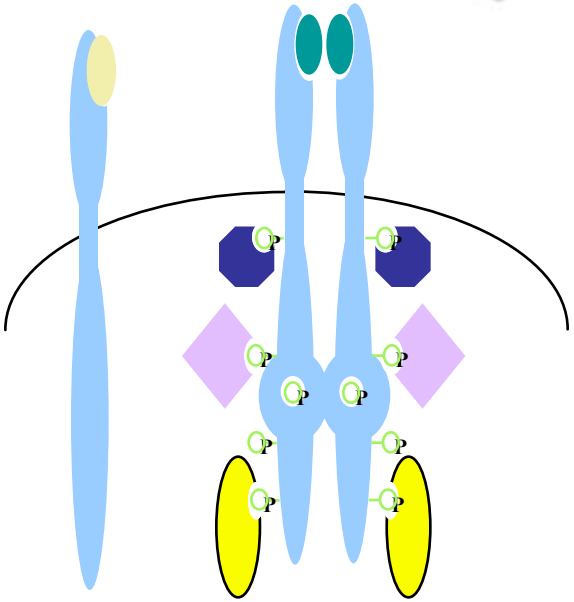
Leucémie MyéloMonocytaire Chronique Juvénile

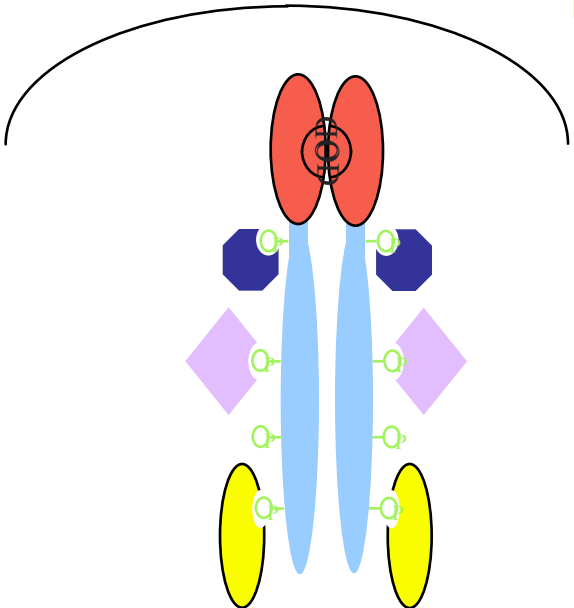
SMP/SMD, inclassable

LMMC & translocation 5;12









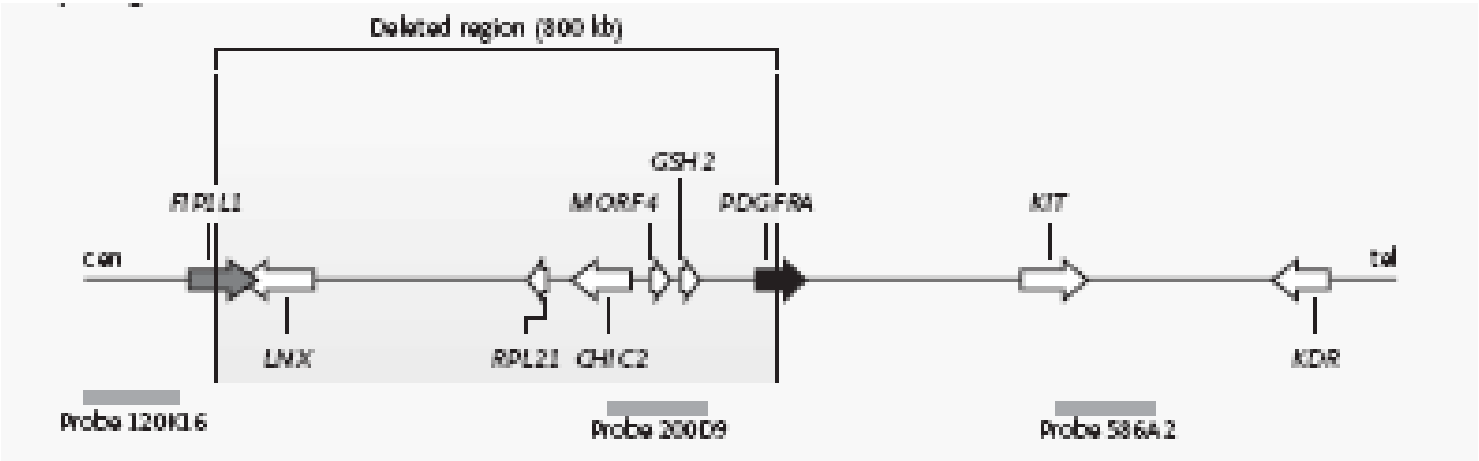
Leucémie Eosinophile Chronique/Syndrome hyperéosinophile :

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 27, 2003 VOL. 348 NO. 13

A Tyrosine Kinase Created by Fusion of the *PDGFRA* and *FIP1L1* Genes as a Therapeutic Target of Imatinib in Idiopathic Hypereosinophilic Syndrome

Jan Cools, Ph.D., Daniel J. DeAngelo, M.D., Ph.D., Jason Gotlib, M.D., Elizabeth H. Stover, M.Phil., Robert D. Legare, M.D., Jorges Cortes, M.D., Jeffrey Kutok, M.D., Ph.D., Jennifer Clark, M.D., Ilene Galinsky, R.N., James D. Griffin, M.D., Nicholas C.P. Cross, Ph.D., Ayalew Tefferi, M.D., James Malone, M.D., Rafeul Alam, M.D., Ph.D., Stanley L. Schrier, M.D., Janet Schmid, M.D., Michal Rose, M.D., Peter Vandenberghe, M.D., Ph.D., Gregor Verhoef, M.D., Ph.D., Marc Boogaerts, M.D., Ph.D., Iwona Wlodarska, Ph.D., Hagop Kantarjian, M.D., Peter Marynen, Ph.D., Steven E. Coutre, M.D., Richard Stone, M.D., and D. Gary Gilliland, M.D., Ph.D.



Hyperéosinophilie

↓

Eliminer les causes secondaires simples
atopie
parasitoses
maladies inflammatoires chroniques

Causes
« hématologiques »

↙ ↘

Primitives :
LMC
SMP
PDGFR-FGFR
Autre anomalie clonale
Blastes (LCE)

Secondaires :
Lymphome - SLP
LAL
Clone T (SHE-L)

?

SHE-I

"Classiques"

Leucémie Myéloïde Chronique

Polyglobulie de Vaquez

Thrombocytémie Essentielle

Splénomégalie Myéloïde
Myélofibrose Primitive

"Plus rares"

Leucémie Neutrophile Chronique

Leucémie Eosinophile Chronique
Syndrome HyperEosinophile

SMP chronique, inclassable

"Formes de chevauchement"

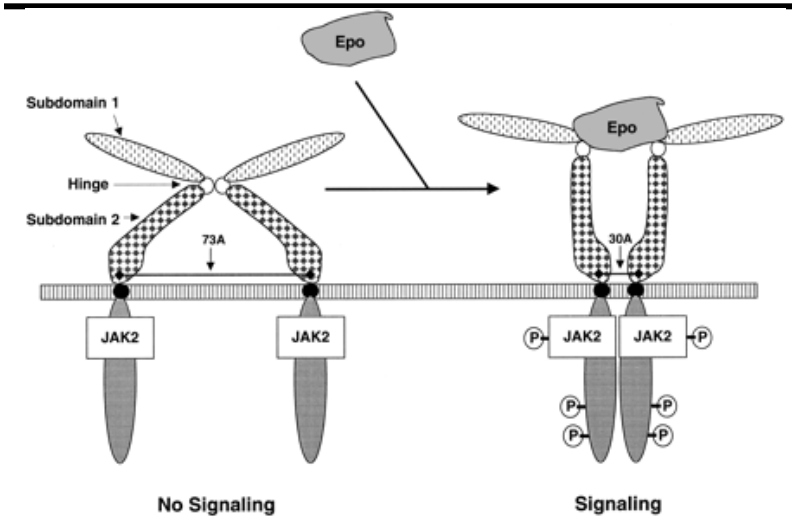
Leucémie MyéloMonocytaire Chronique

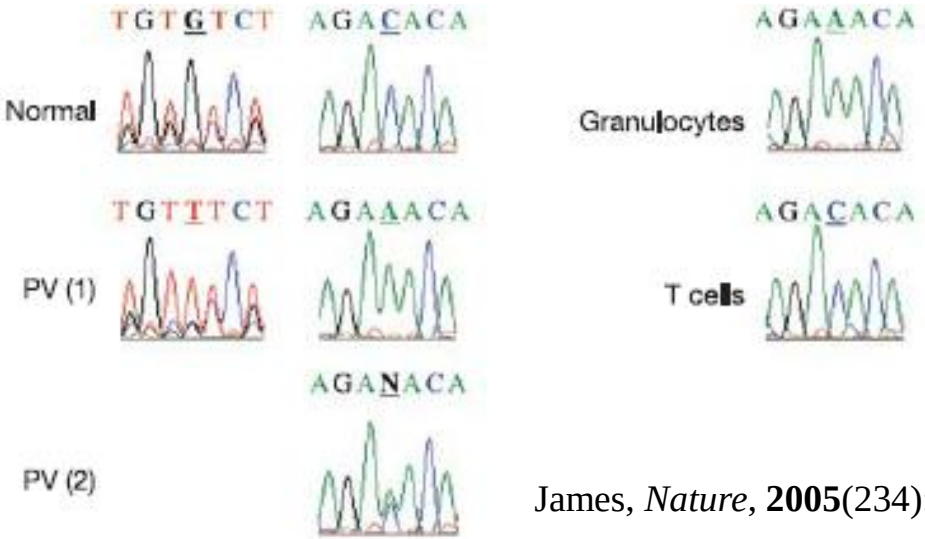
Leucémie Myéloïde Chronique Atypique

Leucémie MyéloMonocytaire Chronique Juvénile

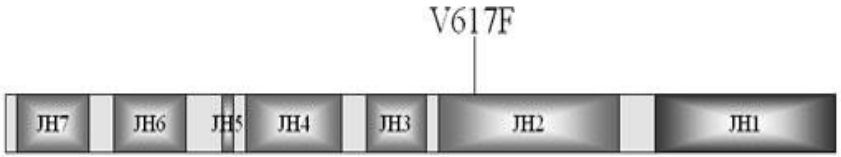
SMP/SMD, inclassable

JAK2-G1849T-V617F

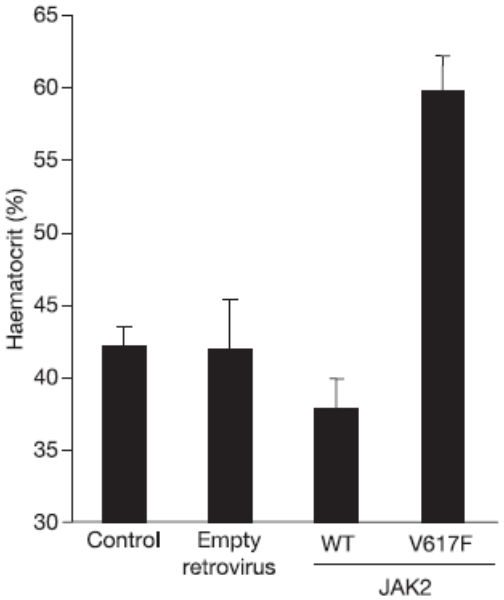


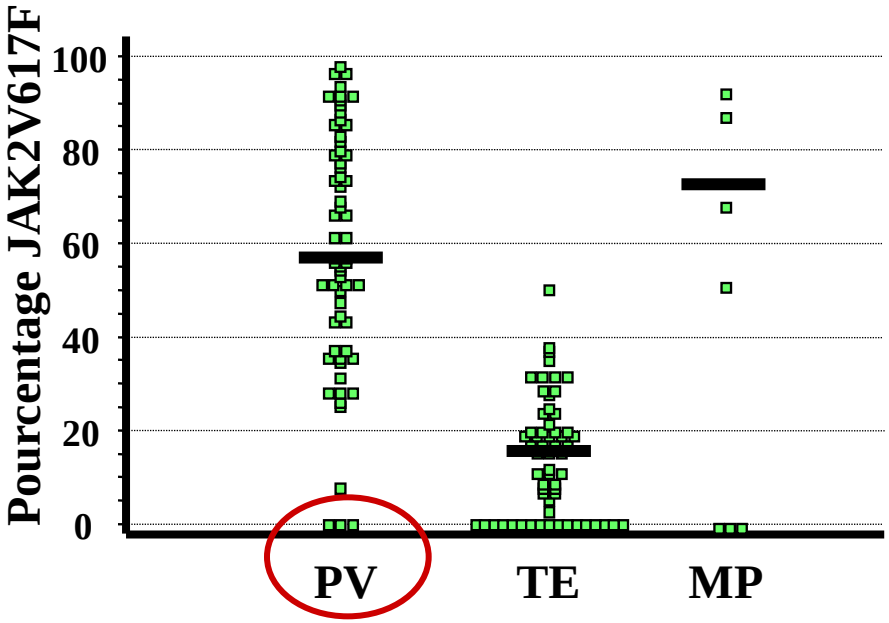


James, *Nature*, **2005**(234):1144



JH1: Kinase domain
JH2: Pseudokinase domain
JH7-4: FERM domain
JH4-3: SH2 domain





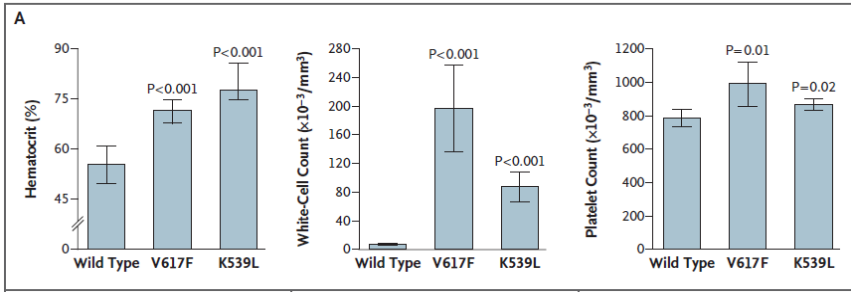
d'après *Blood*, **2006**(108):1865

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

JAK2 Exon 12 Mutations in Polycythemia Vera and Idiopathic Erythrocytosis

Linda M. Scott, Ph.D., Wei Tong, Ph.D., Ross L. Levine, M.D., Mike A. Scott, Ph.D., Philip A. Beer, M.R.C.P., M.R.C.Path., Michael R. Stratton, M.D., Ph.D., P. Andrew Futreal, Ph.D., Wendy N. Erber, M.D., Mary Frances McMullin, F.R.C.P., F.R.C.Path., Claire N. Harrison, M.R.C.P., M.R.C.Path., Alan J. Warren, F.R.C.Path., F.Med.Sci., D. Gary Gilliland, M.D., Ph.D., Harvey F. Lodish, Ph.D., and Anthony R. Green, F.R.C.Path., F.Med.Sci.



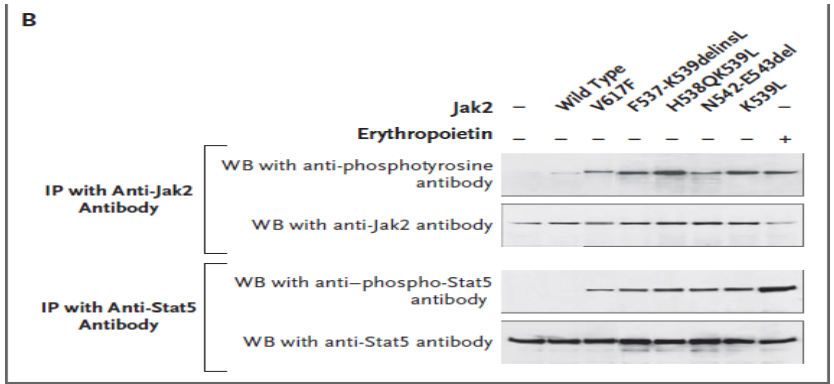


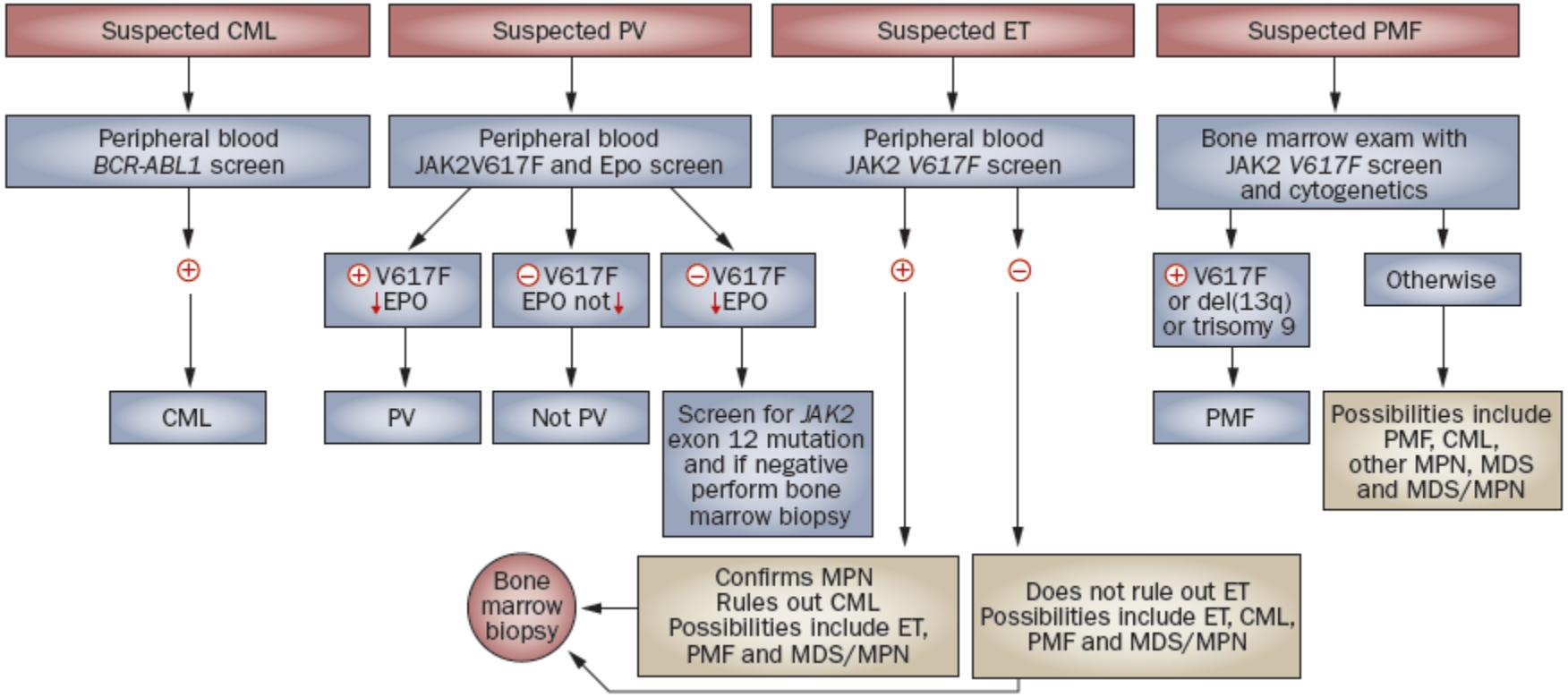
Table 2. Proposed revised WHO criteria for polycythemia vera

Proposed criteria for PV
Major criteria
1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2*617V>F or other functionally similar mutation such as *JAK2* exon 12 mutation
Minor criteria
1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

Diagnosis requires the presence of both major criteria and 1 minor criterion or the presence of the first major criterion together with 2 minor criteria.

*Hemoglobin or hematocrit greater than 99th percentile of method-specific reference range for age, sex, altitude of residence or hemoglobin greater than 17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL from an individual's baseline value that can not be attributed to correction of iron deficiency, or elevated red cell mass greater than 25% above mean normal predicted value.

En pratique :



Tefferi, A. *et al*. *Nat. Rev. Clin. Oncol*. **6**, 627–637 (2009)