DES National d'Hématologie Clinique

Hémopathies Ph positives

28 janvier 2022

Biologie des hémopathies à Ph+

Jean-Michel CAYUELA

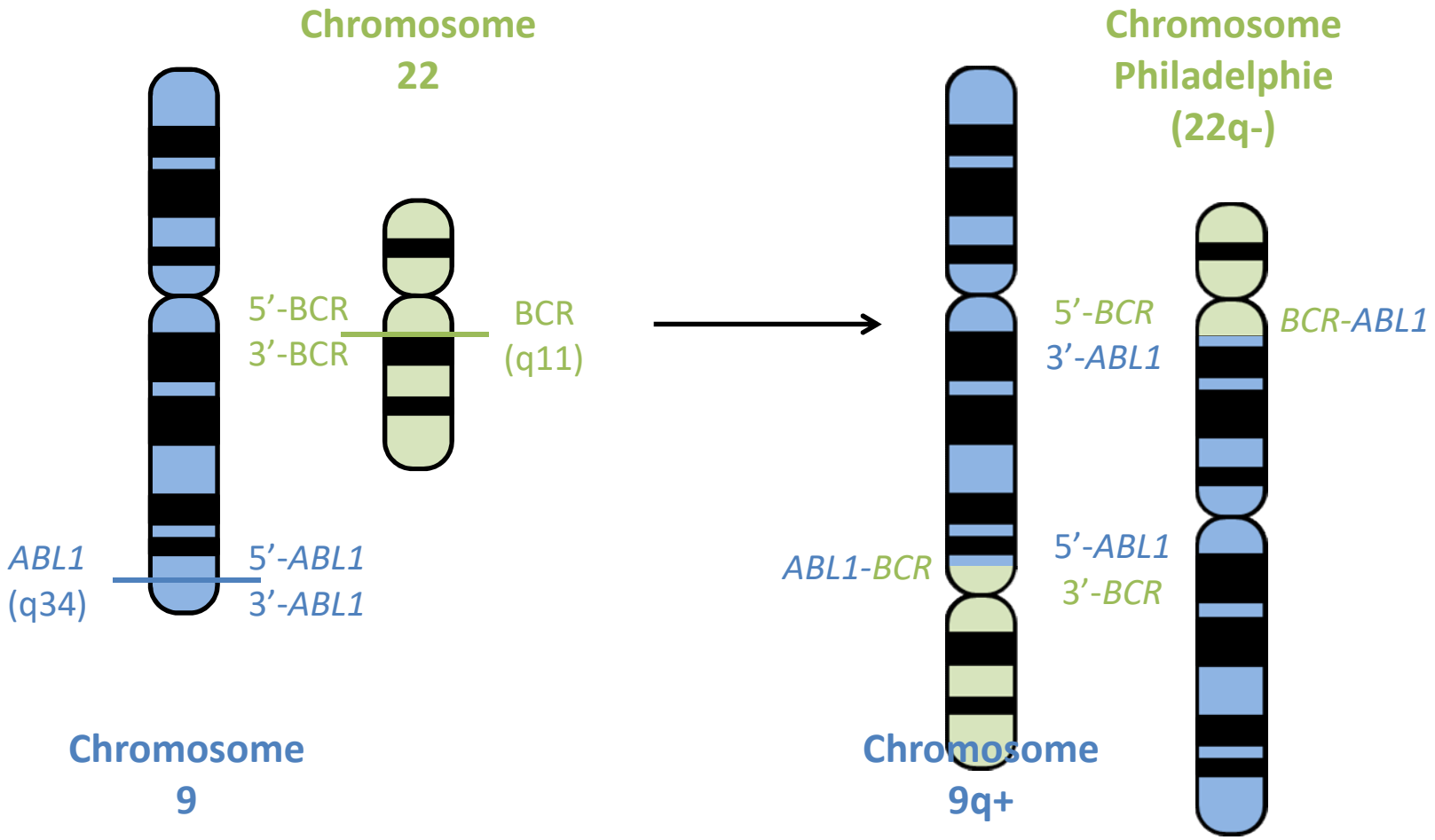
Laboratoire d'hématologie

Hôpital Saint-Louis, Paris

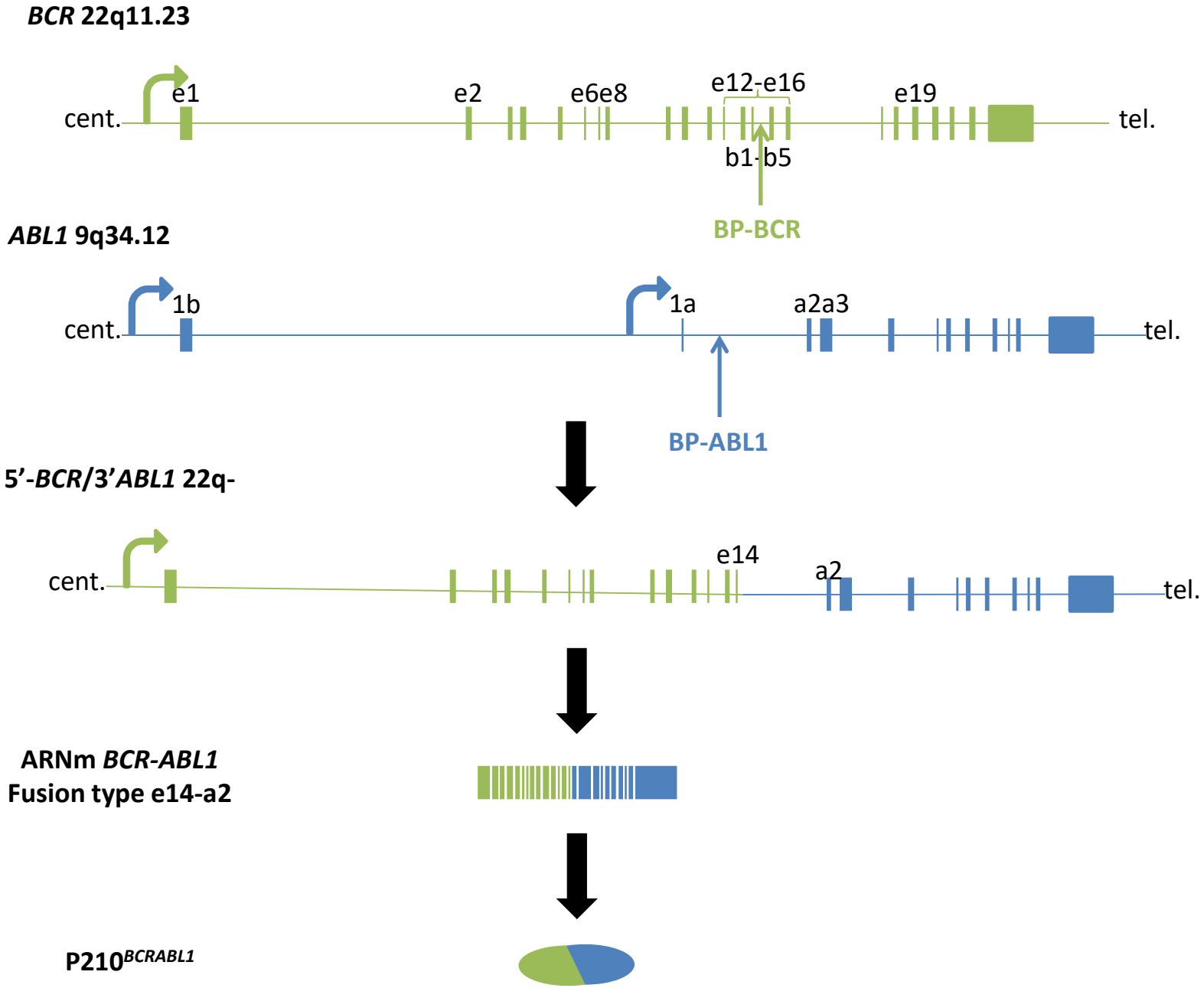
Outlines

- **Biology of CML, CML-BC , Ph+-ALLS**
- Molecular diagnostics
- TKI resistance
  - *BCR-ABL1 Tyrosine kinase domaine mutations*
  - Detection of low level-TKD mutations by NGS

Translocation t(9;22)(q34;q11)

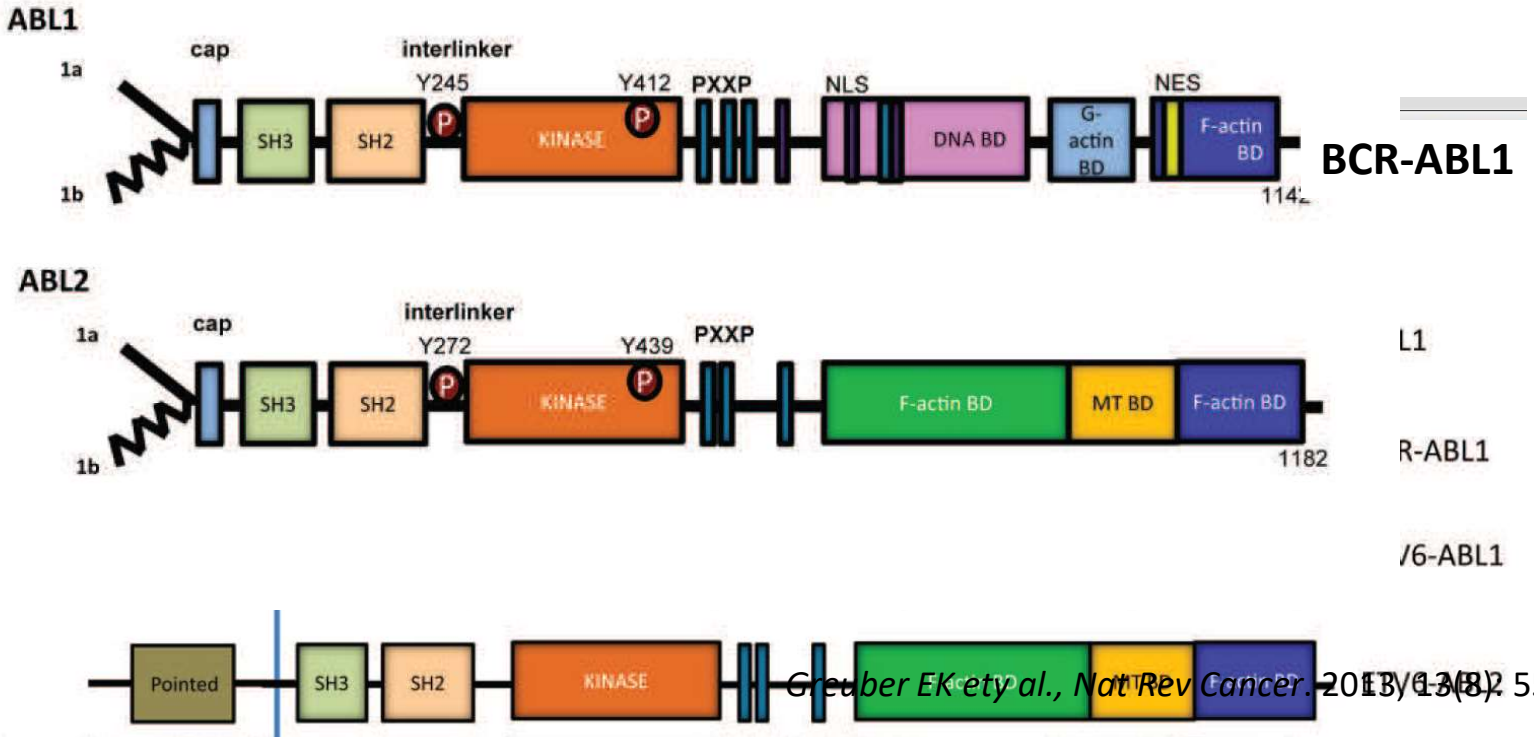


D'après *Rowley JD. Nature 1973;**243**:290*



*D'après Shtivelman E. Nature 1985;**315**:550–4*

ABL1



BCR-ABL1

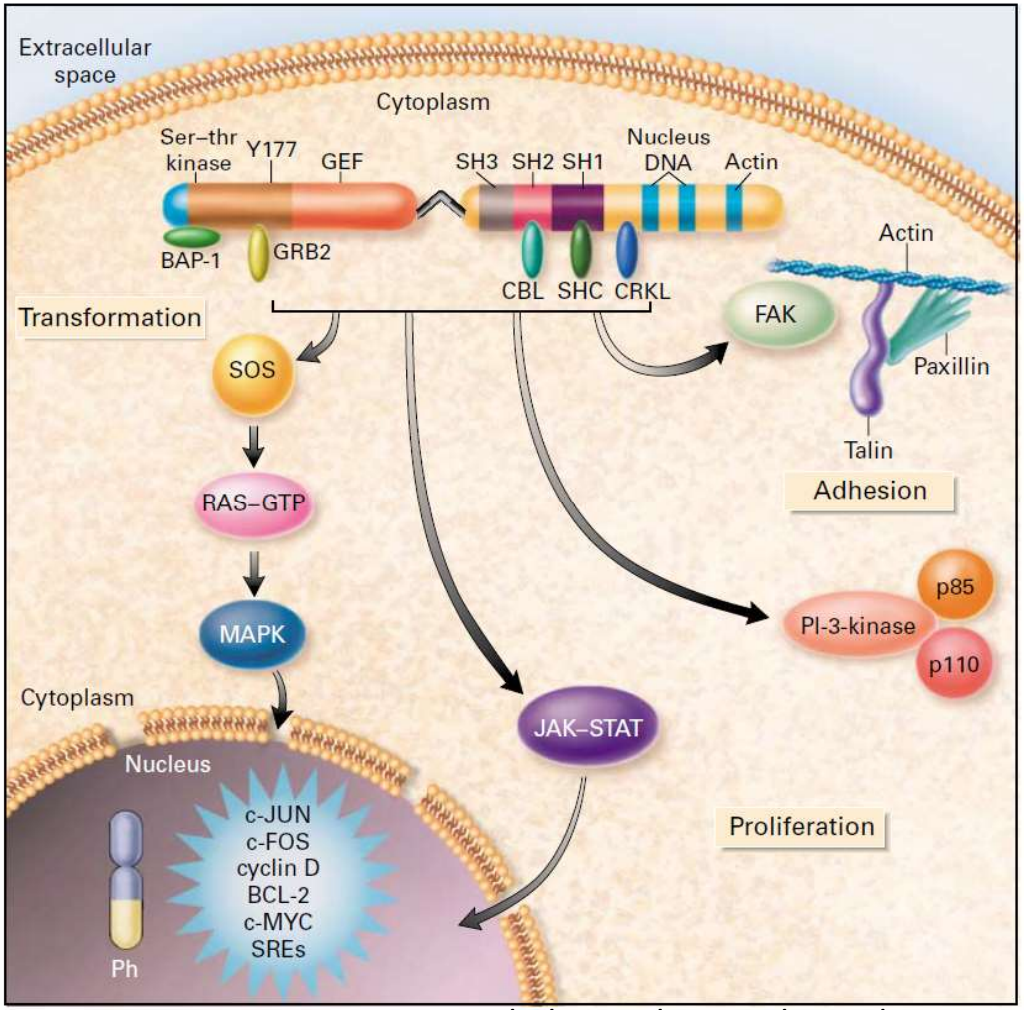
L1

R-ABL1

/6-ABL1

Greuber EK ety al., Nat Rev Cancer. 2013; 13(8): 559–571.

Effets oncogénique de *BCR-ABL1* dans les cellules hématopoïétiques

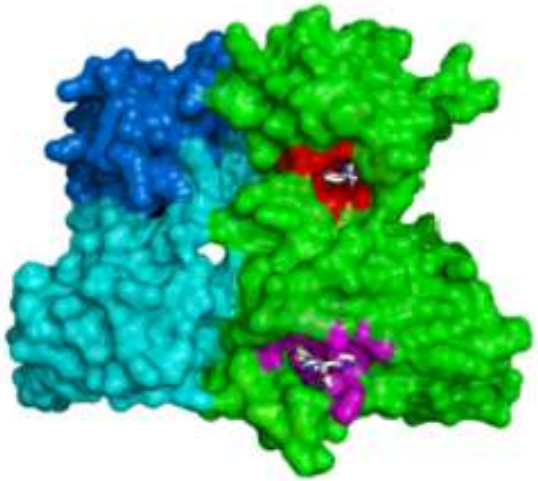


Faderl S et al., N Engl J Med 1999, 341:164-72

BCR-ABL1 inhibitors

Competitive inhibitors of ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib

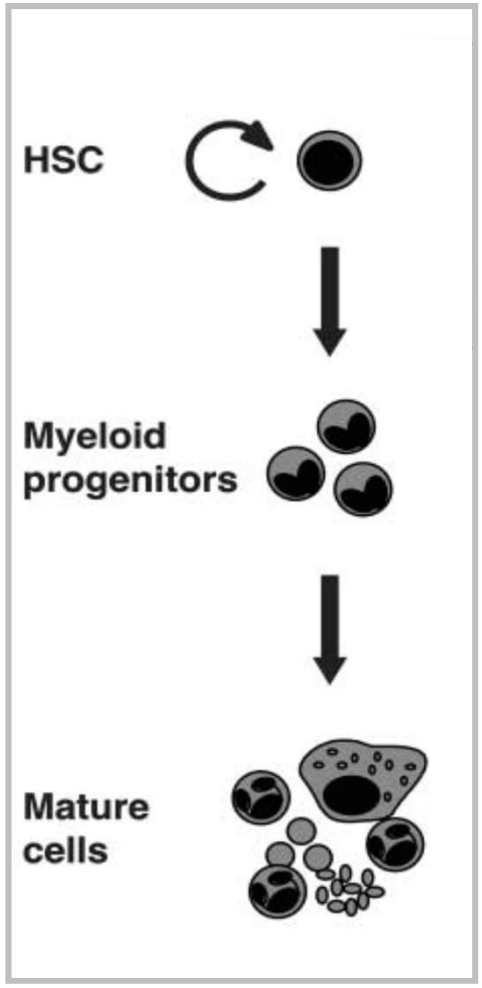


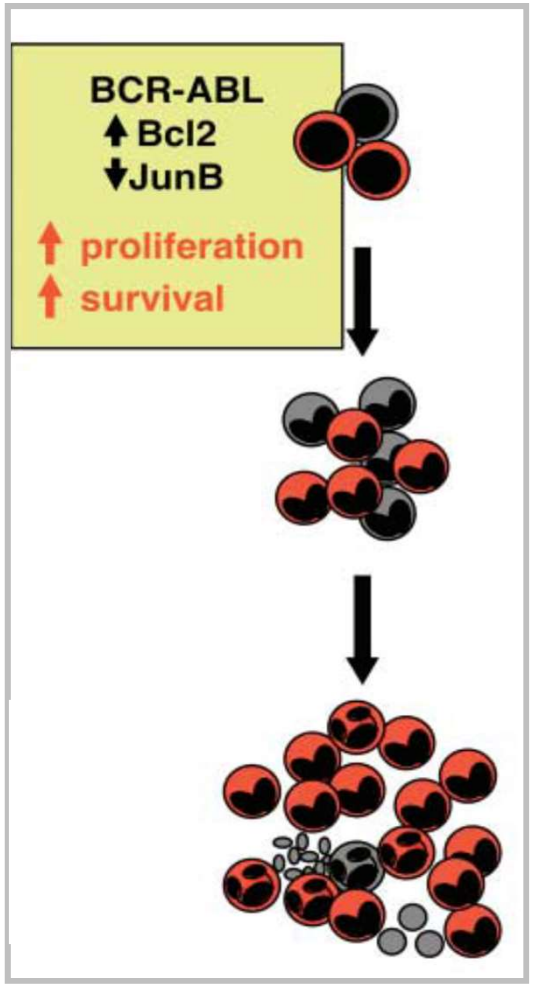
Allosteric inhibitor

- Asciminib

Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

Chronic Myeloid Leukemia (CML)





Adapted from Jamieson CHM, Cancer Cell Dec. 2004:531

Prognostic impact of additional chromosomal abnormalities in newly diagnosed CP-CML

Experience of the *German CML IV* study

	Fréquence **Out of 1151 *de novo CP-CML*** **N (%)**	**PFS / OS at 5 years** **%**
t(9;22)(q34;q11)	1003 (87)	90 / 92
t(v;22)	69 (6)	81 / 87
ACAs	79 (6.9)	
-Y	38 (3.3)	88 / 91
major route*	16 (1.4)	**50/53**
minor route**	25 (2.2)	96/96

**Abnormalities the most frequently detected in advanced phases of CML : +der(22), +8, i(17)(q10), +19*
***Abnormalities rarely detected in AP or BP of CML: t(3;12), t(4;6), t(2;16), t(1;21), t(15;17), inv16 …*

- ✓ « Major route ACAs » are associated with shorter PFS and OS
- ✓ « Minor route ACAs » aren't, but should be considered as warnings according to 2013 ELN recommandations

Fabarius A et al., Blood 2011, 118:6760-68
Baccarani et al., Blood 2013; 122:872-84

Prognostic impact of additional chromosomal abnormalities in newly diagnosed CP-CML

Experience of the *German CML IV* study

	OS at 5 years %
t(9;22)(q3	90 / 92
t(v;22)	81 / 87
ACAs	
	88 / 91
	50/53
	96/96

*Abnormalit -8, i(17)(q10), +19
**Abnorma (15;17), inv16 …

High-risk ACA ELN 2020

: +8
+Ph
i(17q)
+19
-7/7q-
11q23
3q26.2
complex karyotypes

Hochhaus H et al., Leukemia 2020 34, 966–984

- ✓ « Major route ACAs » are associated with shorter PFS and OS
- ✓ « Minor route ACAs » aren't, but should be considered as warnings according to 2013 ELN recommandations

Fabarius A et al., Blood 2011, 118:6760-68
Baccarani et al., Blood 2013; 122:872-84

Integrative genomics in CML

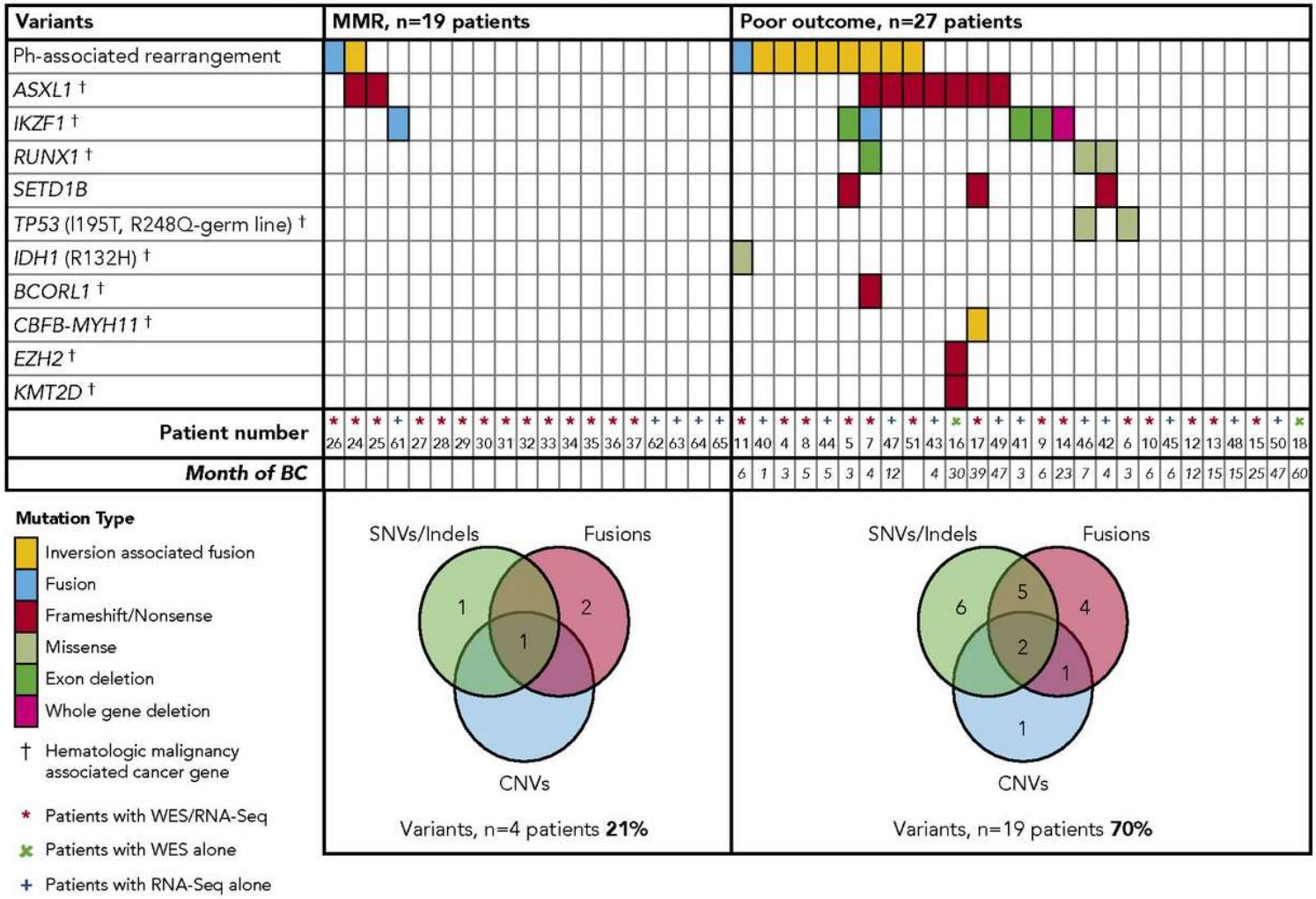
- 65 CML patients treated with 1st line TKI
  - 46 CP
    - 19 durable MMR (median FU 34 mths)
    - 27 poor outcome (26 developed BC after a median of 6 mths)
  - 39 BC, including 20 from the poor outcome series

- Sequencing methods
  - WES: Whole-Exome (DNA) in 38/65 pts
  - RNA-Seq: Whole-transcriptome (RNA) in 59/65 pts
  - Comparison to paired non-tumoral samples

Branford S et al., Blood 2018, 132:948-961

Relevant variants at diagnosis

Relevant variants at diagnosis in chronic phase for patients treated with first-line TKI



Characteristics of variants detected in 3 or more patients at diagnosis in chronic phase

Branford S et al., Blood 2018, 132:948-961

Relevant variants at diagnosis

✓Detected in 23/46 (50%) patients

✓A majority in poor outcome pts

- 4/19 (21%) vs 19/27 (70%)

✓Occured in cancer genes

- ASXL1. Mutated in 9 pts. 6 developed BC and 2 achieved MMR.

Longer time to BC (21 vs 4.5 mths)

- IKZF1. Mutated in 6 pts. 5 developed LBC
- SETD1B. Mutated in 3 pts. 3 developed BC

✓More frequently found in bad responders

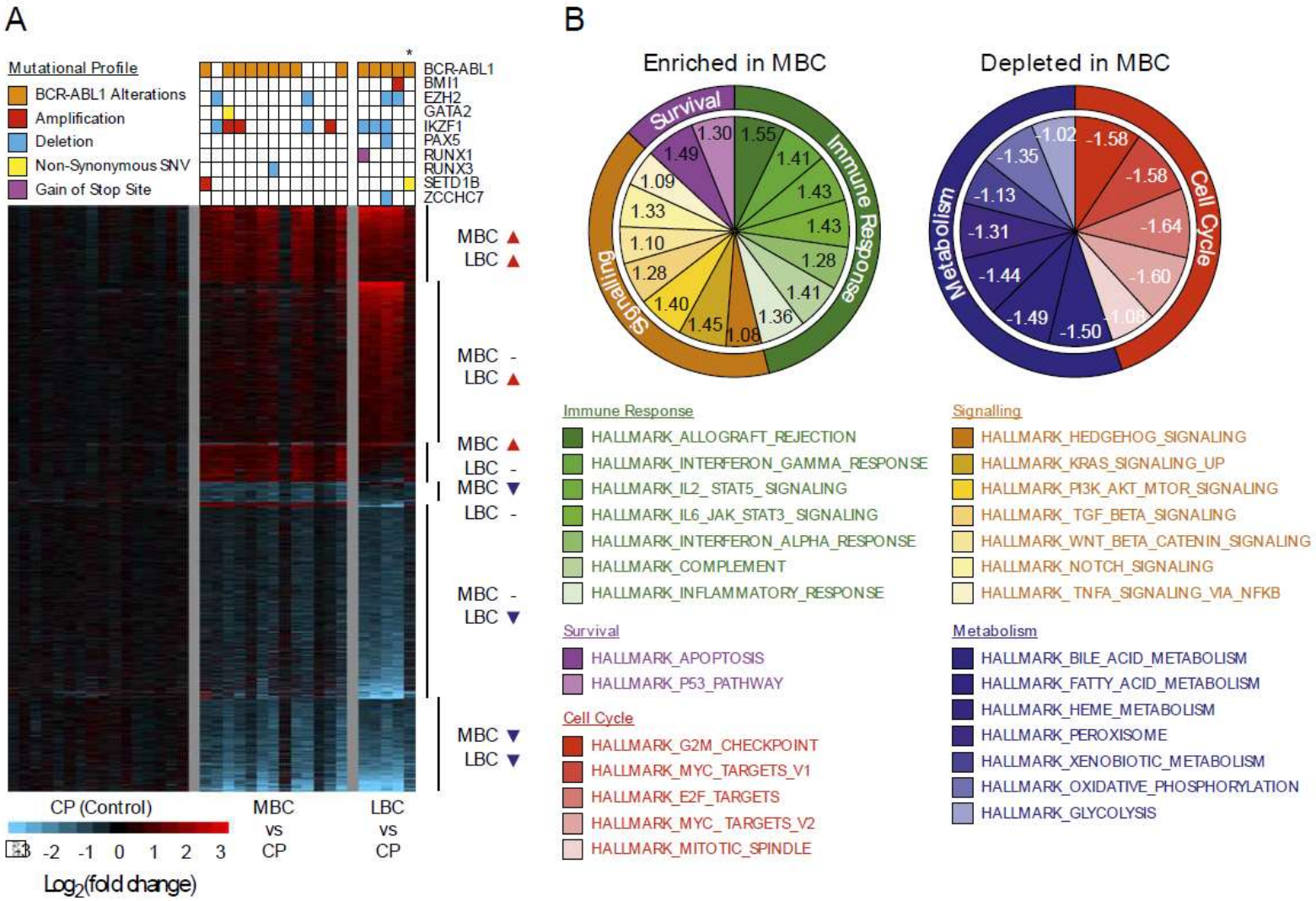
- No MCyR: 8/8; MCyR: 6/9; CCyR: 2/6

✓More frequently found in high risk patients

- High Sokal: 6/7; Int.: 5/12; low: 5/18

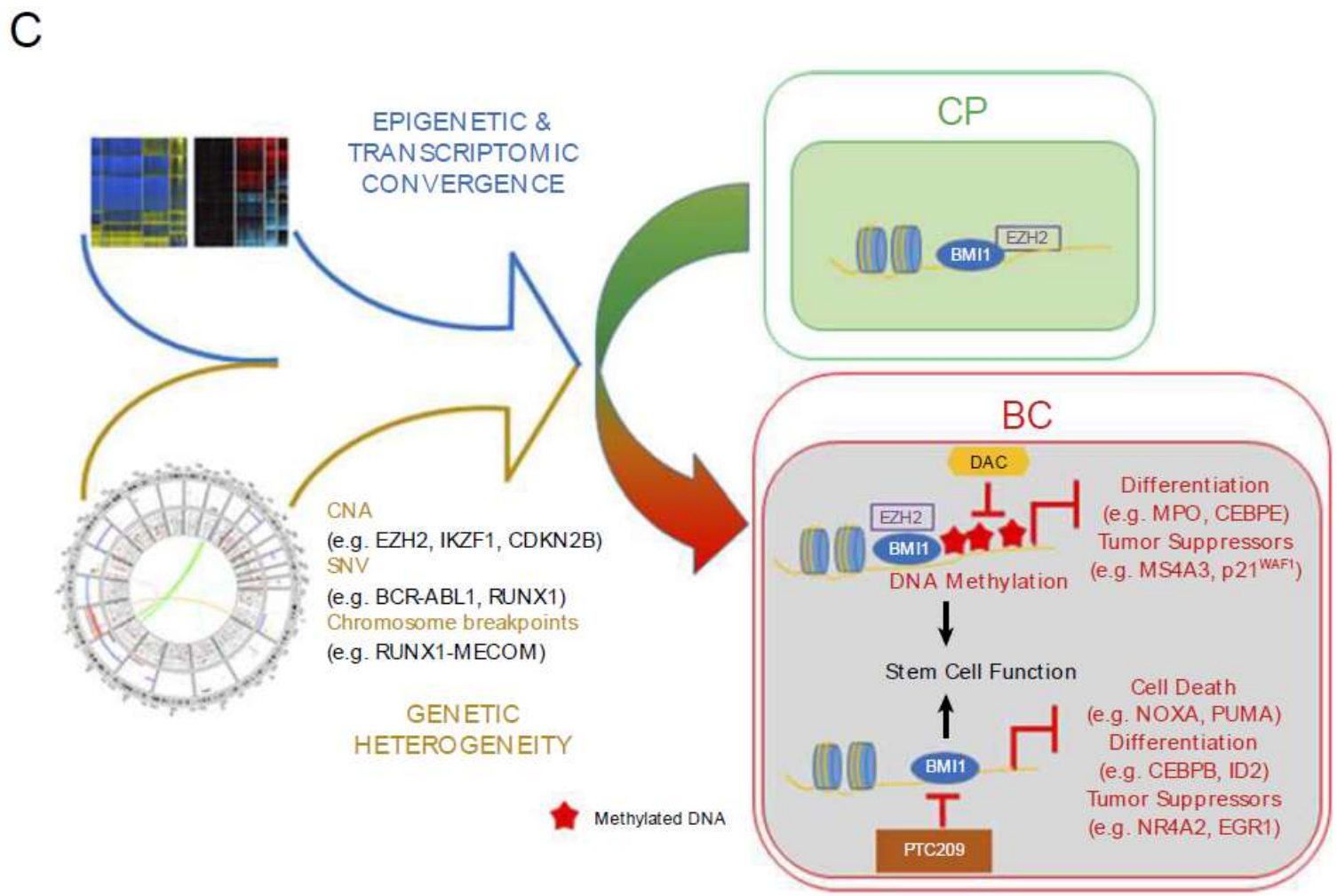
Branford S et al., Blood 2018, 132:948-961

Pathway convergence in CML BC



Ko TK et al; Blood 2020, 135:2337-2353

CP to BC progression model



Ko TK et al; Blood 2020, 135:2337-2353

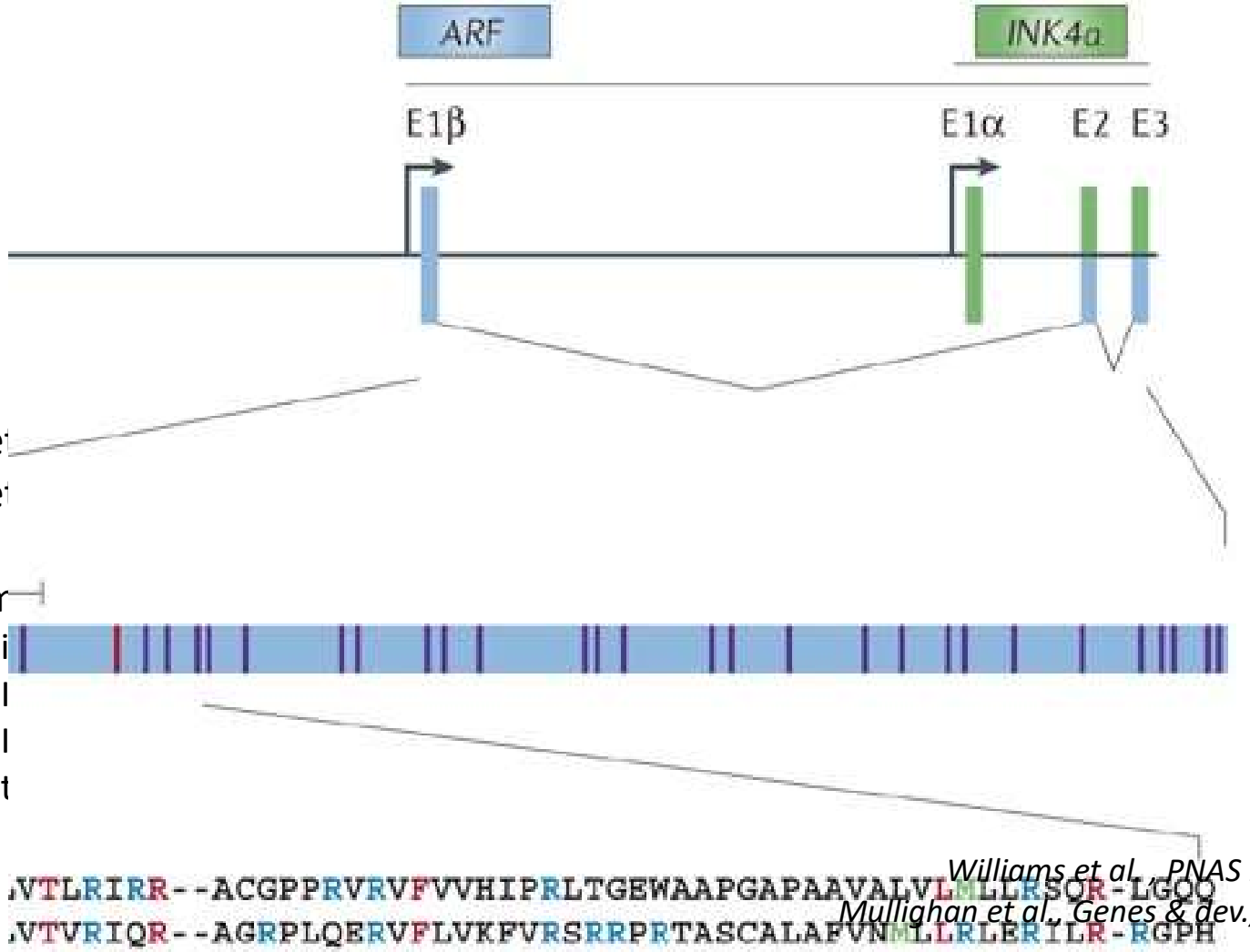
Main copy-number variations in BCR-ABL + ALLs

Locus	Géne	Enfants n = 21	Adultes N = 22	Total (%) N = 43
7p12.2	IKZF1	16	20	36 (84)
9p21	CDKN2A	10	13	23 (53)
9p13.3	PAX5	10	12	22 (51)
20p12.2	C20orf94	7	3	10 (23)
13q14.2	RB1	4	4	8 (19)
5q14.3	MEF2C	2	4	6 (14)
5q34	EBF1	3	3	6 (14)
12q22	BTG1	4	2	6 (14)
13q14	DLEU*	1	3	4 (9)
3p14.2	FHIT	2	2	4 (9)
12p13	ETV6	2	1	3 (7)

*mir16 and mir15

Mullighan et al., Nature 453:110, 2008

Deletion of CDKN2A

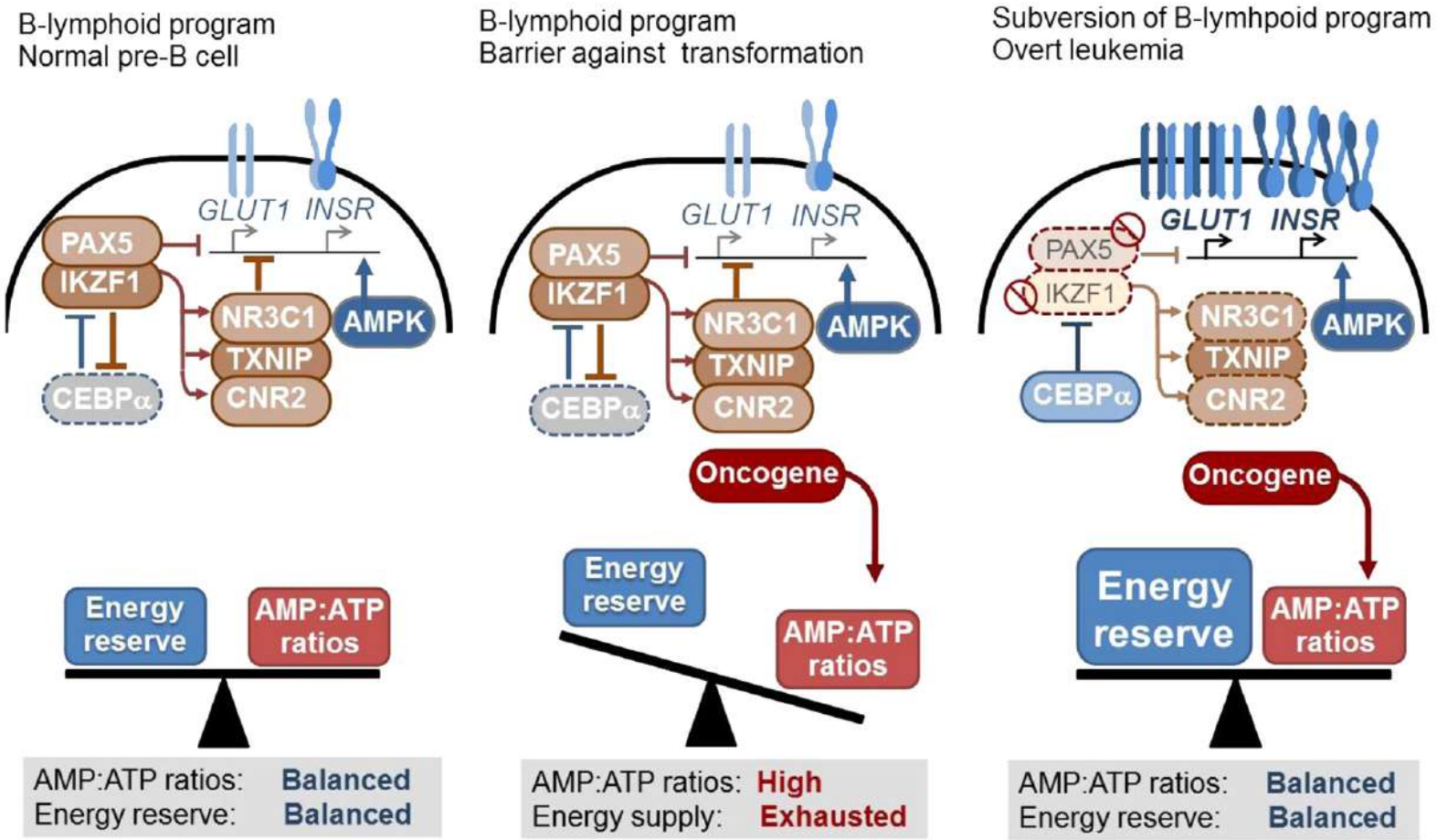


- Dele
- Dele

➢ Perm
  - i
  - I
  - I
  - t

Williams et al., PNAS 103:6688, 2006
Mullighan et al., Genes & dev., 22:1411, 2008

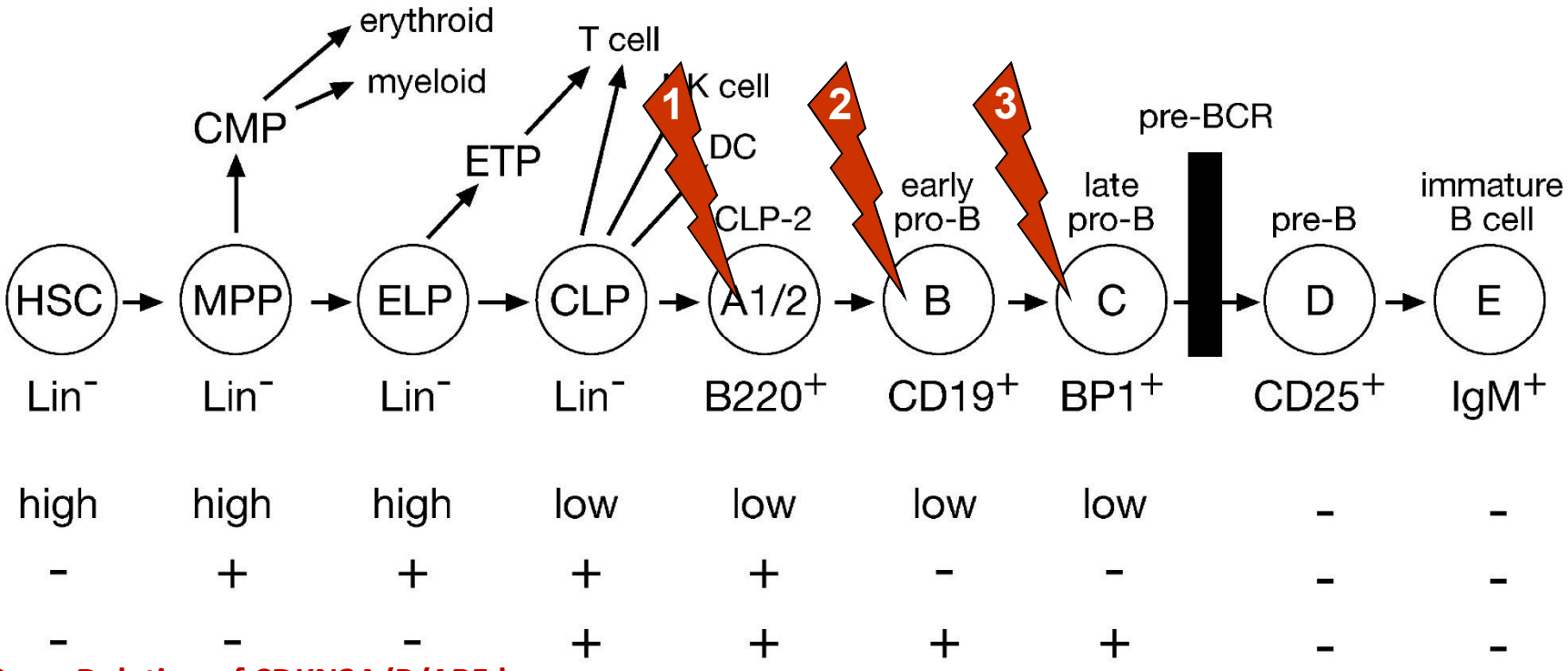
Metabolic gatekeeper function of B-lymphoid transcription factors



1. Metabolic gatekeeper function of B-lymphoid transcription factors. B-lymphoid transcription factors (e.g., PAX5 and IKZF1) promote
-cell development and they oppose myeloid differentiation as an alternative lineage fate through repression of the myeloid transcription factor

Chan L and Muschen M, Nature 2017, 542:479-483; Experimental Hematology 2017, 53:1-6

3 steps model for oncogenesis of BCR-ABL+ ALLs



2. Deletion of CDKN2A/B/ARF locus

- Simultaneous inactivation of TS functions of P53 and Rb.
- B-precursor permissivity to BCR-ABL

3. Inactivation of IKZF1 and PAX5

- blockage of differentiation
- Suppression of the metabolic barrier towards transformation

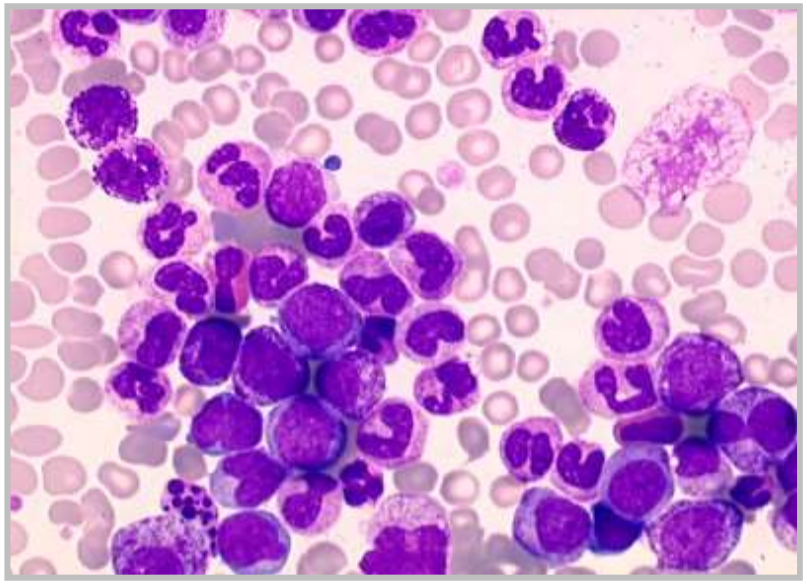
✓ **Pre-B ALL**

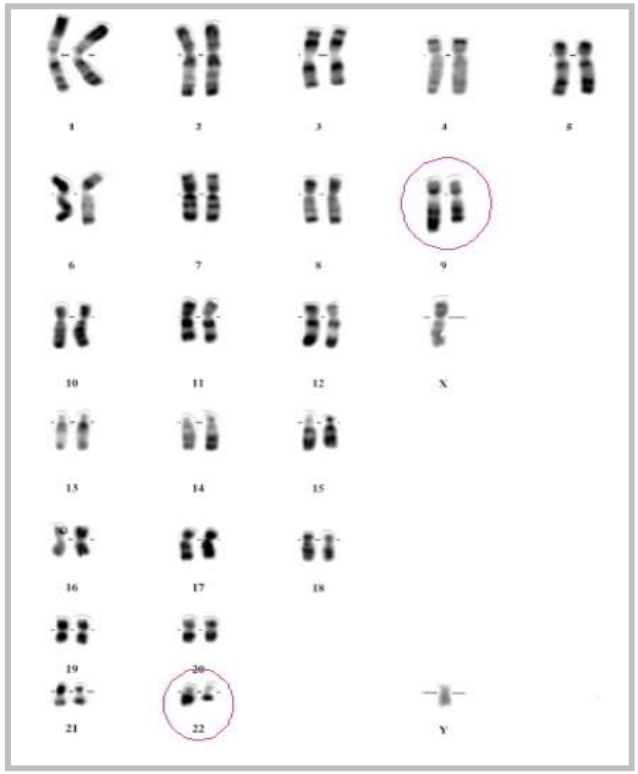
Adapted from
Mullinghan et al., Genes & Dev 22:1411, 2008
Chan L and Muschen M, Nature 2017, 542:479-483; Experimental Hematology 2017,

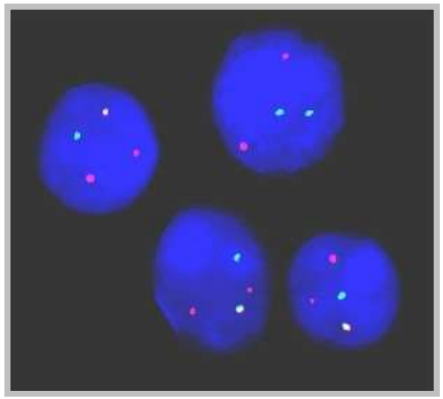
Outlines

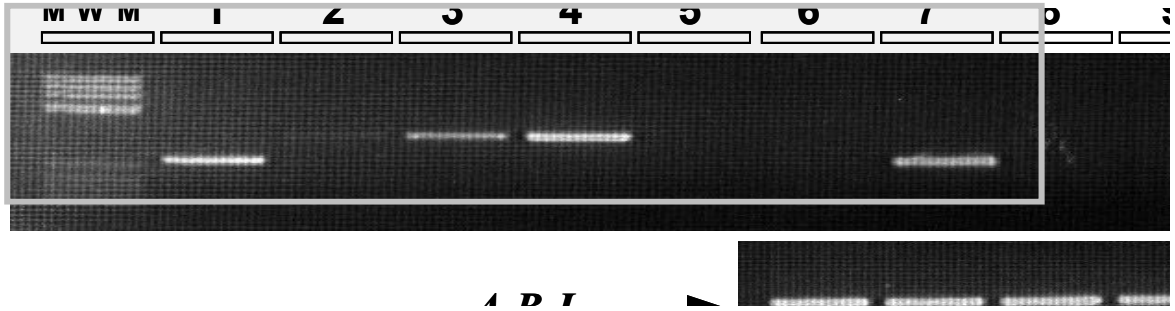
- Biology of CML, CML-BC , Ph+-ALLS
- **Molecular diagnostics**
- TKI resistance
  - *BCR-ABL1 Tyrosine kinase domaine mutations*
  - Detection of low level-TKD mutations by NGS

Morphological and genetic features

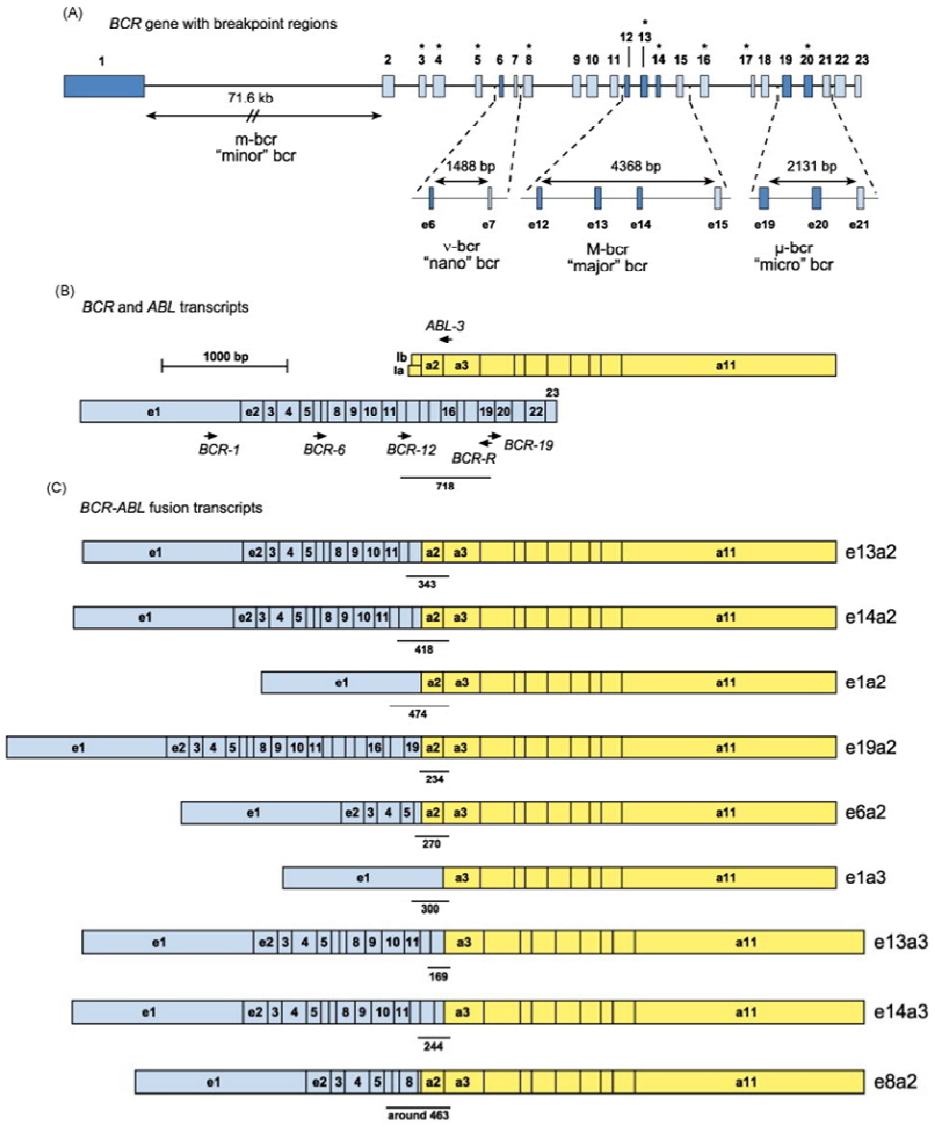






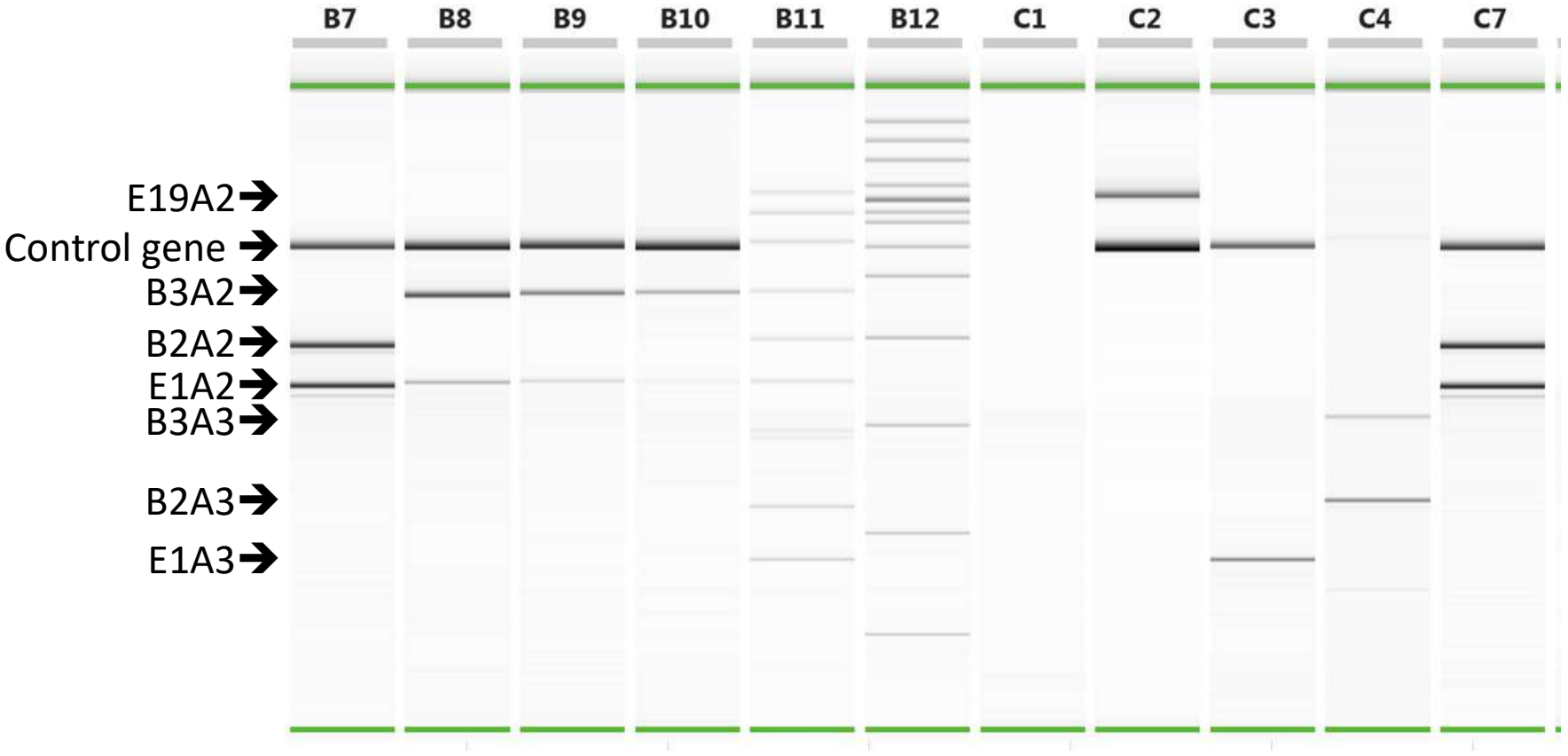


Detection of BCR-ABL1 at diagnosis



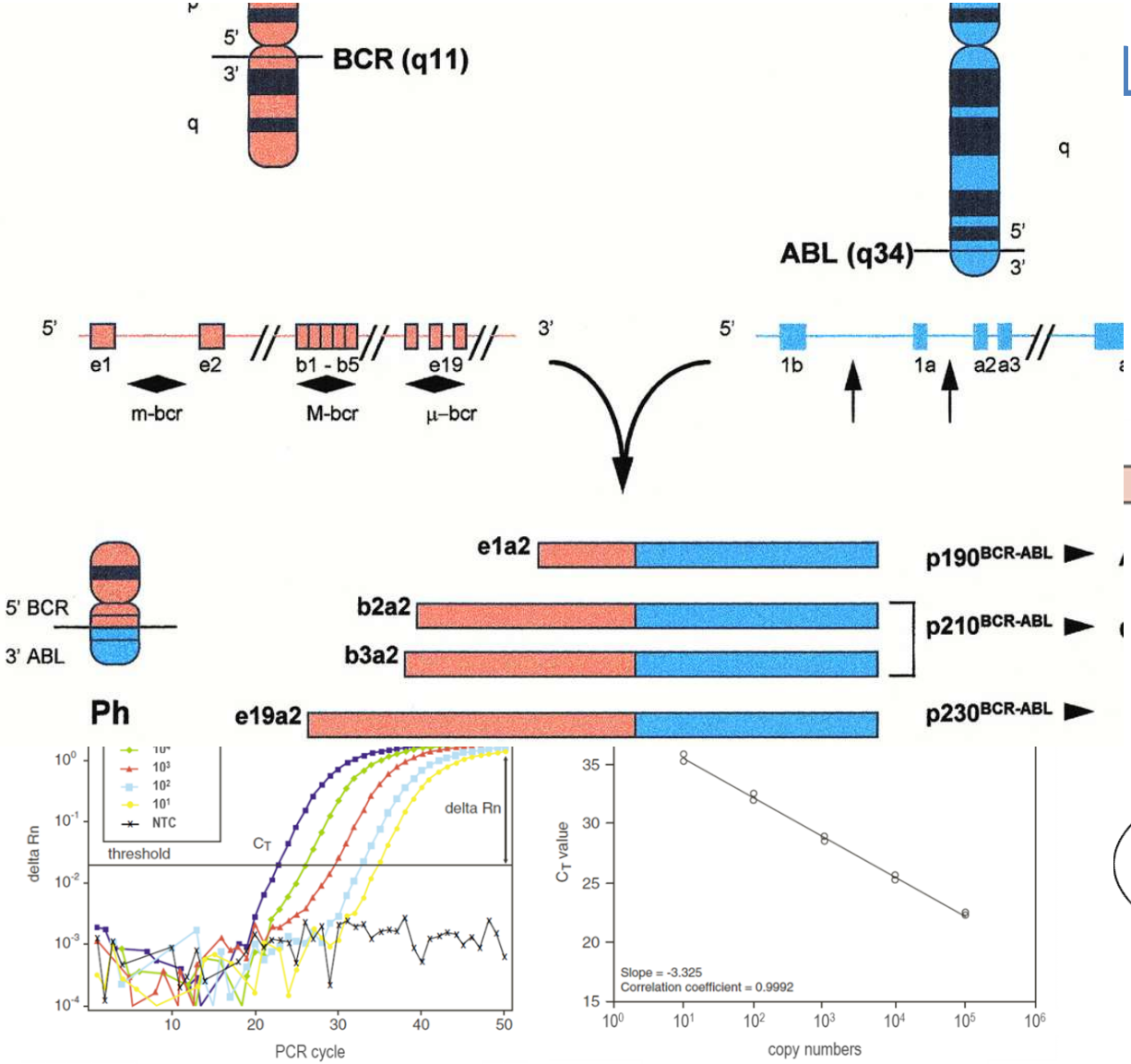
Burmeister et Reinhardt, 2008

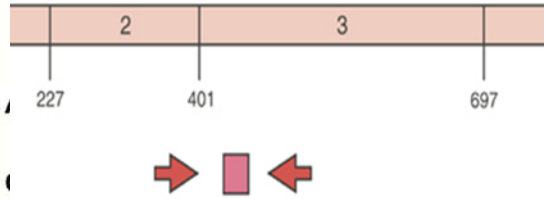
Amplification des transcrits *BCR-ABL1* par RT-PCR



Cayuela JM Unpublished

L1 mRNA





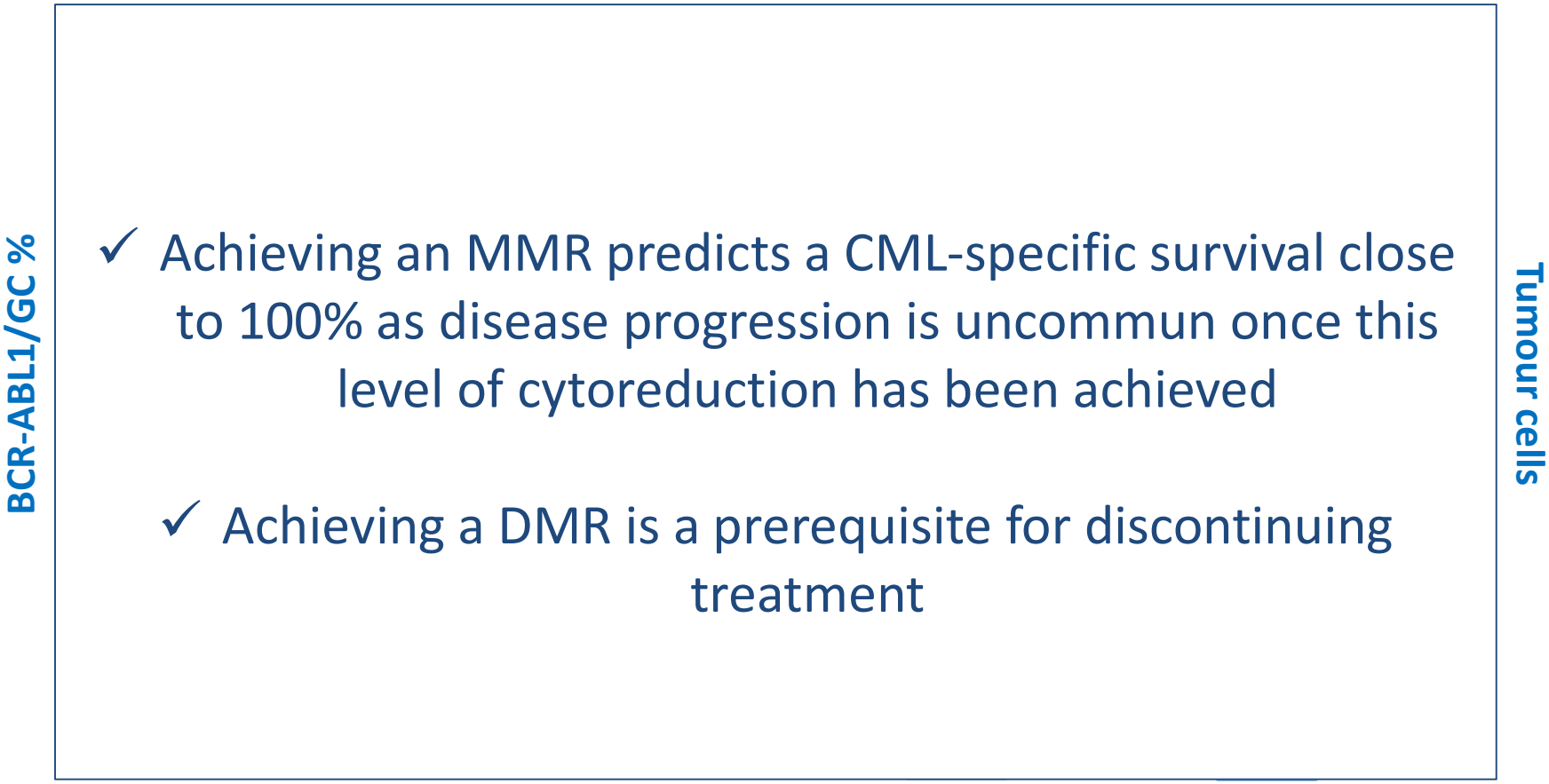
$$\frac{\text{BCR-ABL copy number}}{\text{Control Gene copy number}} \times 100\ \%$$

- **Expression levels normalized for RNA quality and quantity**

Gabert J et al., Leukemia 17, 2318-57, 2003

Beillard et al., Leukemia 17, 2474-86, 2003

International scale (IS)



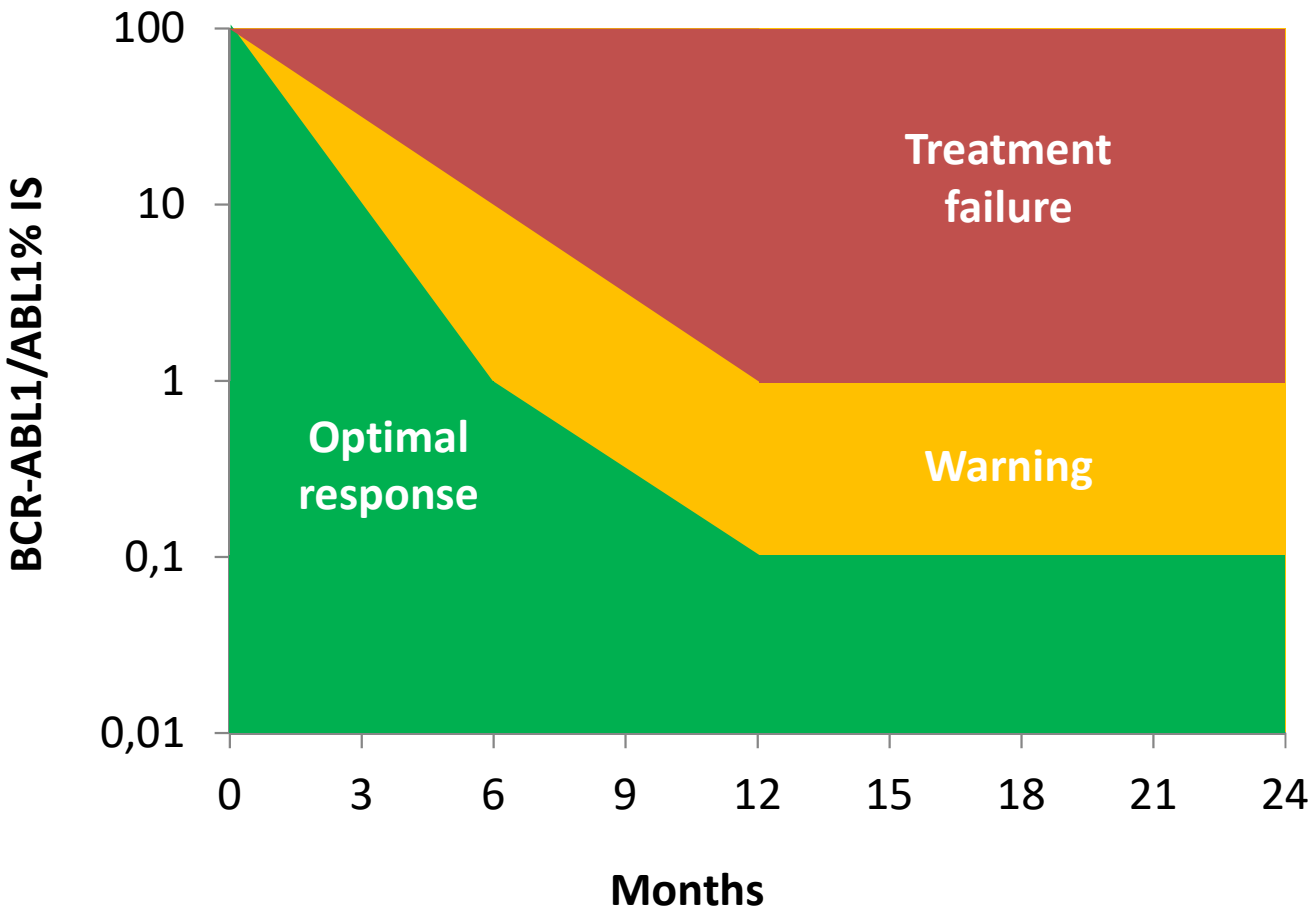
Adapted from Hughes T. et al.; Blood 2006, 108:28-37 and Baccarani M, Blood 2013, 122:872-84

Monitoring

- Blood Cell Counts and differential cell counts every 2 weeks until CHR
- QPCR on blood cells expressed as BCR-ABL1% according to IS every 3 months, even after an MMR is achieved and confirmed, because close monitoring of molecular response is required to assess eligibility for treatment discontinuation
- Cytogenetics or FISH are not sufficiently sensitive to monitor response
  - Should be done with rare or atypical BCR-ABL transcripts that cannot be measured by QPCR
  - Treatment failure/resistance to exclude ACA
  - Progression to AP or BP
- Additional QPCR testing may be indicated if the kinetics of response are not clear, or if toxicity or intolerance cause dose interruptions or reductions
- BCR-ABL KD-mutations
  - Failure : mandatory before changing the treatement
  - Warning : necessary to guide the change in treatment, when decided
  - Intolerance/complications : if possible (BCR-ABL > 0,1%) in case of decision to change

Hochhaus et al. Leukemia, published on line March 2020

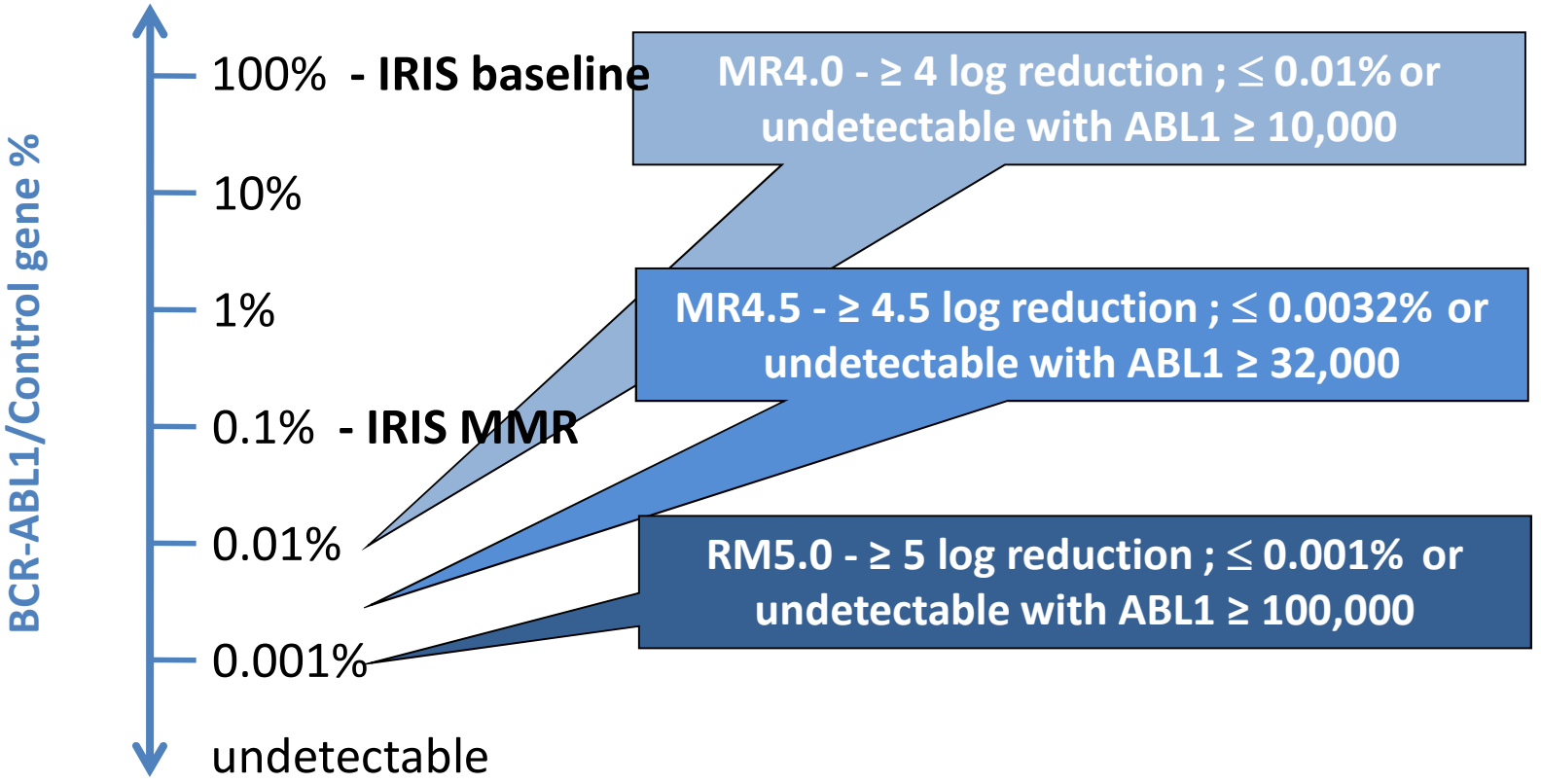
Milestones for treating CML



Adapted from Baccarani M et al., Blood 2013, 122:872-84

And Hochhaus H et al., Leukemia 2020 34, 966–984

Definition of DMR



International Scale

Cross NCP, Leukemia 2012, 26:2172-5
Baccarani M, Blood 2013, 122:872-84
Cross, Leukemia 2015, 29:999-1003
Hochhaus H et al., Leukemia 2020 34, 966–984

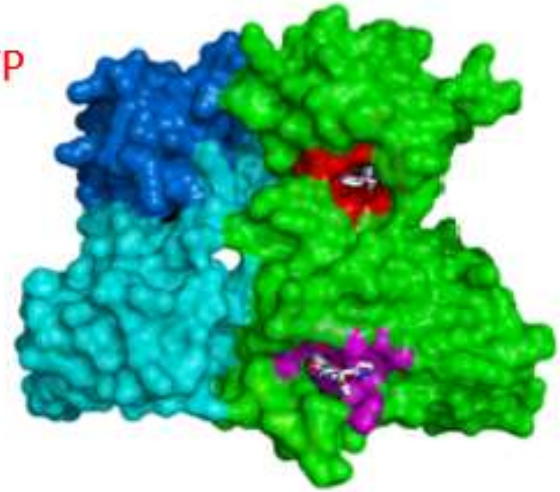
Outlines

- Biology of CML, CML-BC , Ph+-ALLS
- Molecular diagnostics
- **TKI resistance**
  - ***BCR-ABL1 Tyrosine kinase domaine mutations***
  - **Detection of low level-TKD mutations by NGS**

Contexte thérapeutique

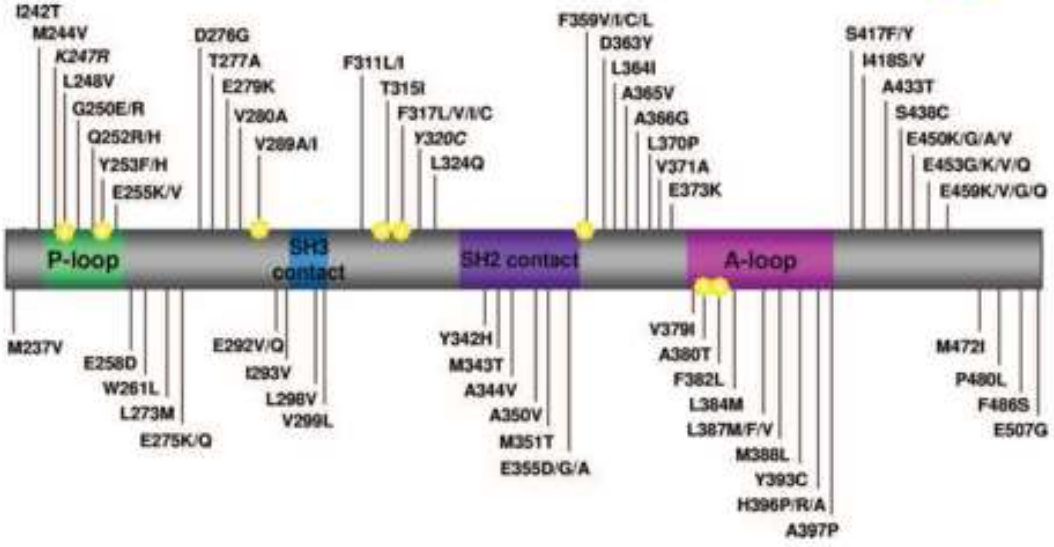
Inihibiteurs compétitifs de l'ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib



Inihibiteur allostérique

- Asciminib



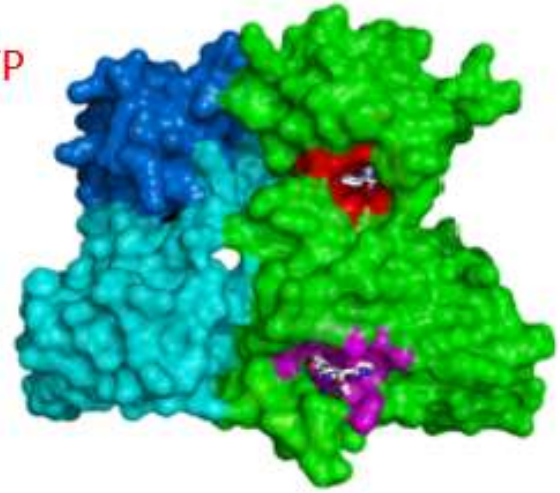
- Mutations d'aa du site de liaison myristoyl : A337V, P465S, V468F, C464W
- Mutation composite A337+M244V

Soverini S et al., Blood 2011, 118:1208
Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

Contexte thérapeutique

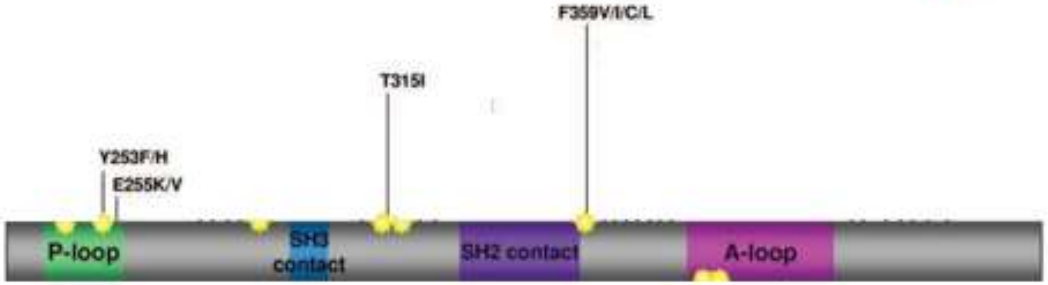
Inihibiteurs compétitifs de l'ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib



Inihibiteur allostérique

- Asciminib



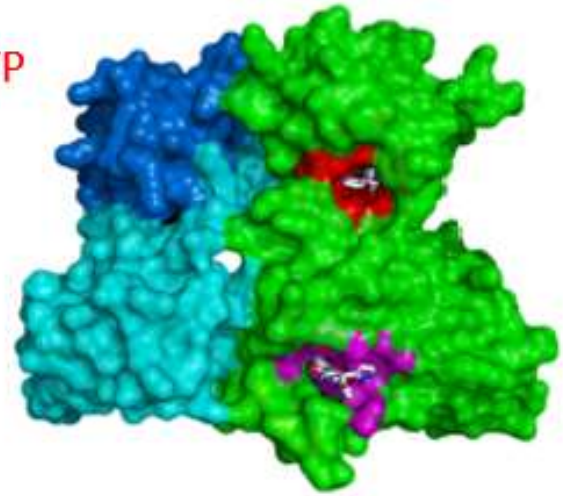
- Mutations d'aa du site de liaison myristoyl : A337V, P465S, V468F, C464W
- Mutation composite A337+M244V

Soverini S et al., Blood 2011, 118:1208
Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

Contexte thérapeutique

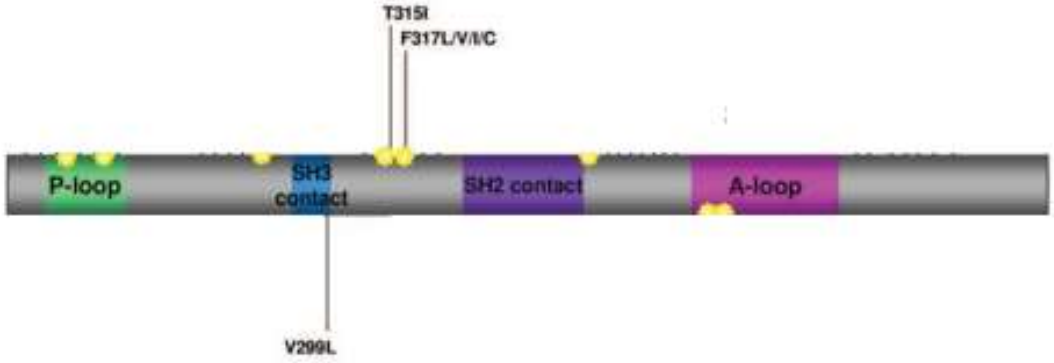
Inihibiteurs compétitifs de l'ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib



Inihibiteur allostérique

- Asciminib



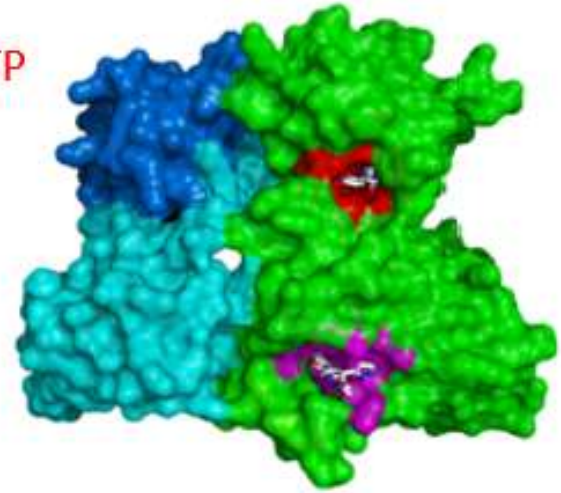
- Mutations d'aa du site de liaison myristoyl : A337V, P465S, V468F, C464W
- Mutation composite A337+M244V

Soverini S et al., Blood 2011, 118:1208
Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

Contexte thérapeutique

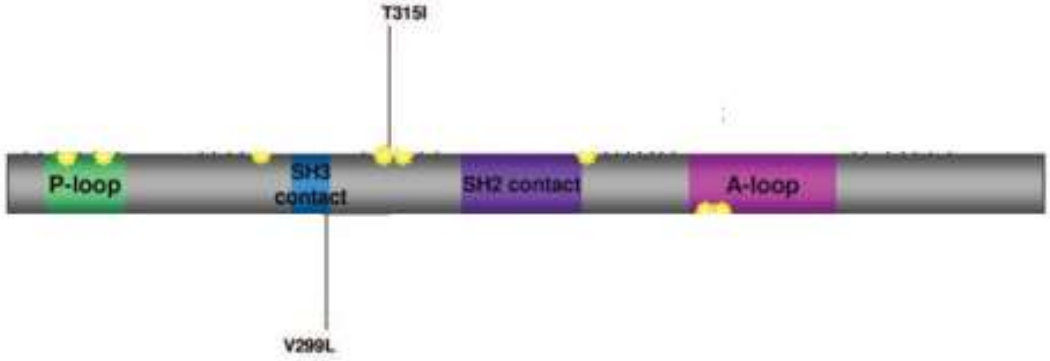
Inihibiteurs compétitifs de l'ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib



Inihibiteur allostérique

- Asciminib



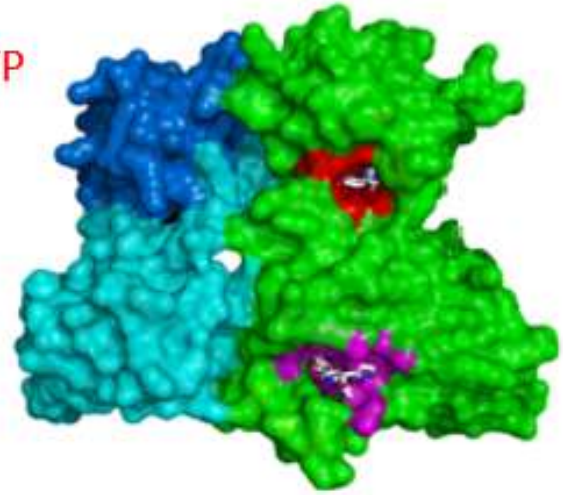
- Mutations d'aa du site de liaison myristoyl : A337V, P465S, V468F, C464W
- Mutation composite A337+M244V

Soverini S et al., Blood 2011, 118:1208
Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

Contexte thérapeutique

Inihibiteurs compétitifs de l'ATP

- Imatinib
- Nilotinib
- Dasatinib
- Bosutinib
- Ponatinib



Inihibiteur allostérique

- Asciminib

- Mutations d'aa du site de liaison myristoyl : A337V, P465S, V468F, C464W
- Mutation composite A337+M244V

Soverini S et al., Blood 2011, 118:1208
Willie A et al. Nature 2017, 543:733-7
Qiang W et al., Leukemia 2017, 31:2844-47

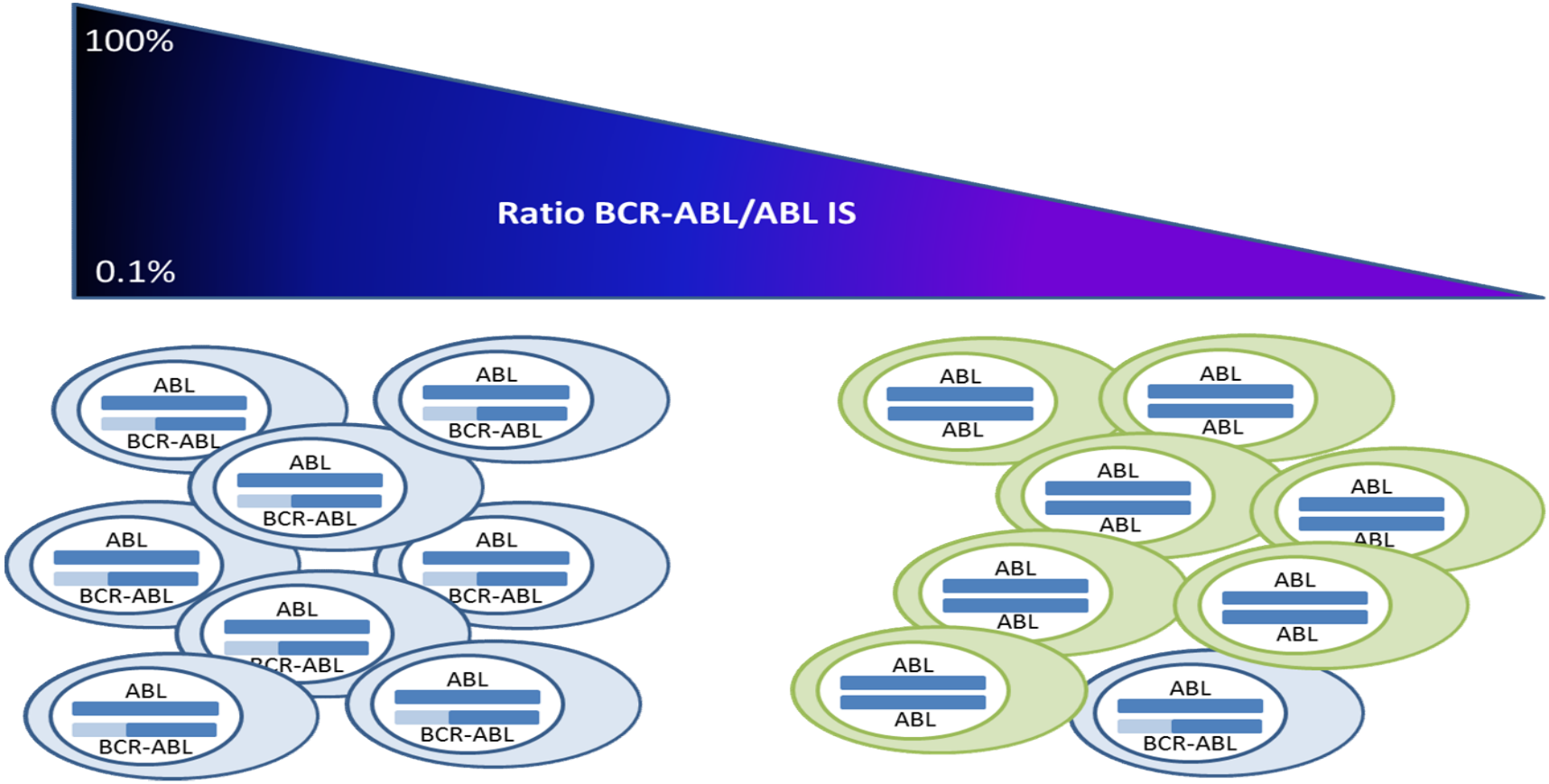
TKD mutations and therapeutic options

Mutations	Therapeutic options
T315I	Ponatinib, HSCT, investigational drugs
T315A, F317L/V/I/C	Consider nilotinib, bosutinib or ponatinib rather than dasatinib
Y253H, E255K/V, F359V/C/I	Consider dasatinib, bosutinib or ponatinib rather than nilotinib
V299L	Consider nilotinib or ponatinib rather than dasatinib or bosutinib
Autres mutations	Clinical significance unclear: consider high-dose TKI, alternative TKI, HSCT, investigational drugs

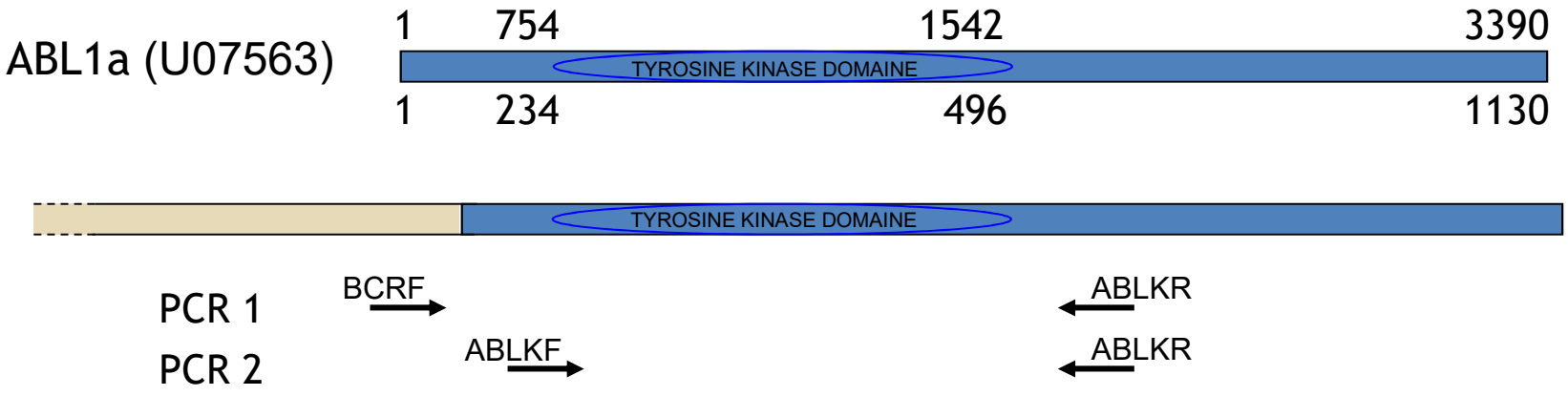
Adapted from Soverini S, Blood 2011

NCCN 2017 Guidelines CML V1.2018

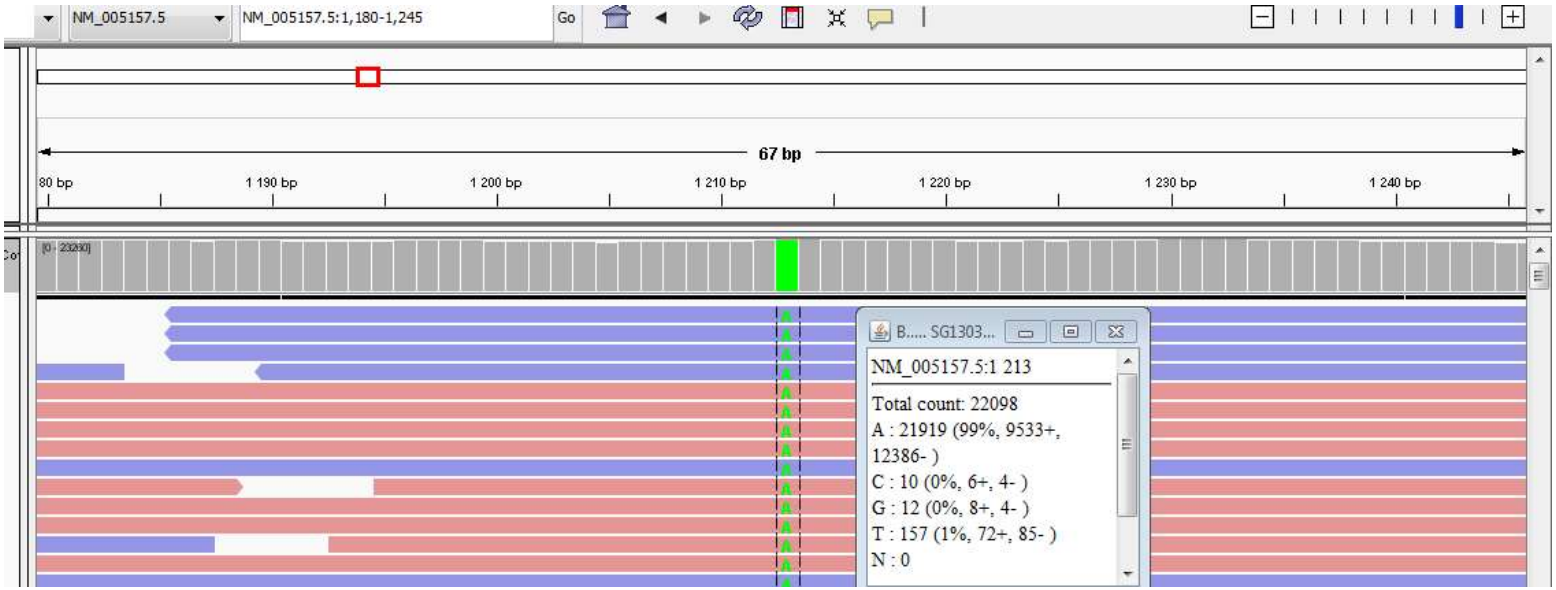
Dilution of *BCR-ABL1* mRNA



BCR-ABL1 TKD amplification and Sanger Sequencing

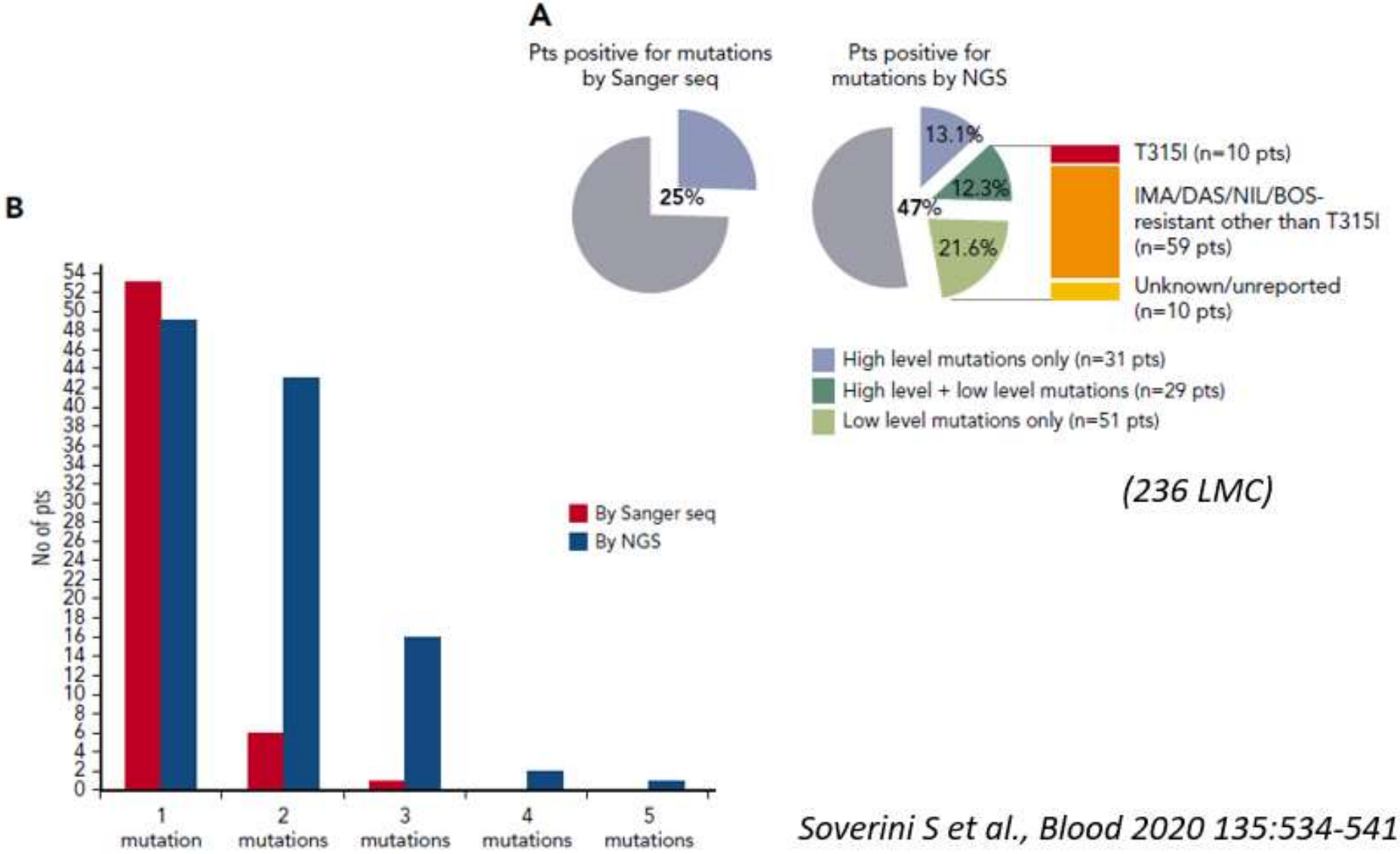


Branford et al. Blood 2002



Comparison between Sanger and NGS

NEXT-in-CML study



(236 LMC)

Soverini S et al., Blood 2020 135:534-541

Comparison between Sanger and NGS

NEXT-in-CML study

	Patients positive for mutations by SS	Patients positive for mutations by NGS
First-line failure	13/57 (23)	27/57 (47)
First-line warning	7/68 (10)	23/68 (34)
Second-line failure	15/39 (38)	20/39 (51)
Second-line warning	6/37 (18)	17/37 (49)
Third-line failure	14/21 (67)	17/21 (80)
Third-line warning	1/7	3/7
Fourth-/fifth-line failure	4/7	4/7
Total	60/236 (25)	111/236 (47)

Soverini S et al., Blood 2020 135:534-541

Pour en apprendre d'avantage

https://www.thinktesttreat.com/