

# Cellular and molecular mechanisms of imatinib resistance

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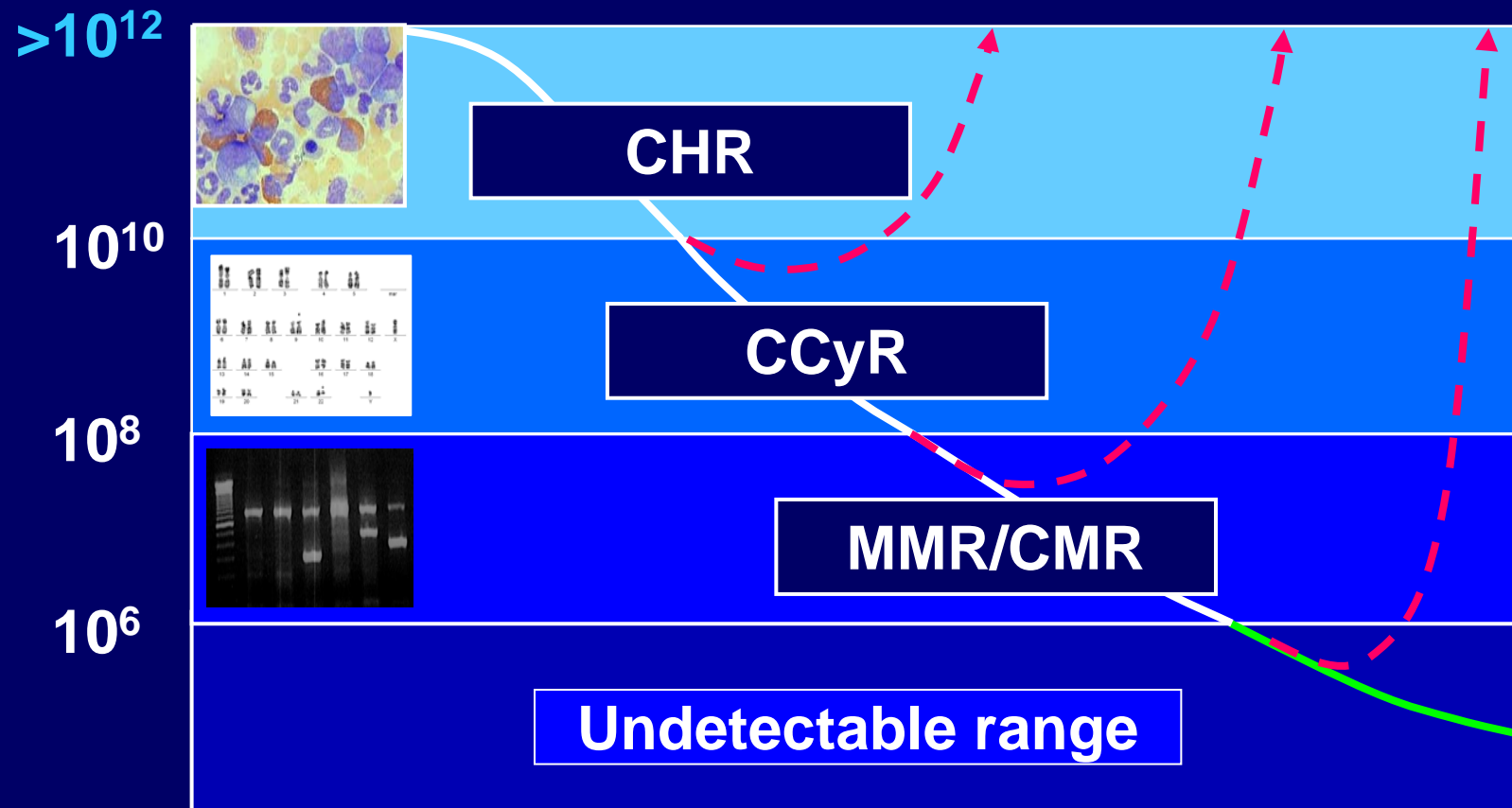
EUTOS for CML



*European Treatment and Outcome Study*

# Goals of CML therapy

Leukemia cells



CML = chronic myelogenous leukemia; CHR = complete hematologic response; CCyR = complete cytogenetic response; MMR = major molecular response; CMR = complete molecular response

# Levels of resistance / relapse

1° resistance: Hematologic resistance

Cytogenetic resistance

(Continuous PCR positivity = MRD Persistence)

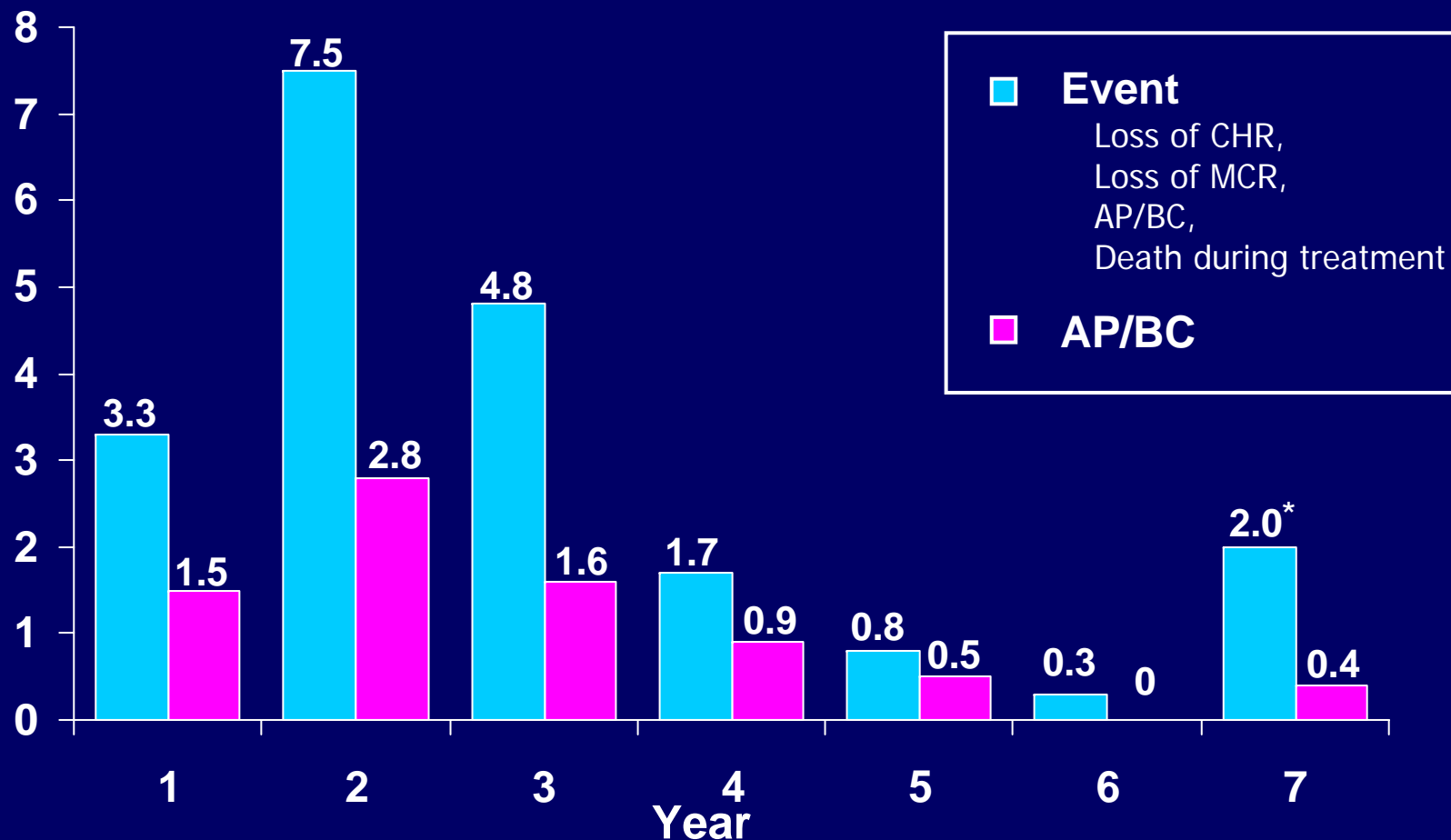
2° relapse: Hematologic relapse / Progressive disease

Cytogenetic relapse

(Molecular relapse)

## Annual event rates: imatinib arm

- KM estimated EFS at 7 years = 81%
- KM estimated rate without AP/BC at 7 years = 93%



\*Total events (n=5) including loss of MCR (n=3) and deaths (n=2, one of which was coded as progression to AP/BC in a patient in CMR 6 months prior to death).

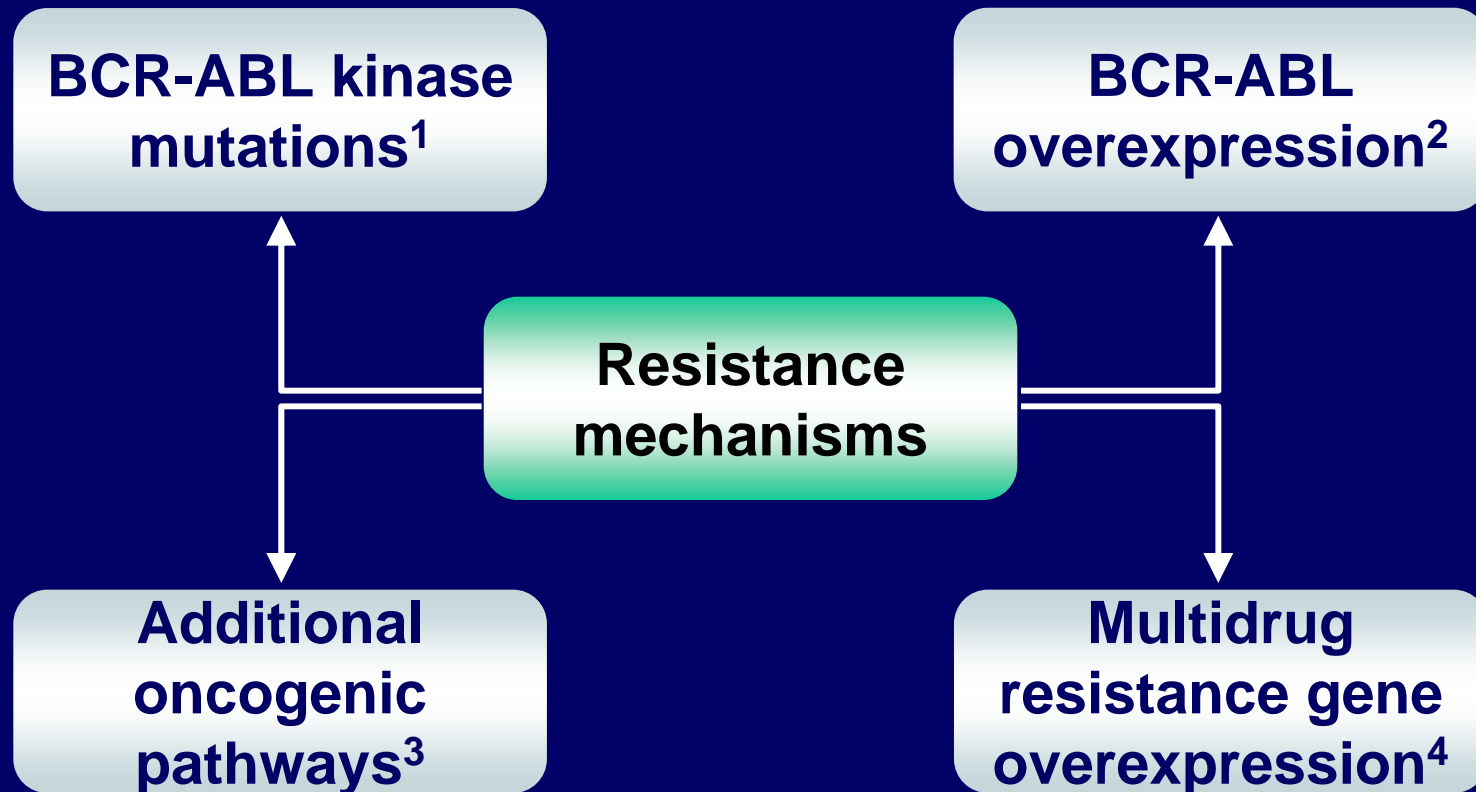
**Definition of failure and suboptimal response (Baccarani et al. submitted 2009)**

Time	Failure	Subopt Resp	Warnings
Diagnosis	-	-	High risk ACA in Ph+ cells
3 mos	No CHR	No CgR	
6 mos	< CHR No CgR	< PCgR	
12 mos	< PCgR	< CCgR	< MMoIR
18 mos	< CCgR	< MMoIR	
Anytime	ACA in Ph+ cells Loss of CHR Loss of CCgR Mutation (IM-insensit.)	Loss of MMoIR Mutation (IM-sensit.)	Any ↑ BCR-ABL transcript level OCA in Ph- cells

# Proposed management of CML depending on response

<b>Response</b>	<b>Management</b>
<b>Failure</b>	<b>Continuing IM at current dose no longer appropriate</b> <b>Use other treatment options</b>
<b>Suboptimal</b>	<b>May still have substantial benefit from IM but long-term outcome not likely as favorable</b> <b>Eligible for other treatments</b>
<b>Warning</b>	<b>Standard dose IM may not be best choice</b> <b>Should be monitored very carefully</b>

# Mechanisms of resistance *in vitro*

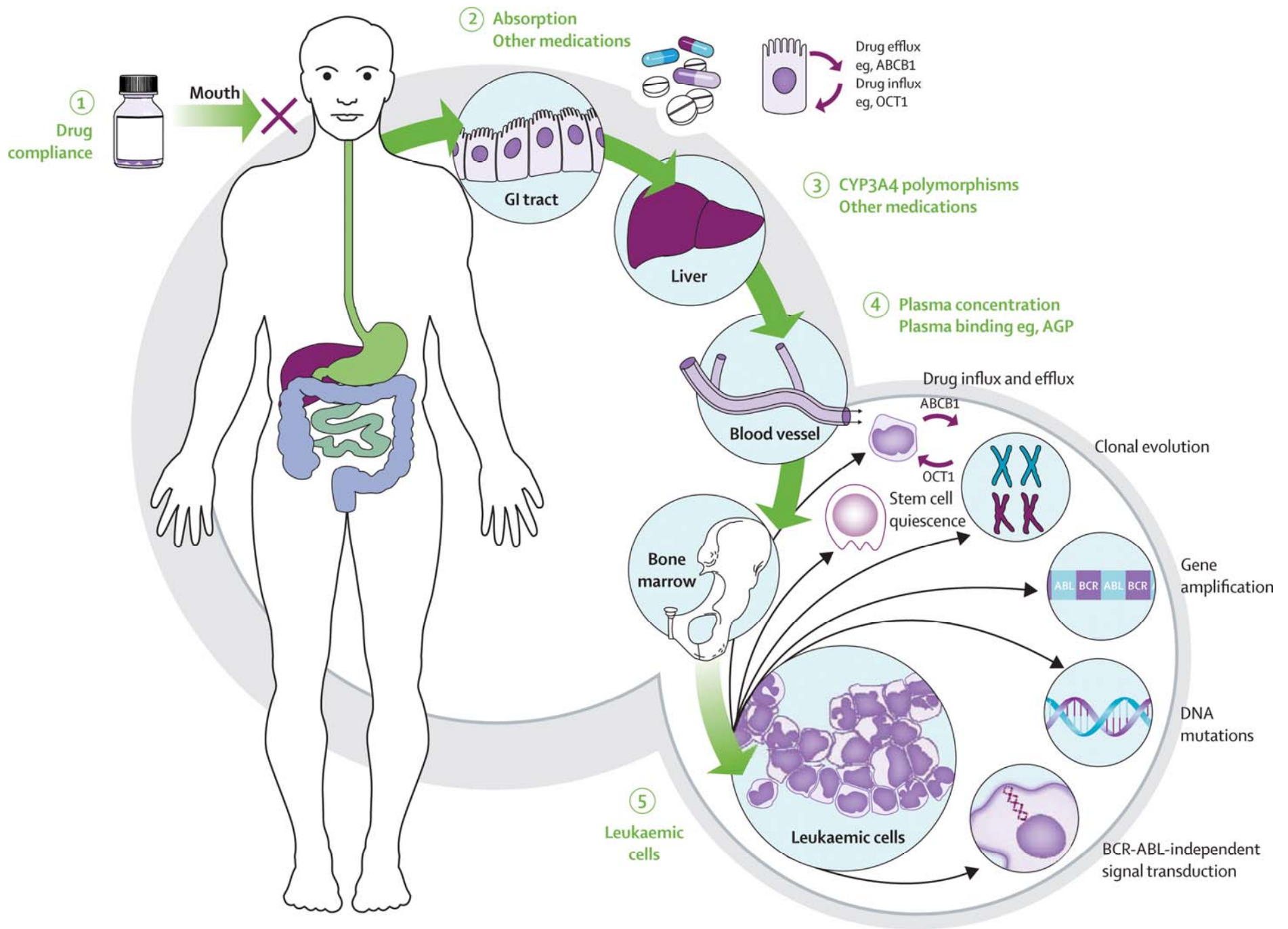


1. Gorre et al. *Science*. 2001

2. Weisberg and Griffin. *Blood*. 2000

3. Donato et al. *Blood*. 2003

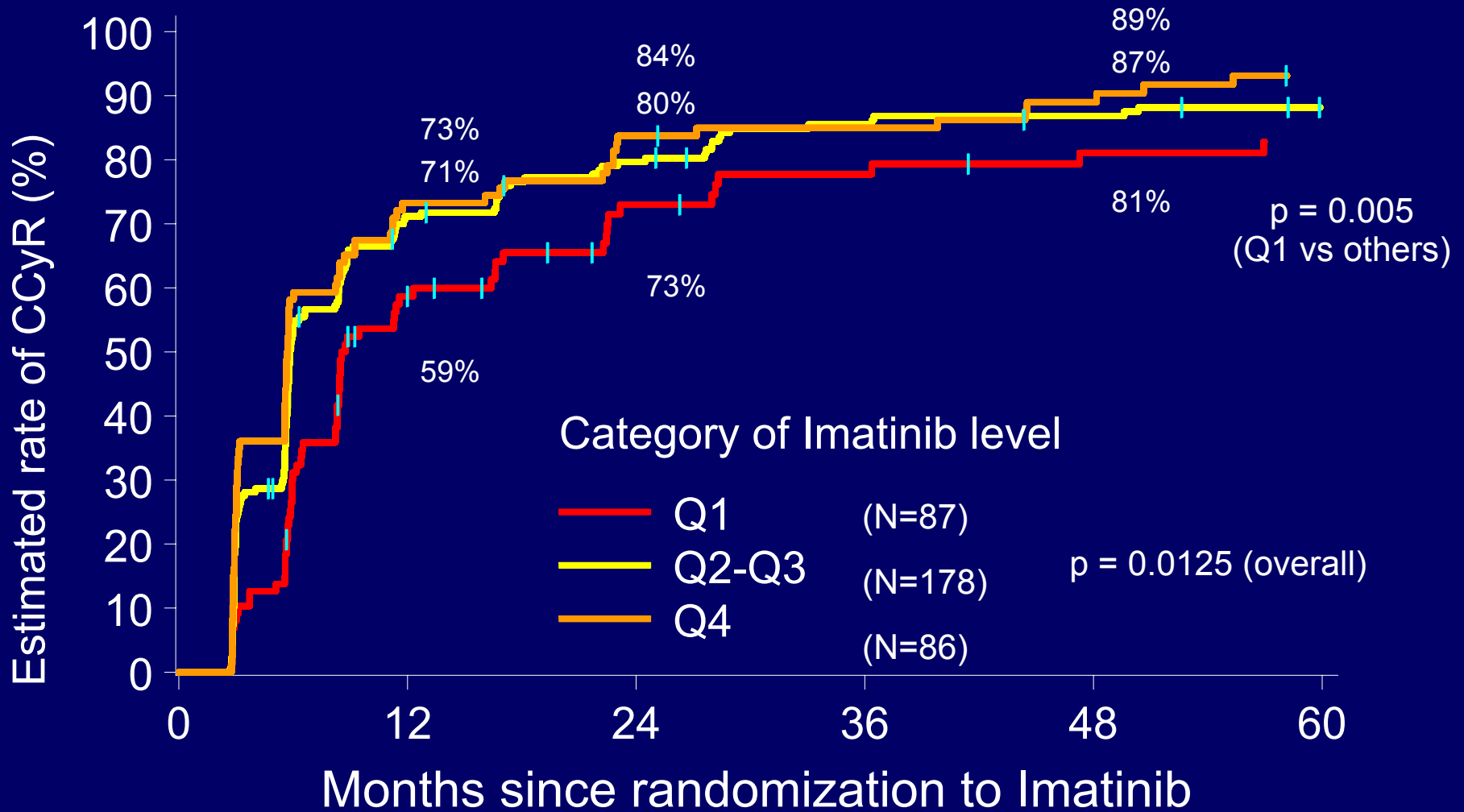
4. Mahon et al. *Blood*. 2003



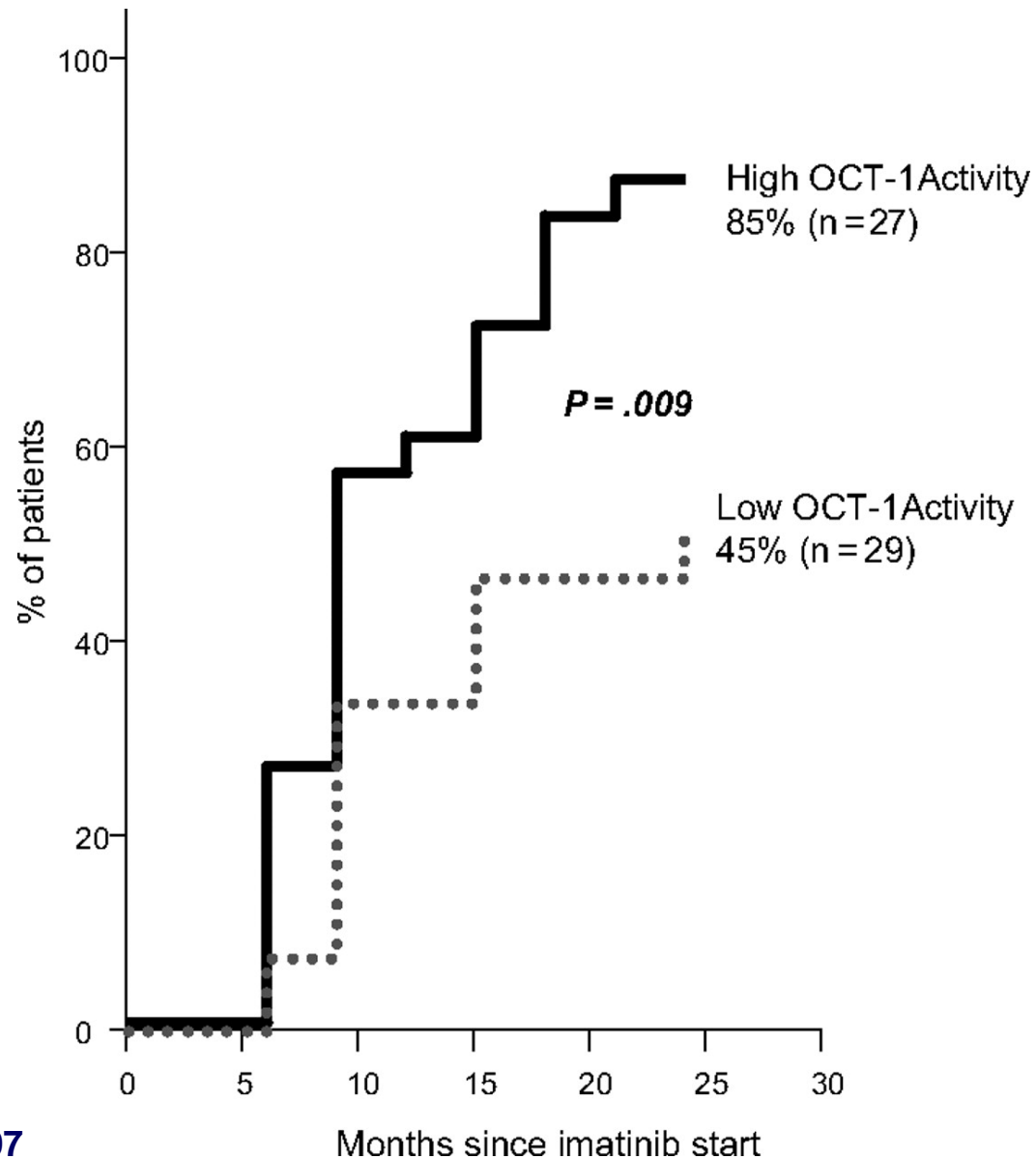
# Defined mediators of imatinib resistance

- BCR-ABL–dependent
  - Point mutations, amplification
- BCR-ABL–independent
  - Clonal evolution, aneuploidy, loss of p53
  - SRC family kinases, NF- $\kappa$ B, PTP (SHP-1)
  - Differential expression of transporter proteins (hOCT1, *MDR1*)
  - Pharmacologic barriers, CYP3A4/5 inducers (carbamacepine, dexamethasone, phenytoin, St John's Wort)

# Higher imatinib trough levels at day 29 correlates with better CyR



# High OCT-1 activity is associated with faster MMR in imatinib treated CML patients



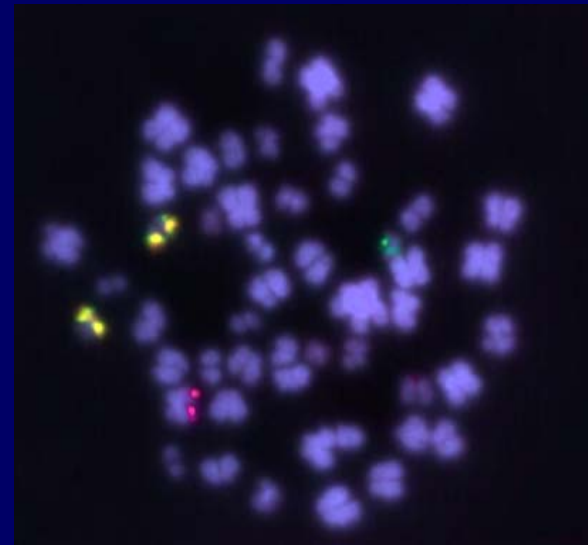
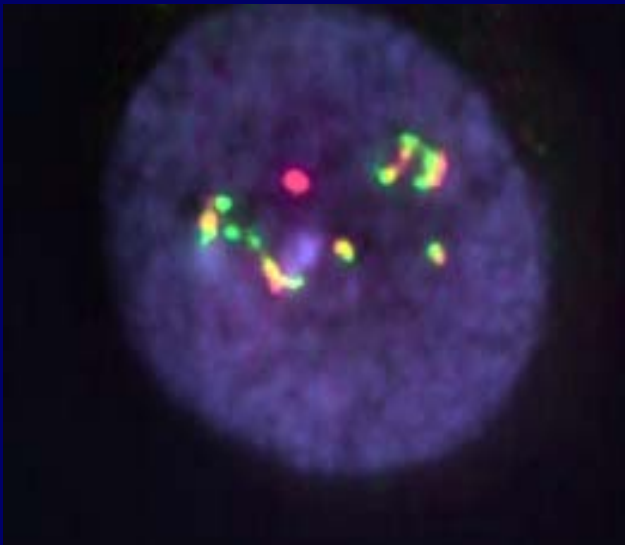
# Molecular - Cytogenetic Causes of Resistance

Patients With Hematologic Resistance/Relapse	Chronic phase (n=35)	Accelerated phase (n=33)	Blastic phase (n=66)	All (n=134)
<b><i>BCR-ABL</i> mutations (%)</b>	10/20 (50)	13/21 (62)	10/33 (30)	<b>33/74 (45)</b>
<b>Clonal evolution (%)</b>	15/29 (52)	8/16 (50)	16/22 (73)	<b>39/67 (58)</b>
<b>Combination (%)</b>	5/17 (29)	2/9 (22)	4/17 (24)	<b>11/43 (26)</b>

# Genomic BCR-ABL amplification

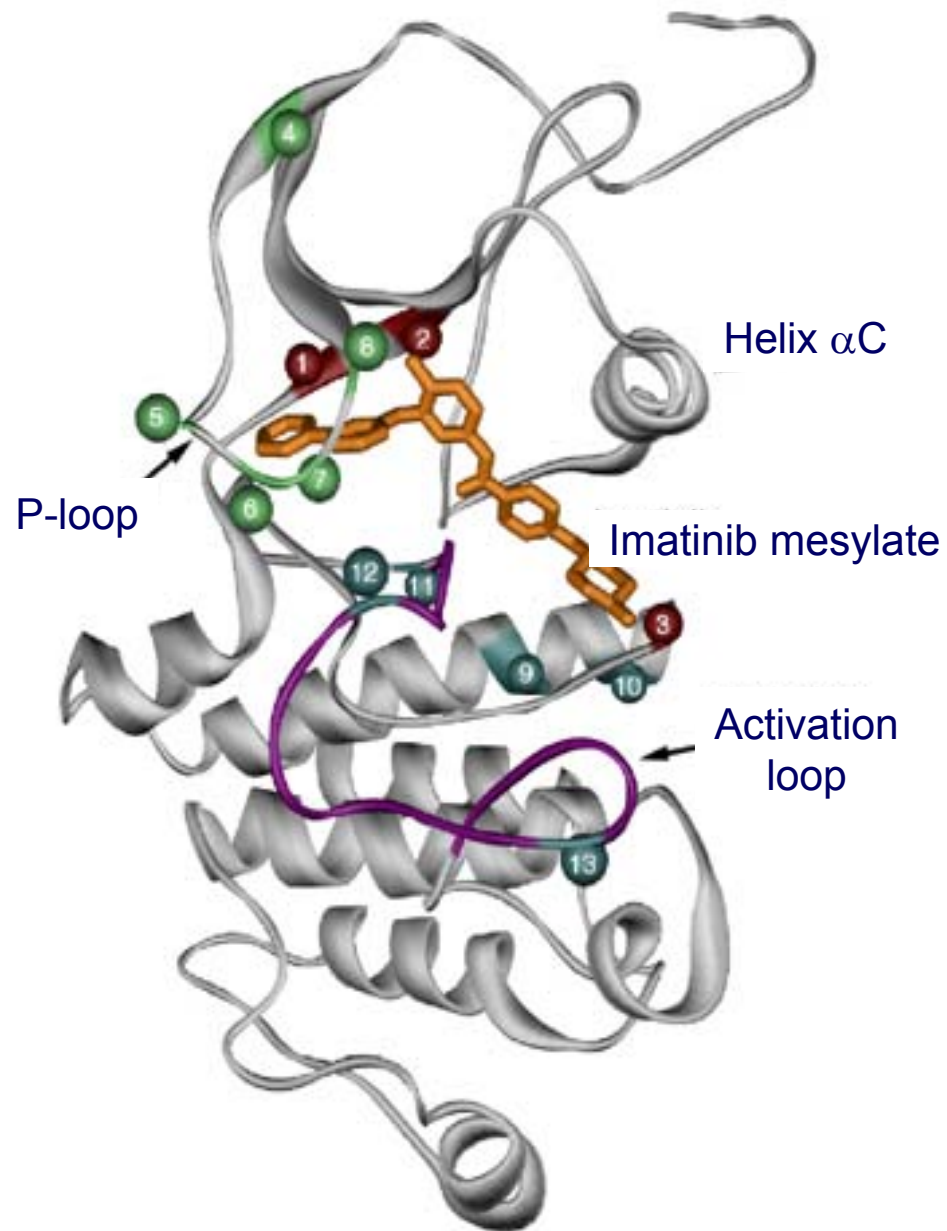
## Fluorescence *in situ* hybridization

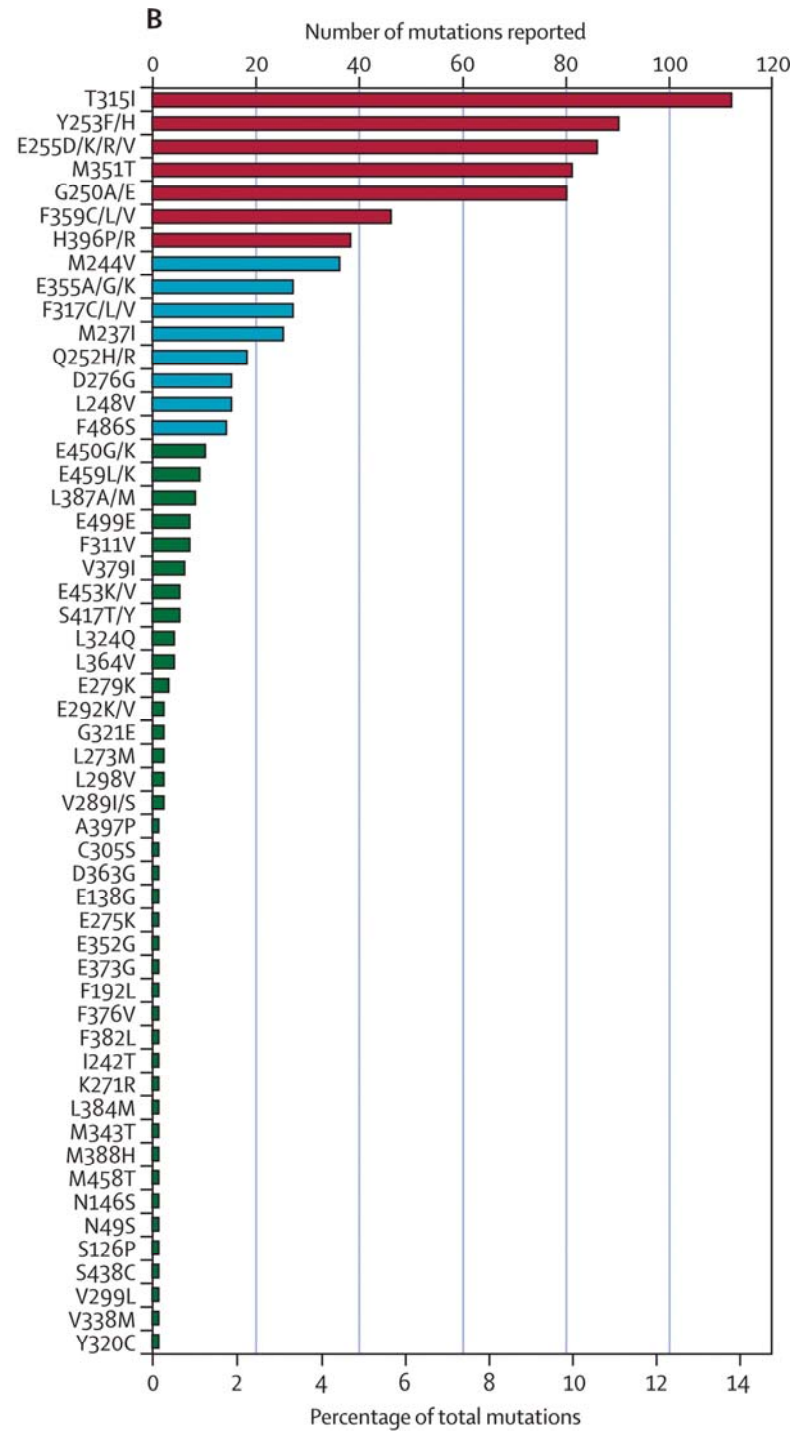
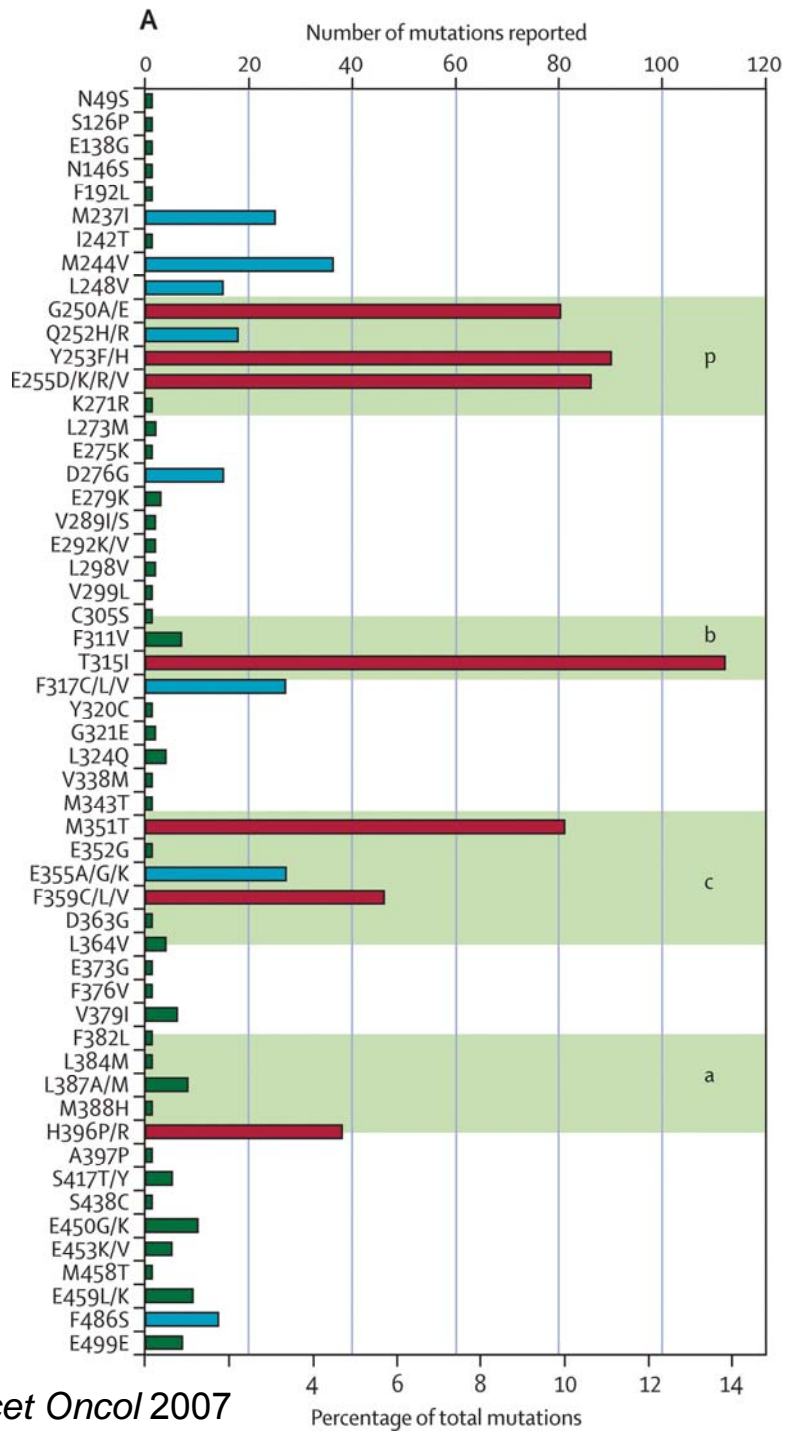
32 sample pairs (pre-therapy / resistance)



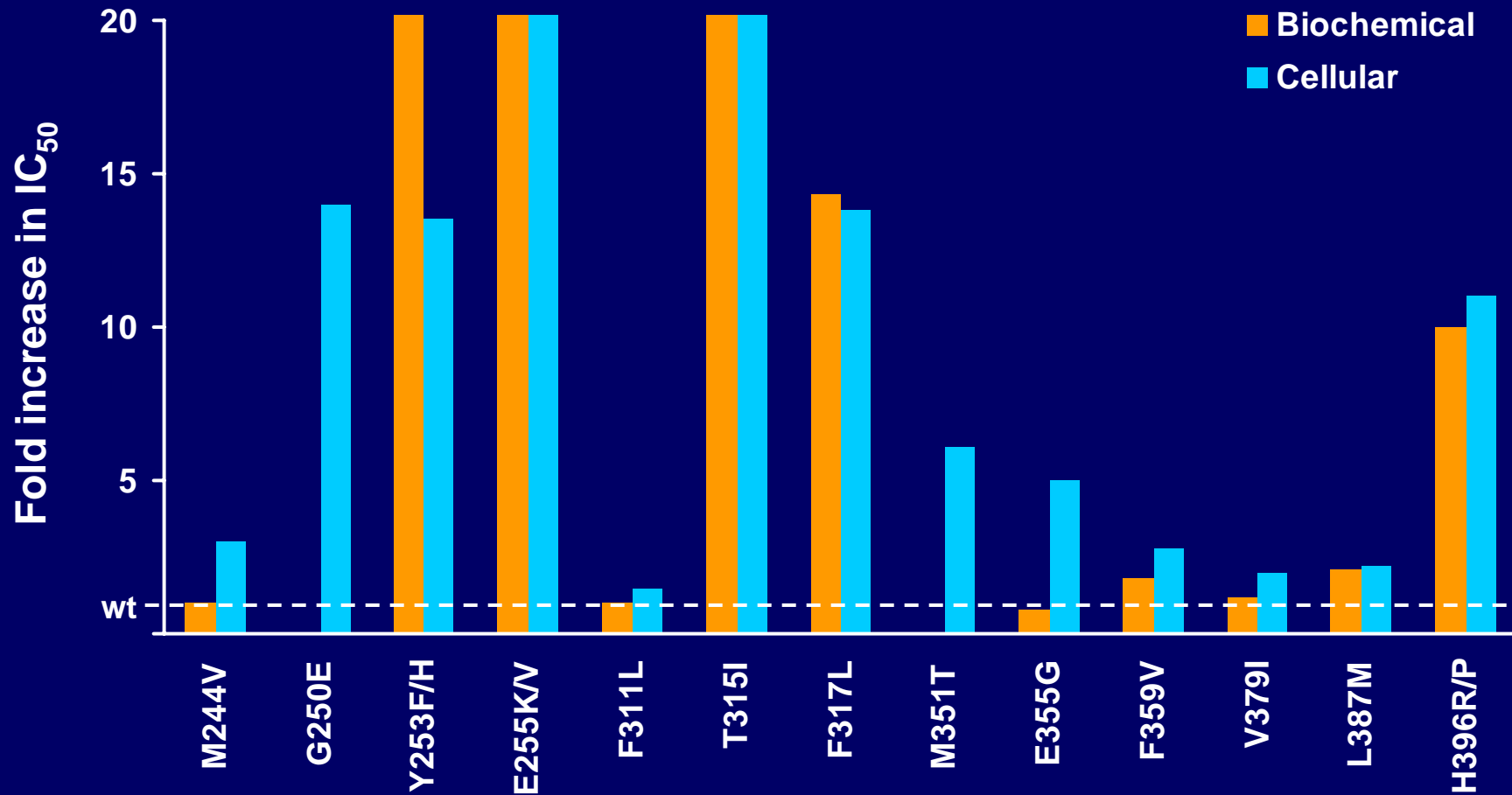
2/32 ( 6%) multiple BCR-ABL copies

7/32 (22%) 2 BCR-ABL copies





# Mutant *BCR-ABL* Have Increased $IC_{50}$ Values for Imatinib



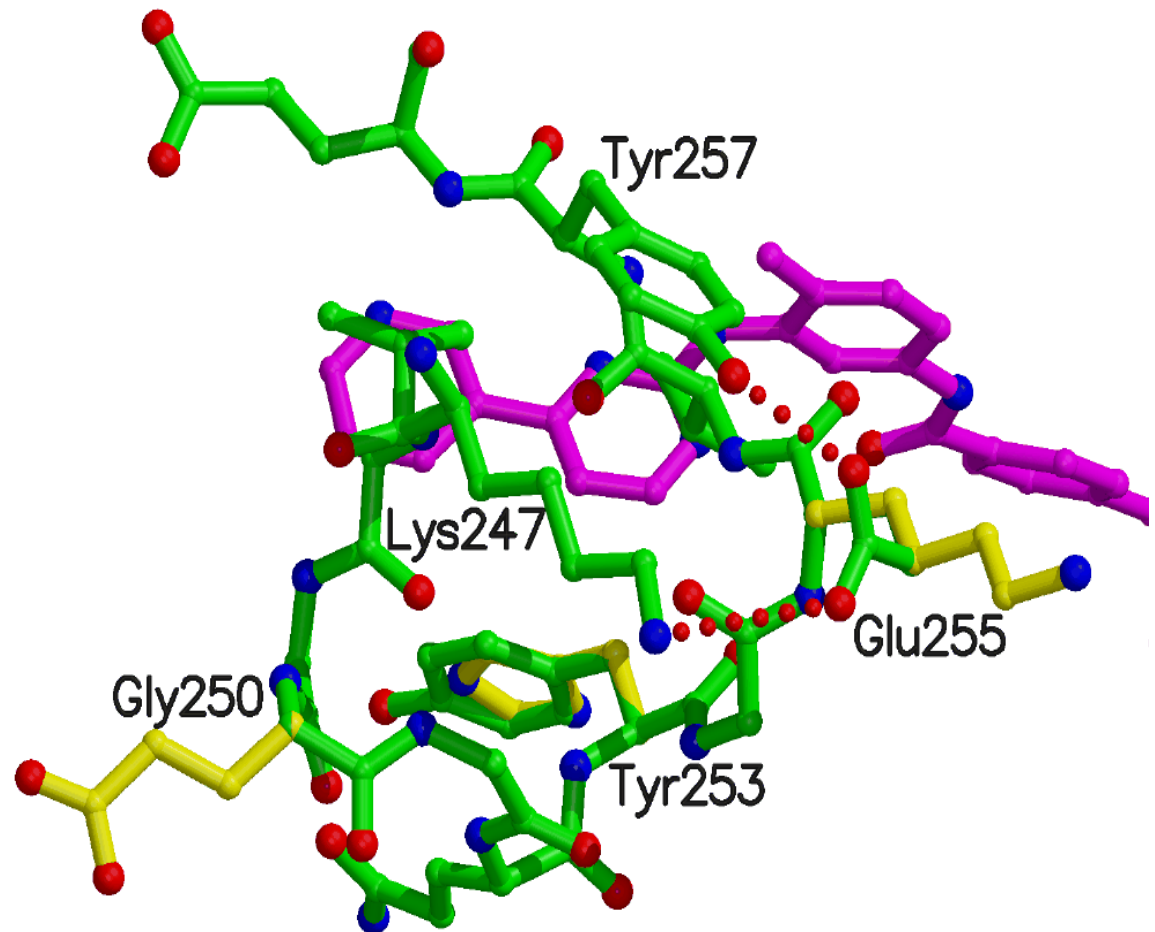
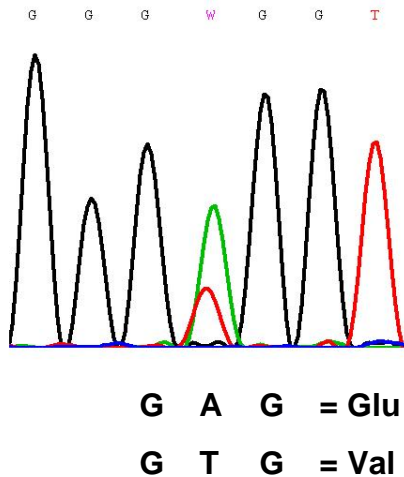
$IC_{50}$  = 50% inhibitory concentration.

Corbin et al. *Blood*. 2003;101:4611.

# E255L/V mutation

Glu255Lys/Val (IC<sub>50</sub> 6.7 μM):

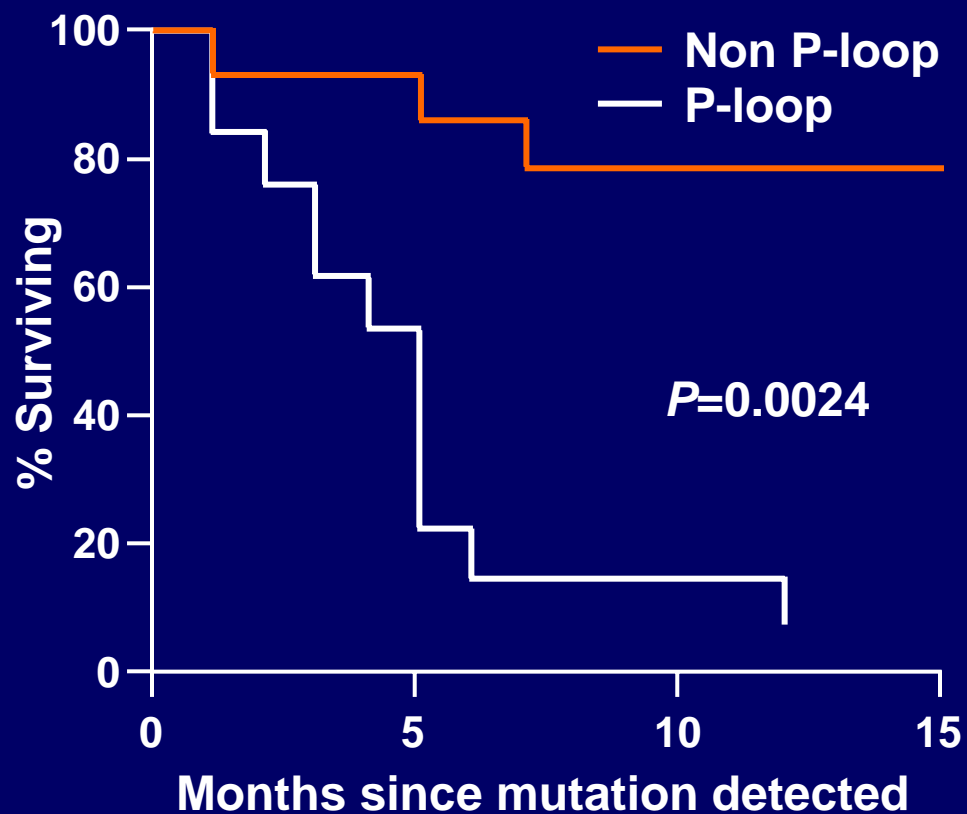
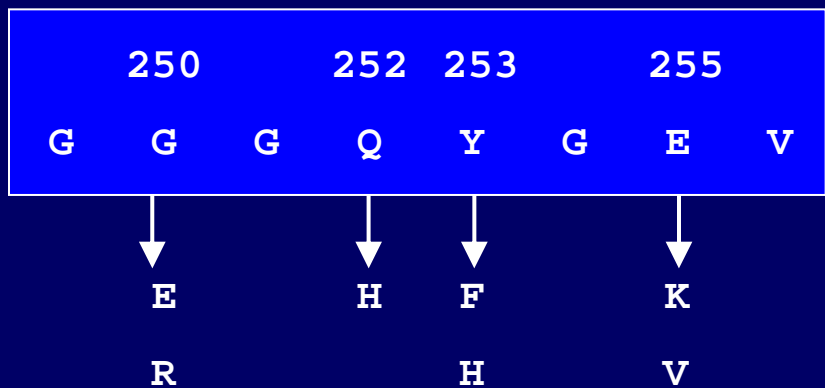
- Loss of H bonds to Tyr257 & Lys247



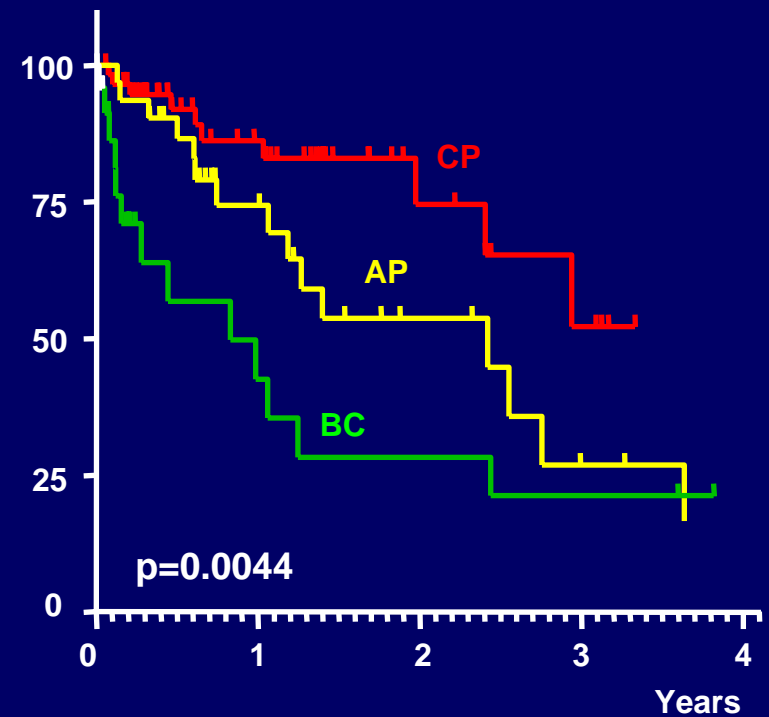
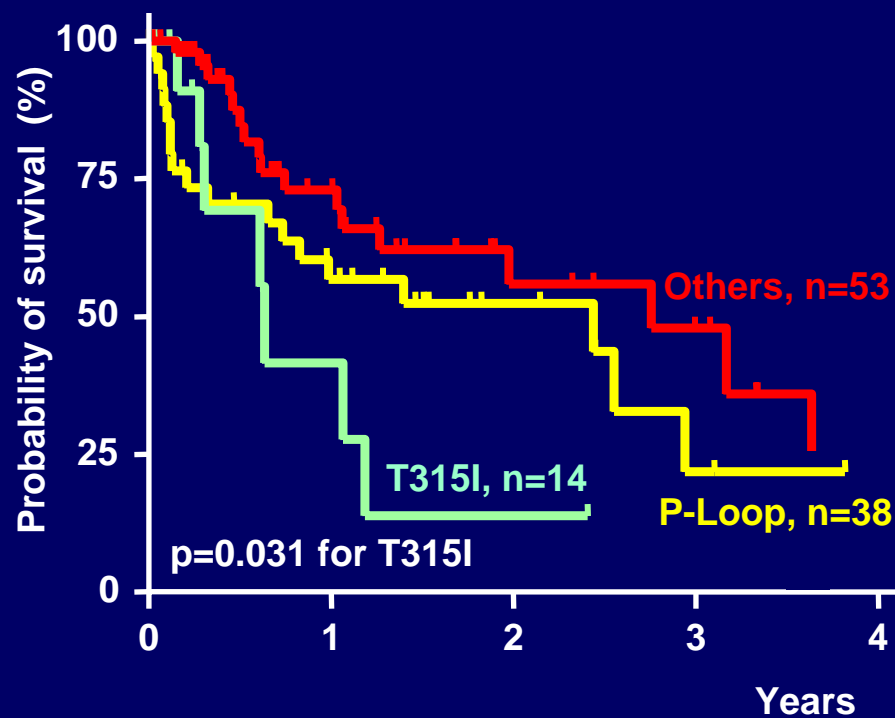
# Survival After Imatinib Resistance

## Initial Australian experience, n=27

P-Loop: Highly conserved area

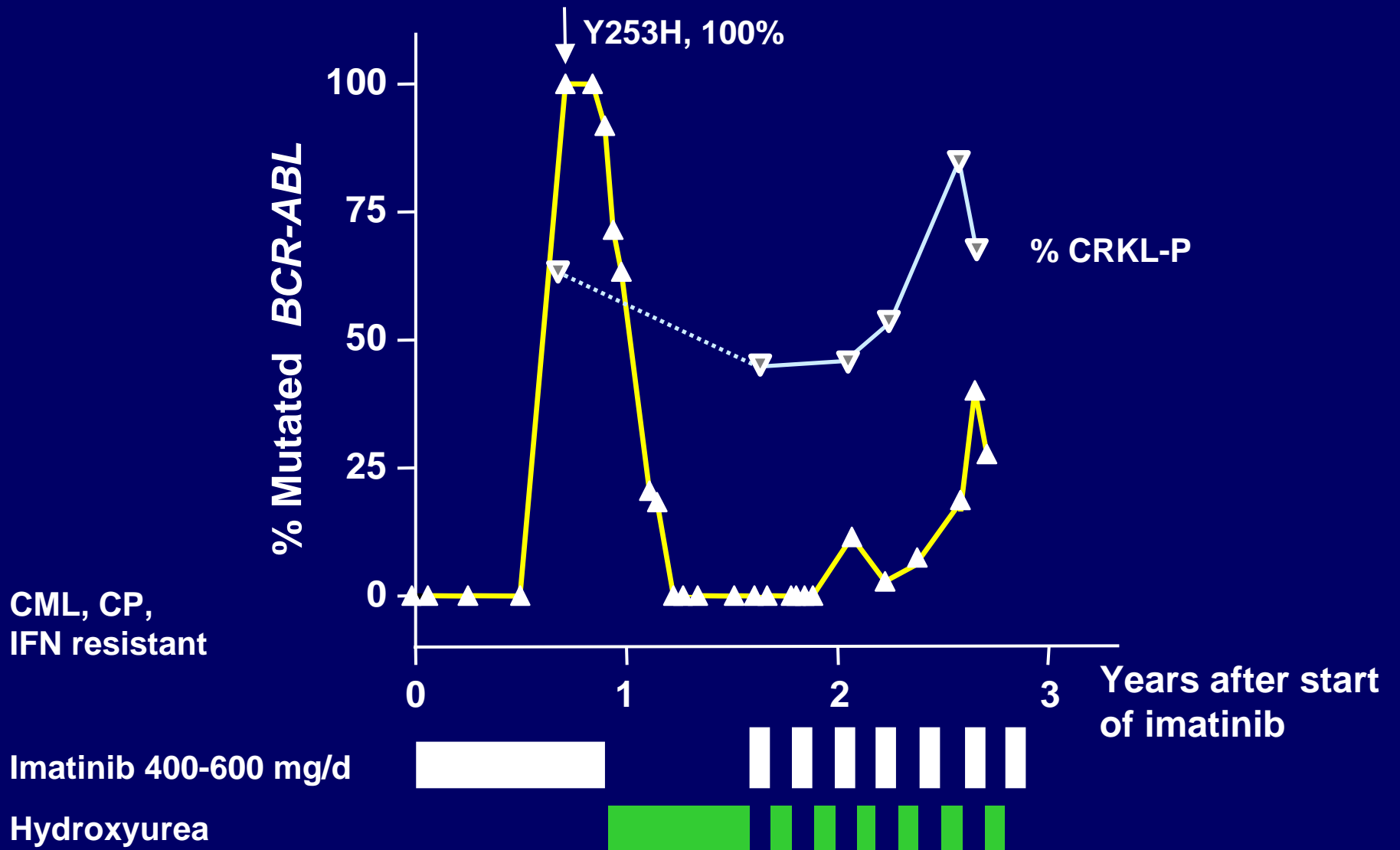


# Outcome after Imatinib resistance associated with BCR-ABL mutations, n=105

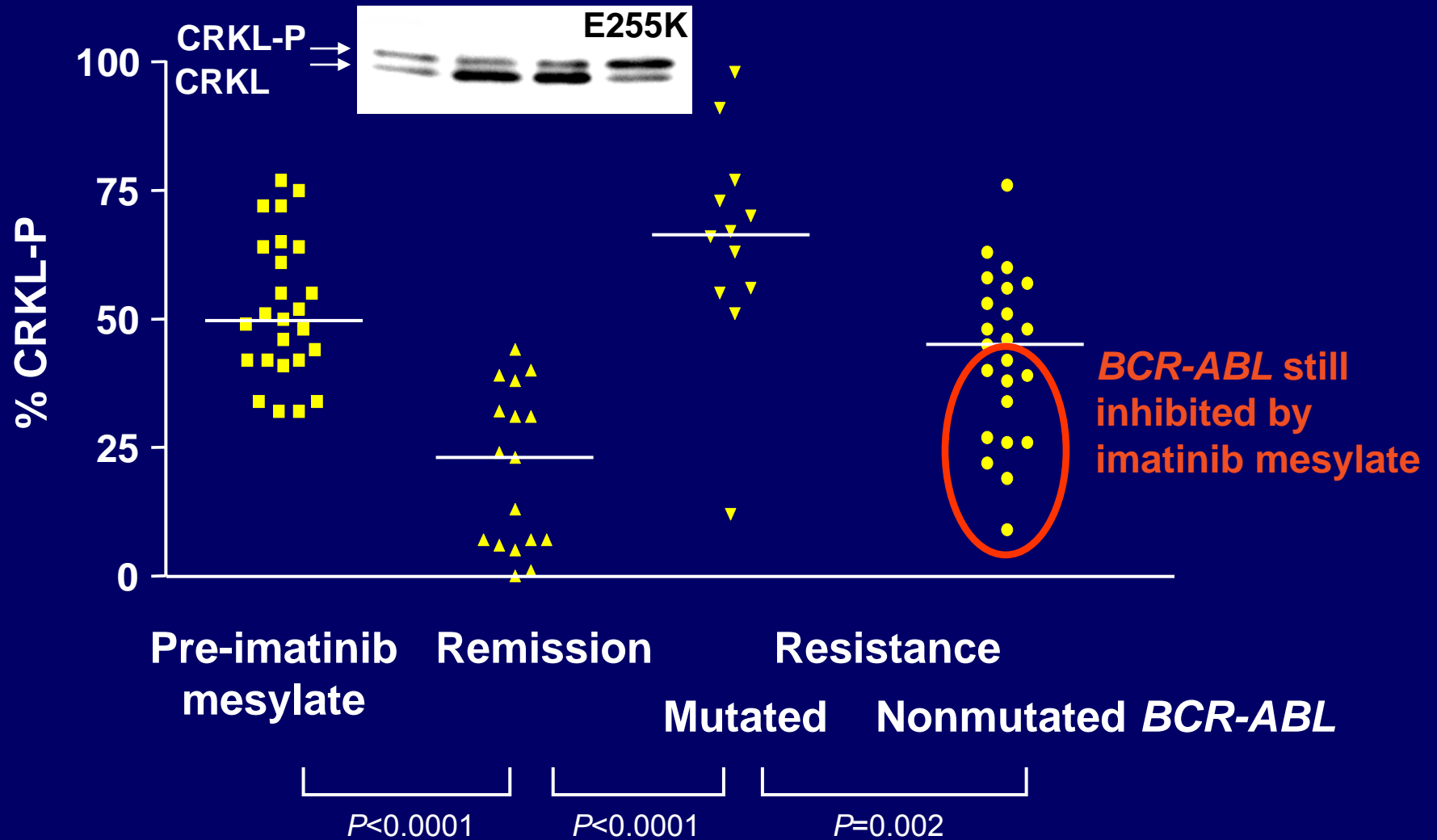


Censored @ SCT, dasatinib, or nilotinib.

# Dynamics of resistance clones

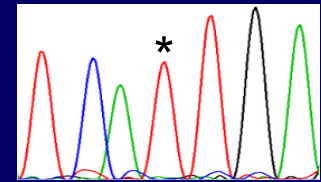


# CRKL-P in Resistant Patients

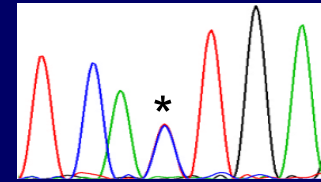
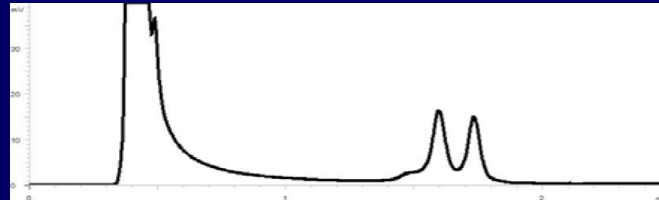


**mutant BaF3<sup>T315I</sup>**

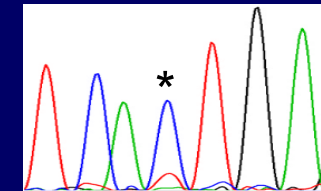
**100%**



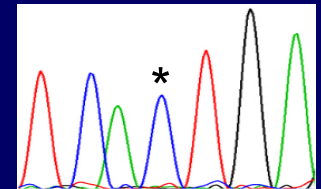
**50%**



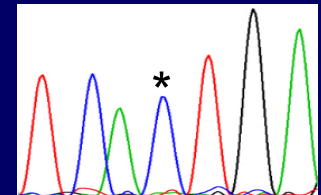
**10%**



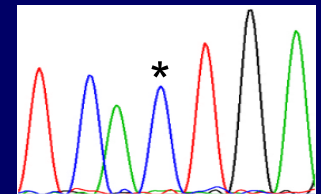
**1%**



**0.1%**



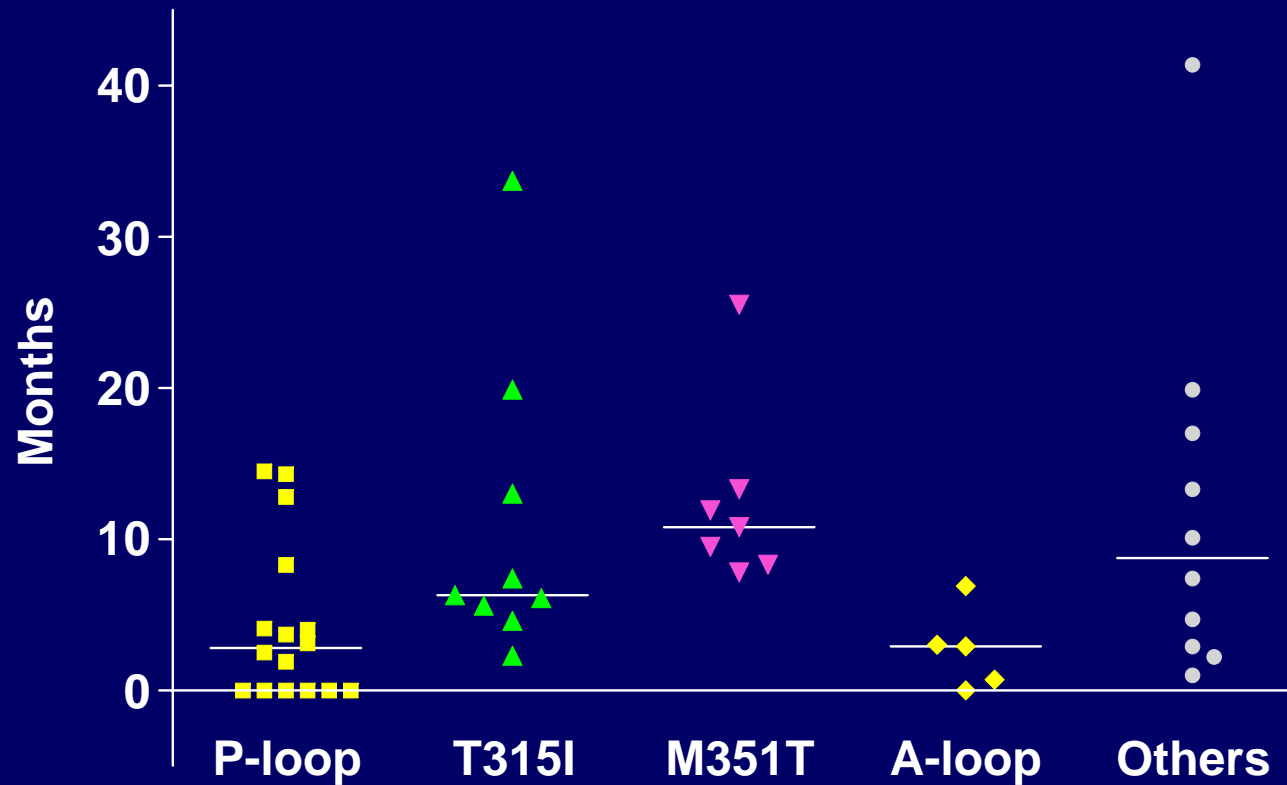
**normal BaF3<sup>BCR-ABL</sup>**



**Sensitivity of  
D-HPLC**

**0.1-1%**

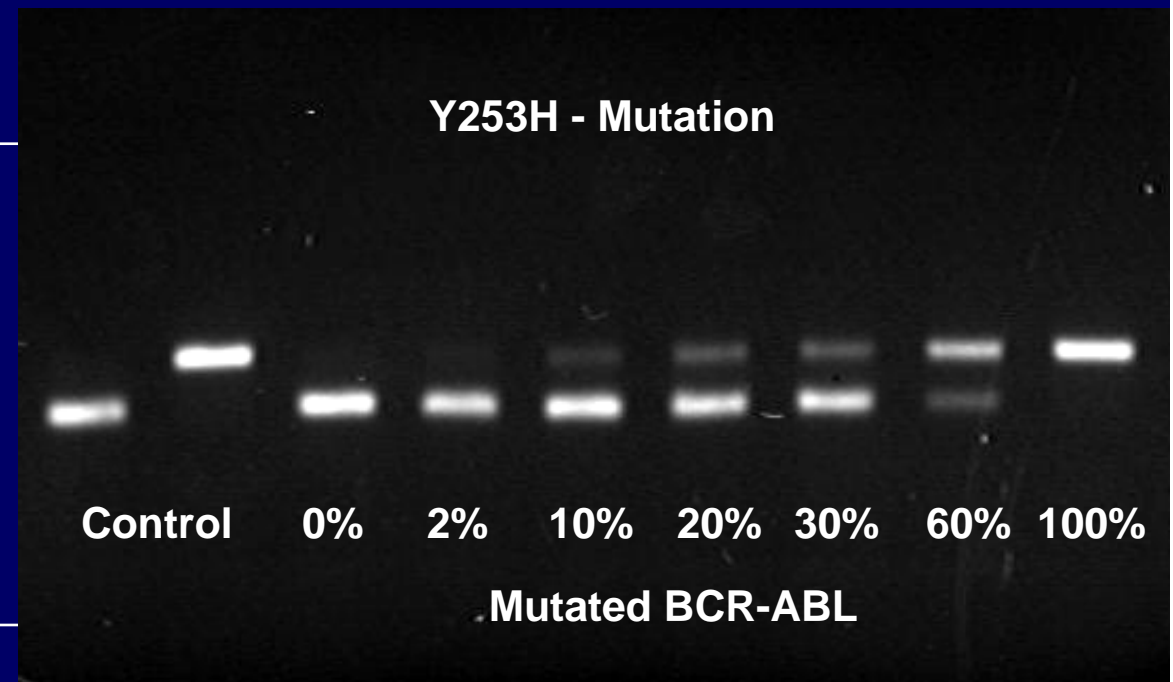
# Interval-Positive D-HPLC: Hematologic Relapse



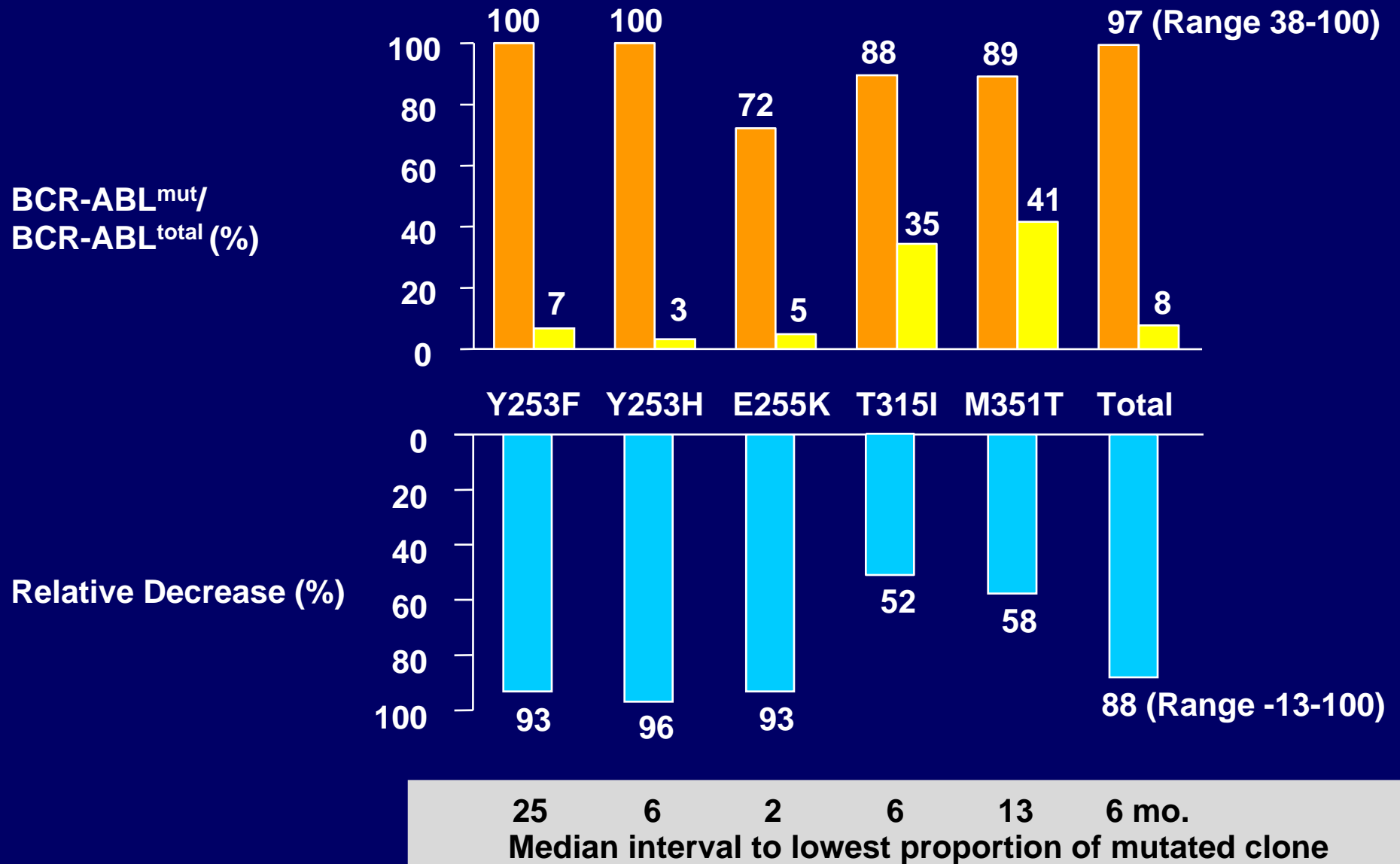
**Median (mo):**    2.8            6.3            10.8            2.9            8.7

# Mutation specific restriction to estimate proportion of mutated BCR-ABL

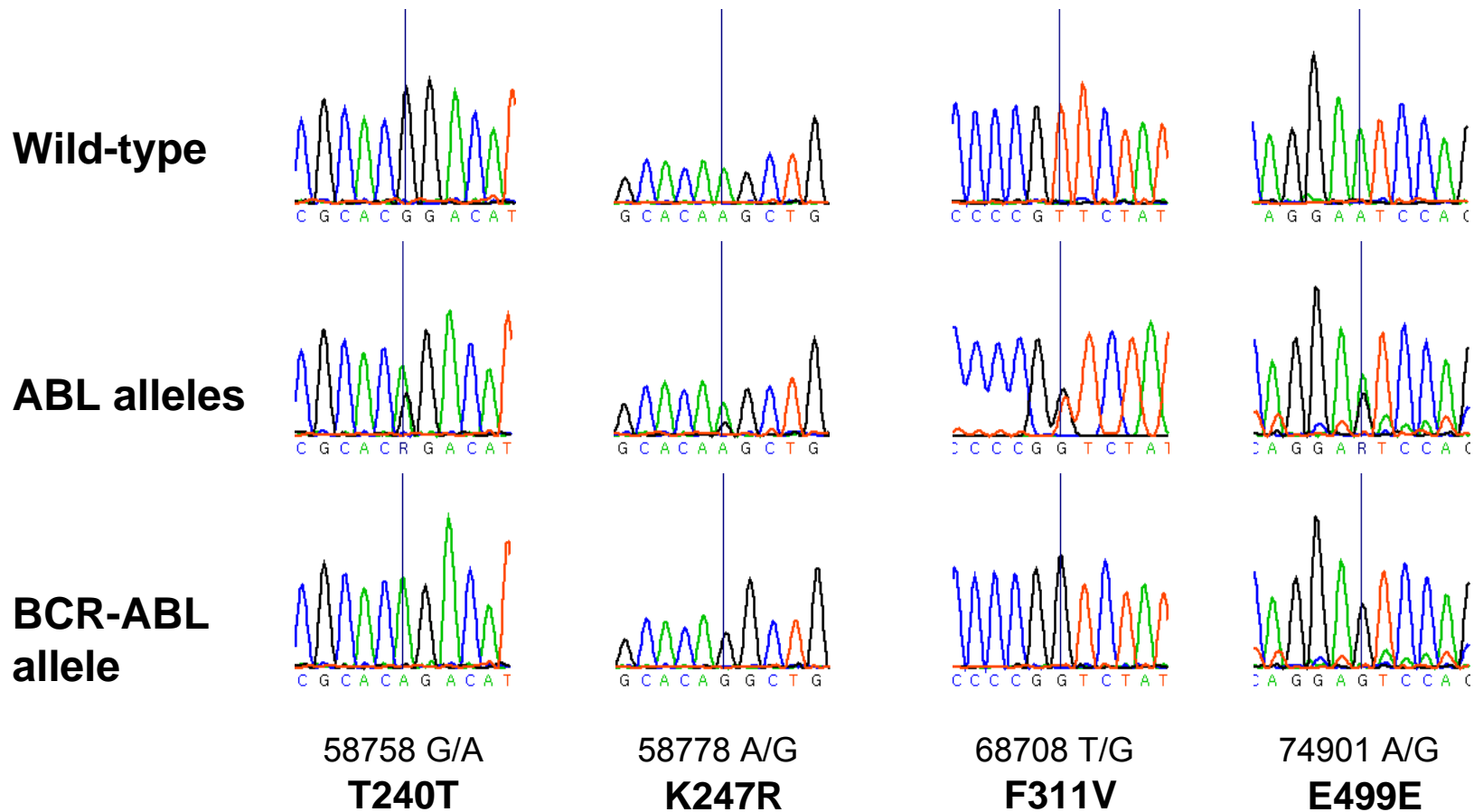
AA	Enzyme
M244	<i>NlaIII</i>
Y253	<i>RsaI</i>
E255	<i>MnI</i>
T315	<i>DdeI</i>
M351	<i>NcoI</i>



# Deselection of mutated clones after TKI discontinuation (n=20; CP n=12, AP n=4, BC n=4)



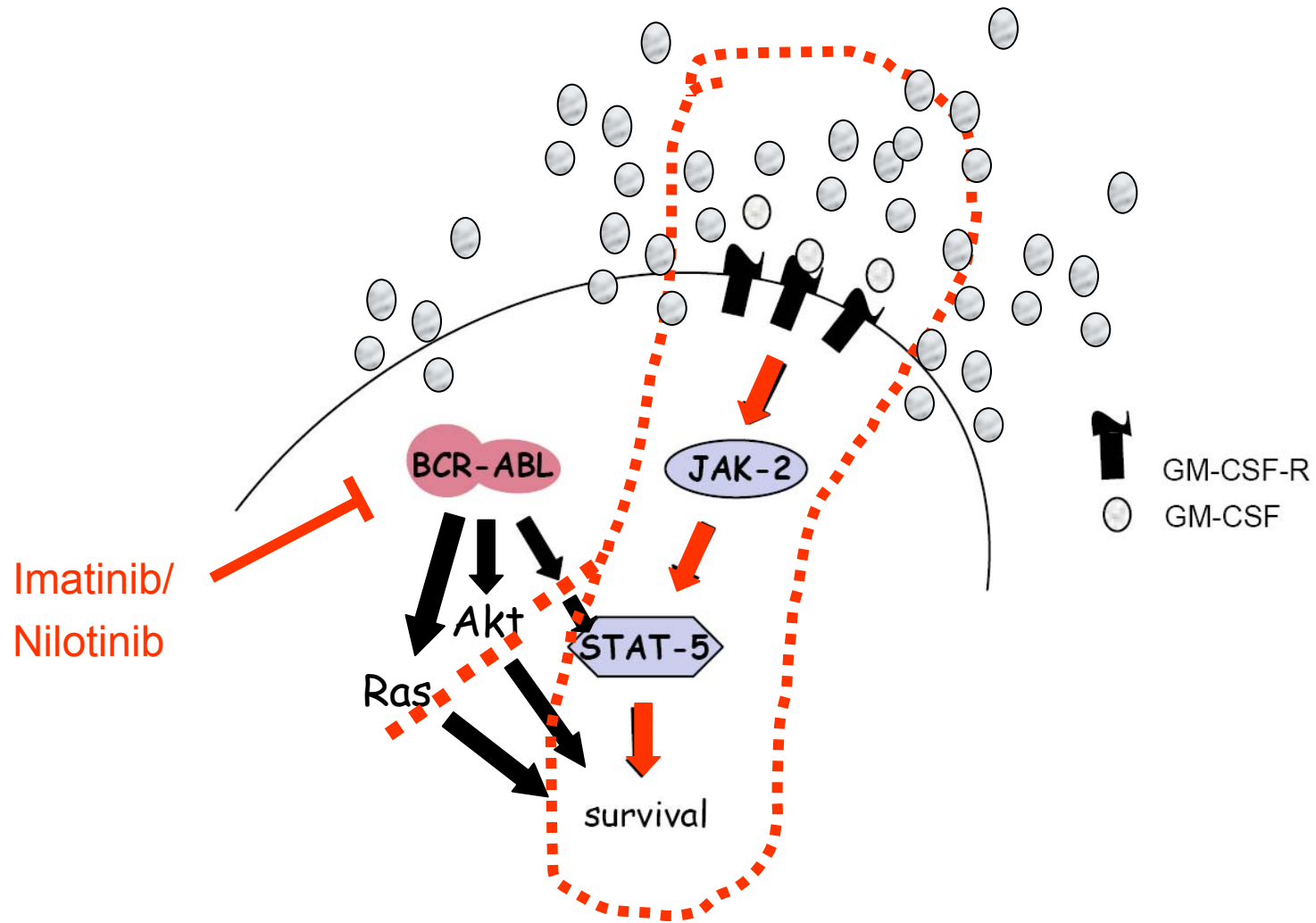
# Not all “mutations“ are related to resistance!



## Allele frequencies of polymorphisms within the BCR-ABL kinase domain in n=911 analyzed CML patients

Nucleotide position	Nucleotide polymorphism	Amino acid change	N	Allele frequency (%)
58758	A	T240T	1	0.1
58778	G	<b>K247R</b>	9	1.0
68708	G	F311V	2	0.2
68722	G	T315T	1	0.1
68736	G	Y320C	1	0.1
74901	G	E499E	73	8.0

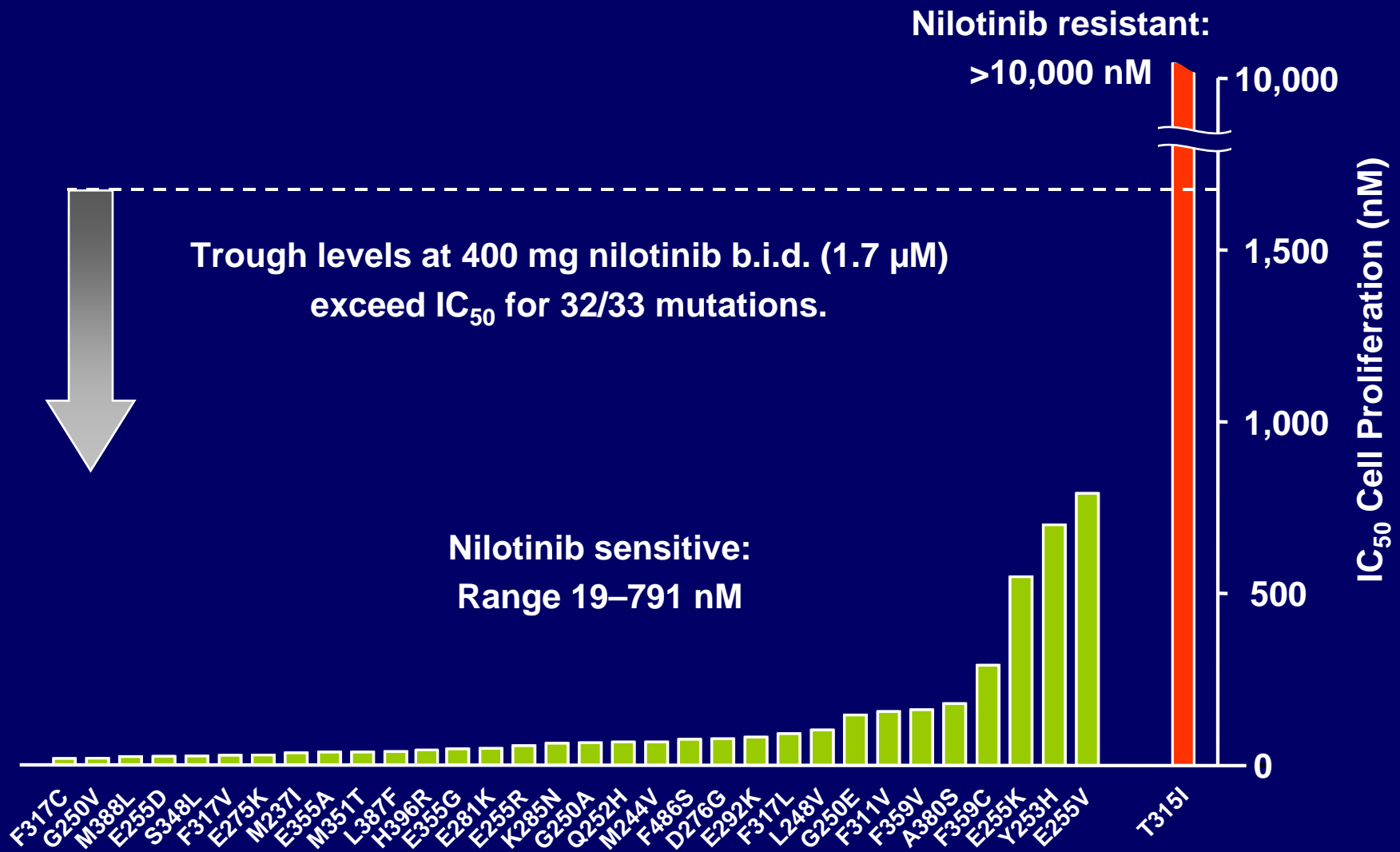
# Autocrine mechanism of Imatinib resistance via GM-CSF



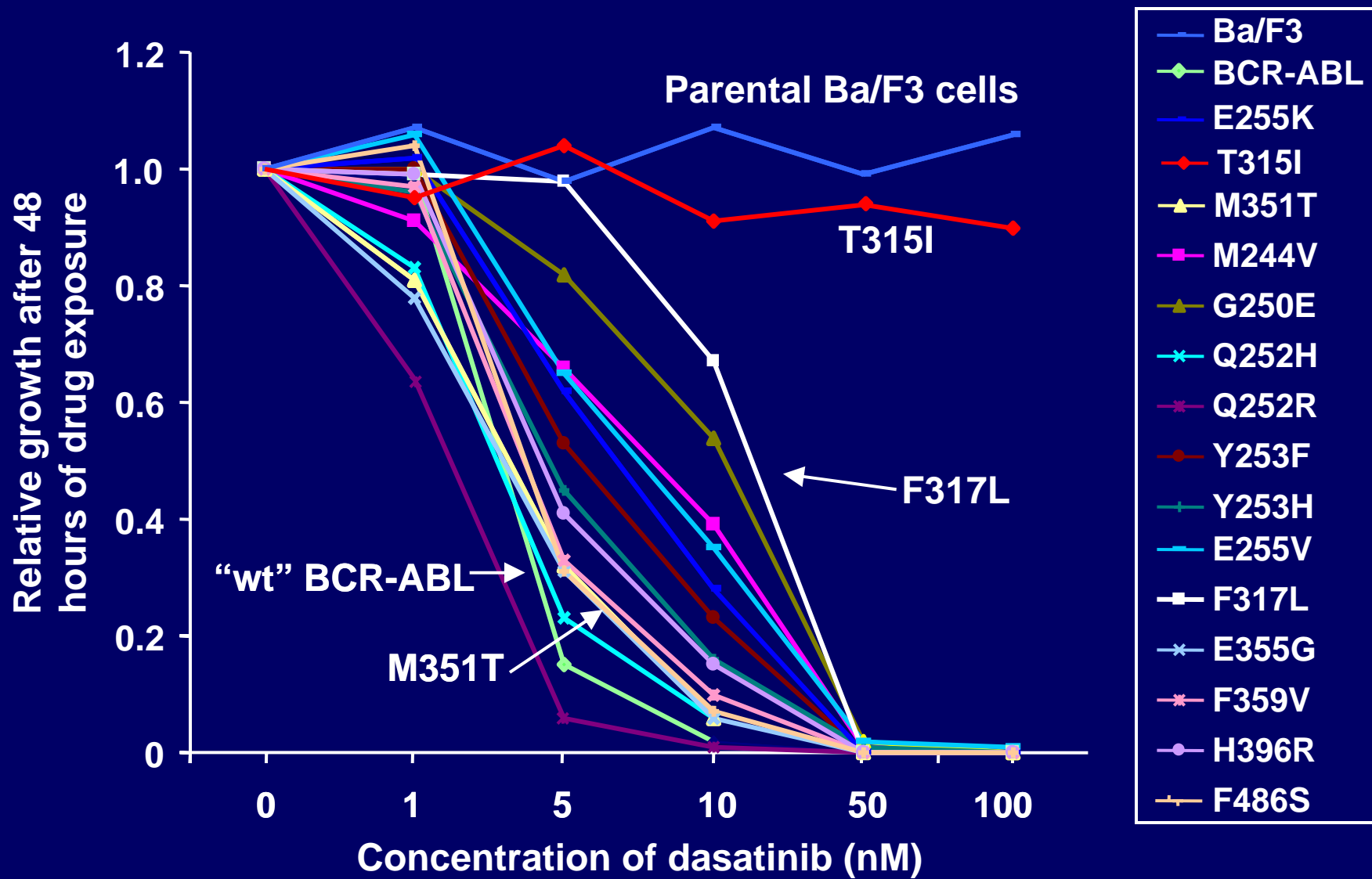
# Comparison of ABL TK Inhibitors

	<b>Imatinib</b>	<b>Dasatinib (BMS)</b>	<b>Bosutinib (SKI)</b>	<b>Nilotinib (AMN)</b>	<b>INNO-406 (NS-187)</b>
ABL	X 1	X 300	X 30	X 20	X 55
active ABL	(-)	(+++)	(+++)	(-)	(+)
inactive ABL	(+)	(+++)	(+++)	(++)	(++)
PDGFR	(+)	(+++)	(-)	(+)	(+)
C-KIT	(+)	(+++)	(-)	(+)	(+/-)
SRC	(-)	(+++)	(++++)	(-)	(-)
LYN	(-)	(++)	(+++)	(-)	(+)
CNS leukemia	(-)	+?	NA	(-)	Effective
Status	Approved	Approved	Phase I/II	Approved	Phase I

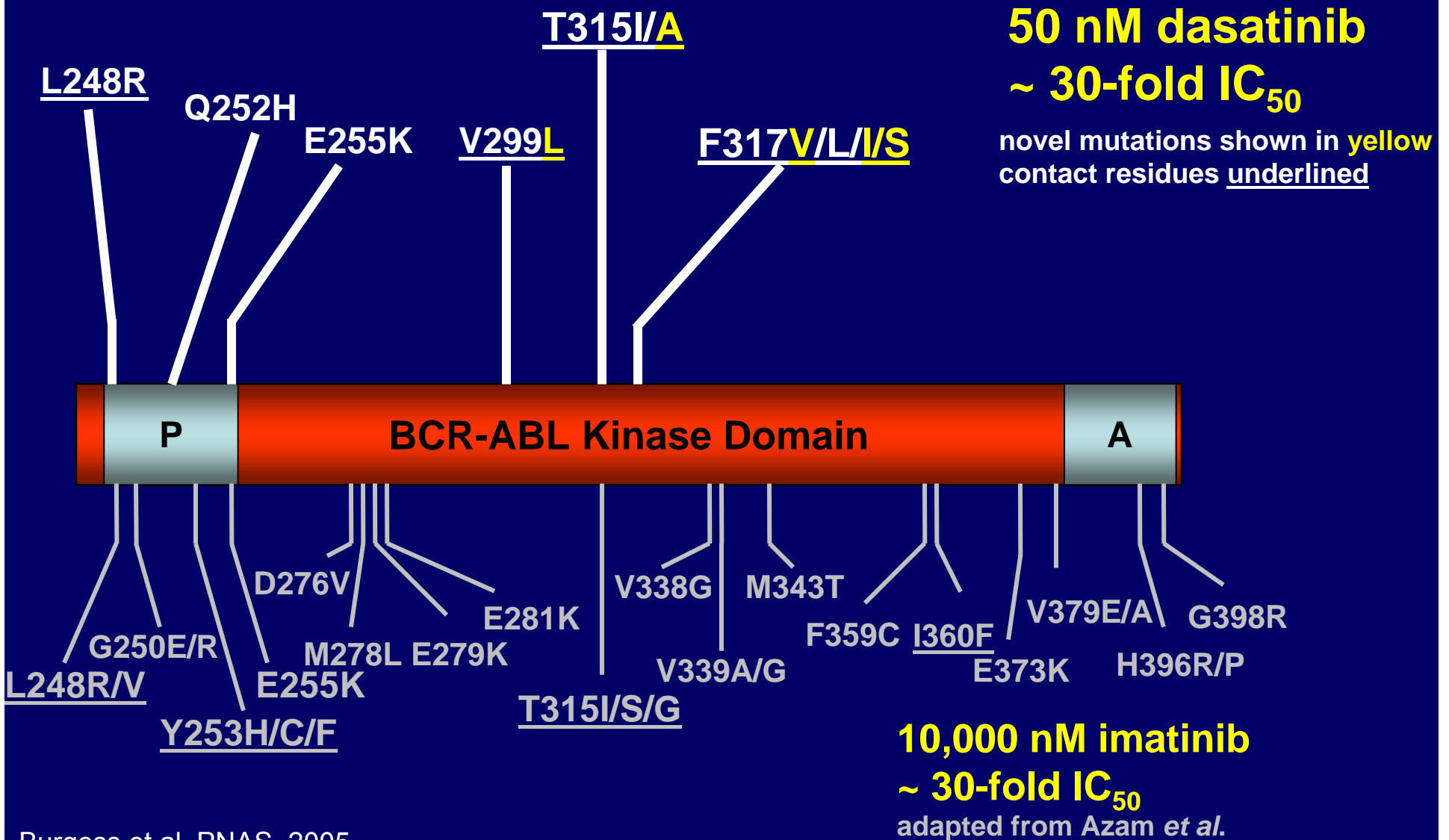
# Nilotinib Sensitivity of Cells Expressing Imatinib-Resistant BCR-ABL With Point Mutations



# Dasatinib Inhibits Growth of 14/15 Imatinib-resistant BCR-ABL-expressing Ba/F3 Cell Lines In Vitro



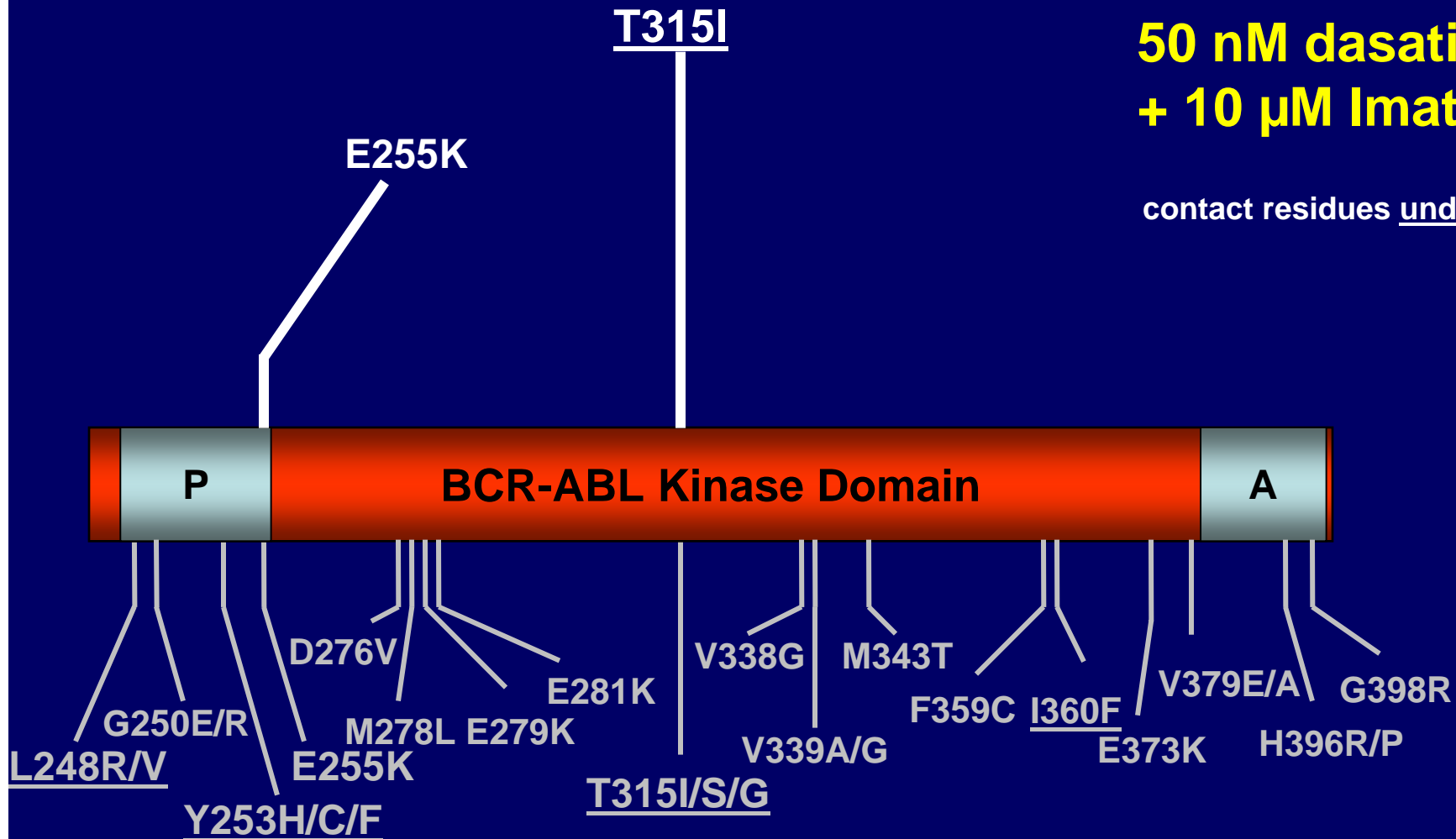
# Resistance to dasatinib *in vitro* is primarily caused by BCR-ABL mutations at contact residues



# Combination of imatinib and dasatinib further reduces the range of resistant clones

50 nM dasatinib  
+ 10  $\mu$ M Imatinib

contact residues underlined



10,000 nM imatinib

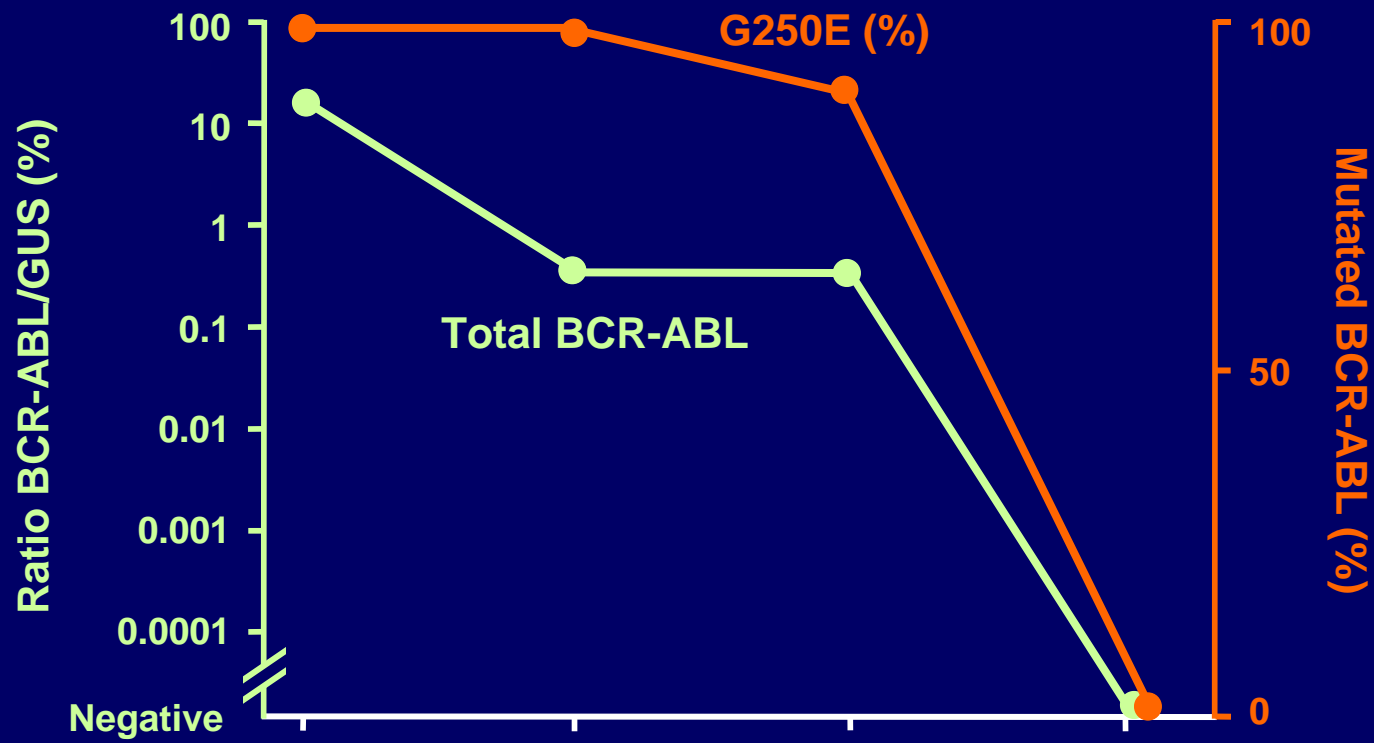
~ 30-fold IC<sub>50</sub>

adapted from Azam *et al.*, Cell 112:831-843

# Course of mutated and unmutated BCR-ABL – 3 patterns –

Pattern 1:

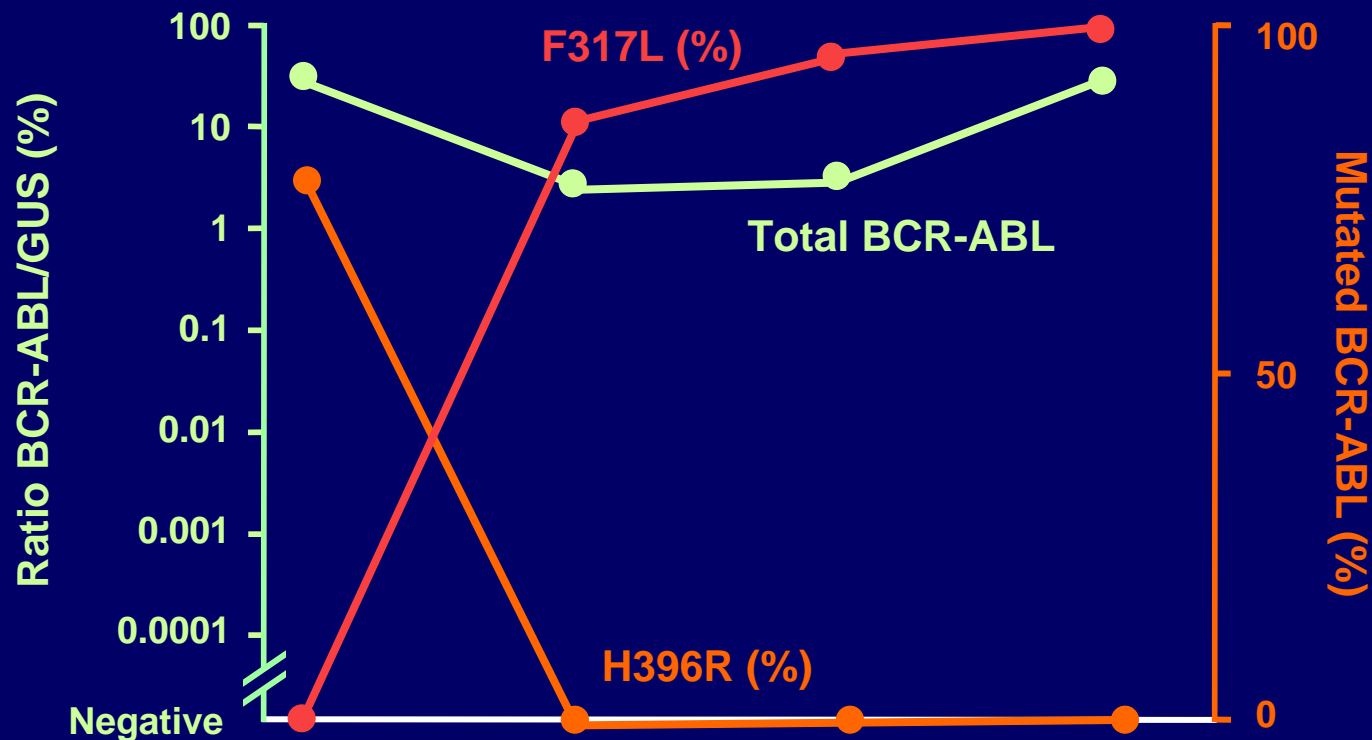
⇒ Baseline mutation disappeared **AND** BCR-ABL decreased



# Course of mutated and unmutated BCR-ABL – 3 patterns –

## Pattern 2:

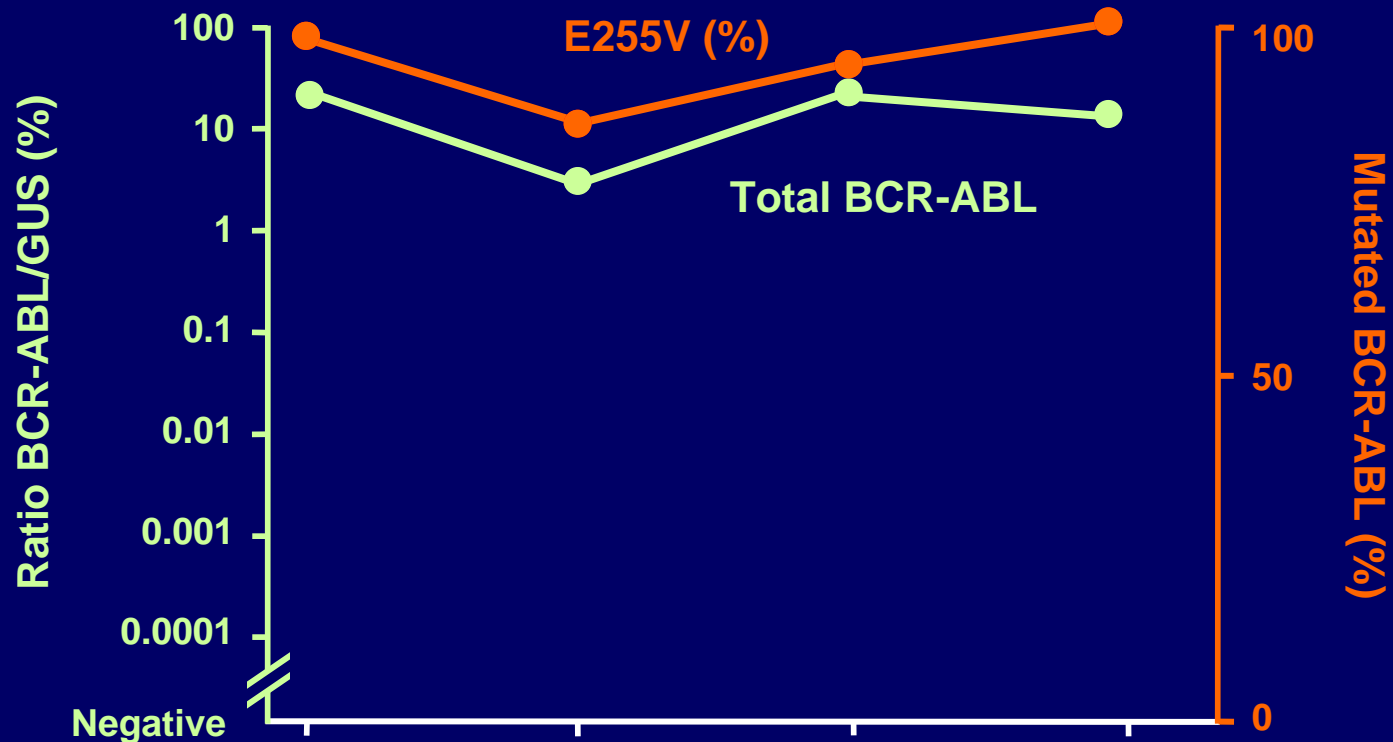
- ⇒ Baseline mutation disappeared **AND** BCR-ABL persisted
  - new mutations evolved in n=12 patients  
(T315I n=3; F317L n=7; M351T n=2; Y320C n=1)



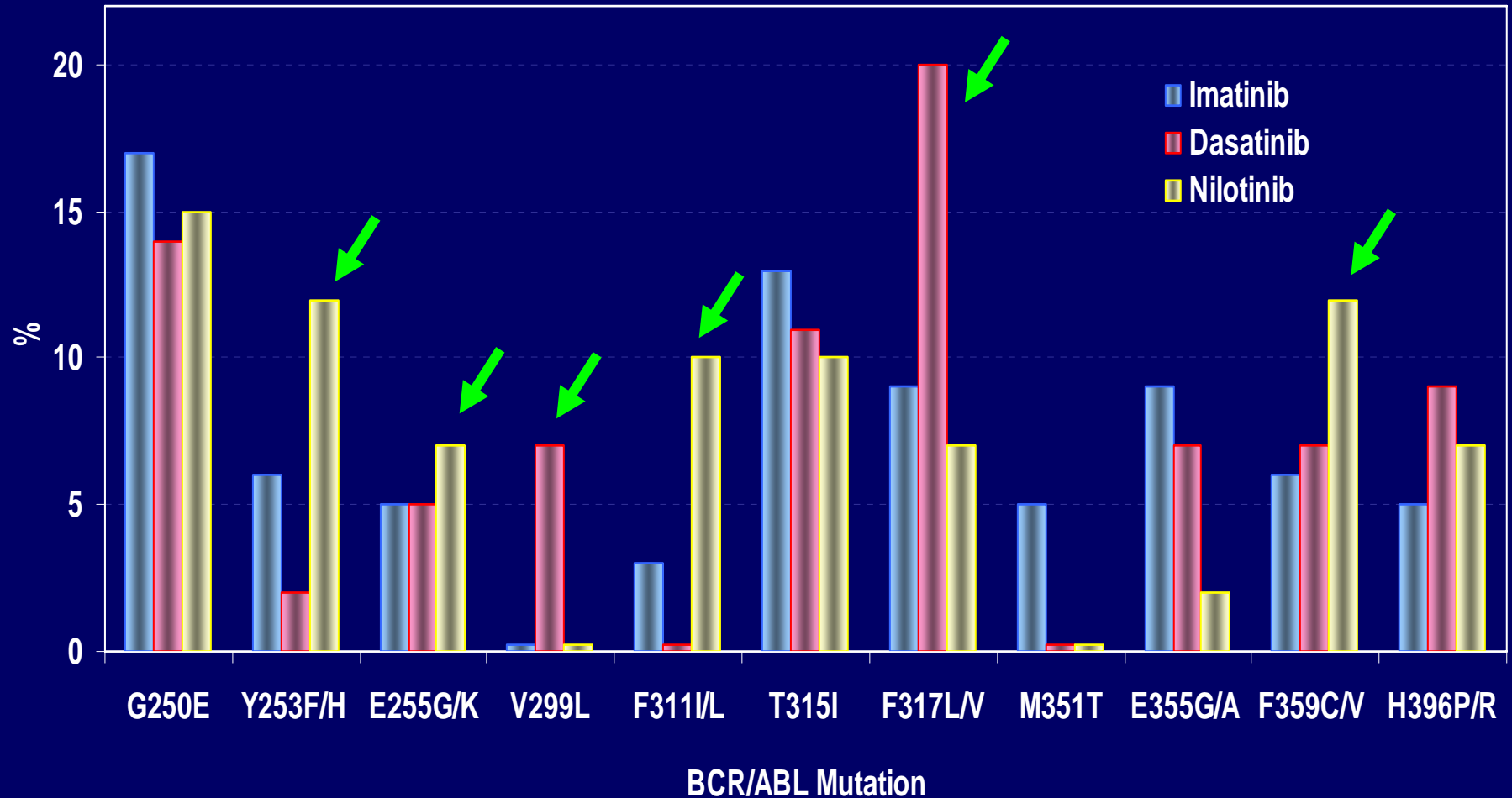
# Course of mutated and unmutated BCR-ABL – 3 patterns –

Pattern 3: n=18

- ⇒ Baseline mutation persisted **AND** BCR-ABL persisted
  - additional mutations evolved in n=3 patients (T315A n=1; V299L n=2)

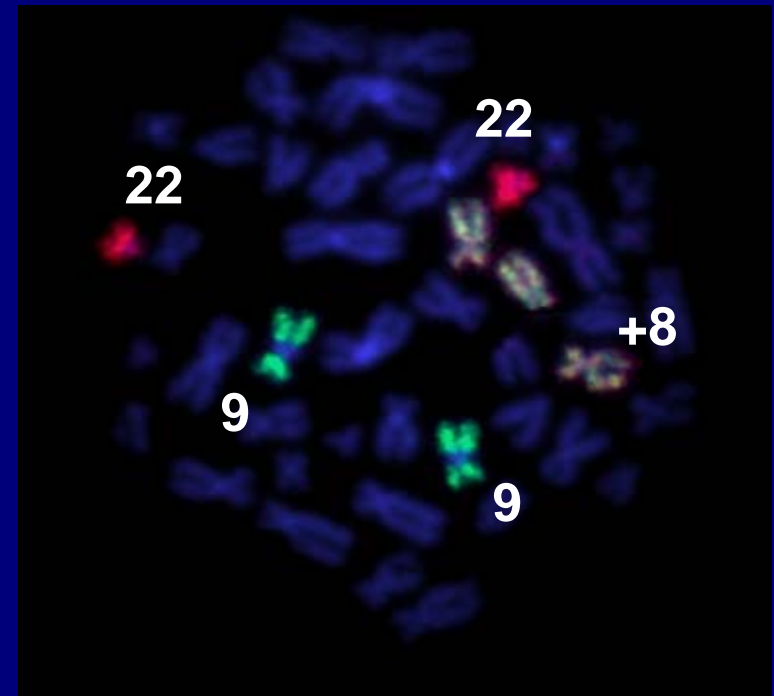
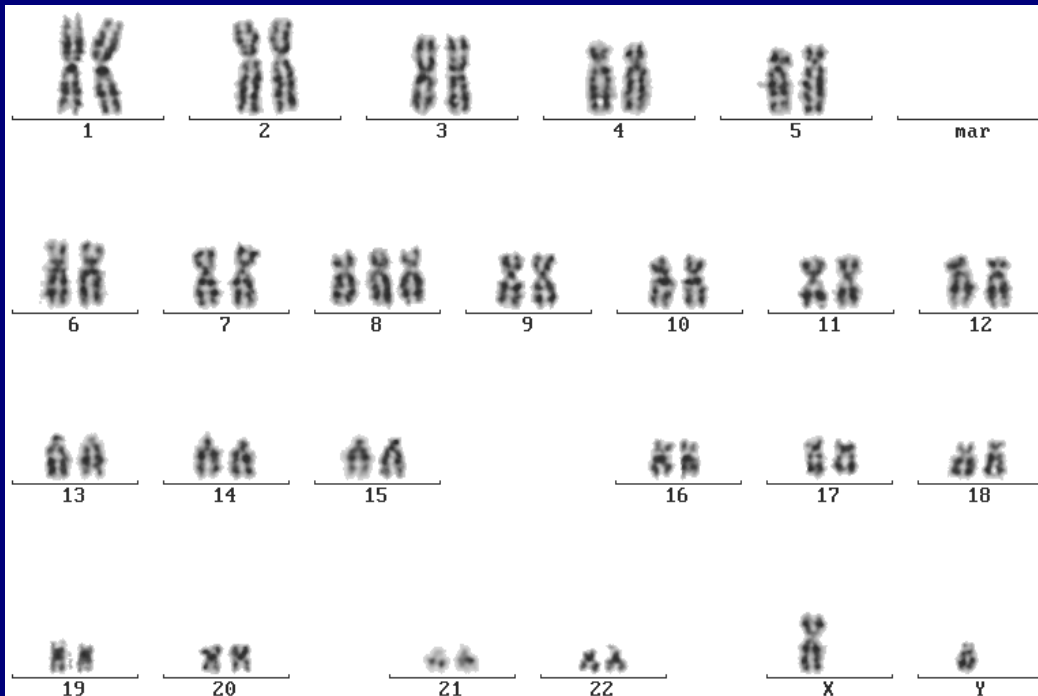


# Spectrum and frequency of BCR-ABL KD mutations recovered after TKI therapy



- T315I and F359V recovered after treatment with Bosutinib

# Clonal cytogenetic aberrations in Ph-negative hematopoiesis

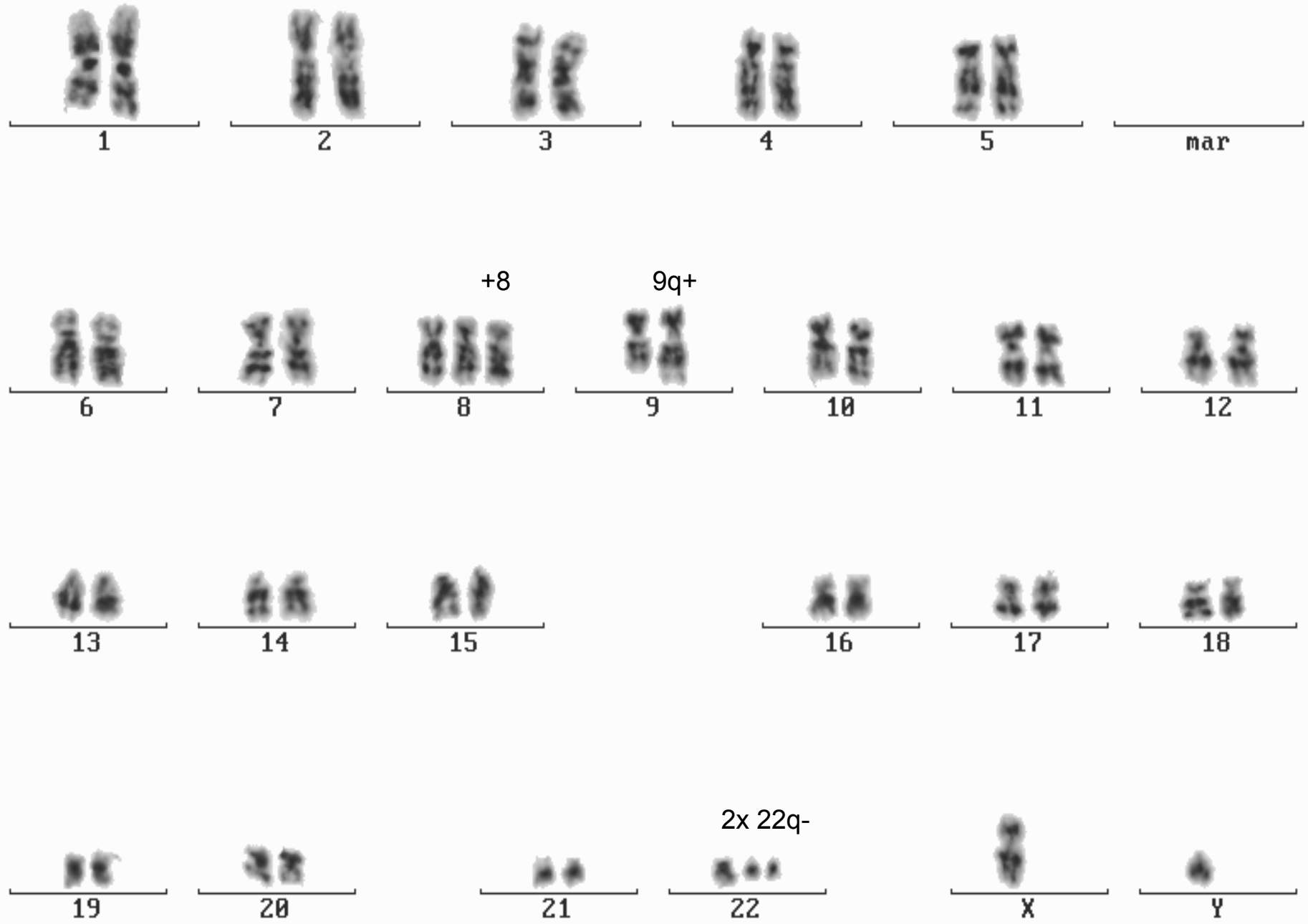


**47,XY,+8**

**Prevalence:  
5-10% of cytogenetic responders**

Bacher et al. *Leukemia* 2005

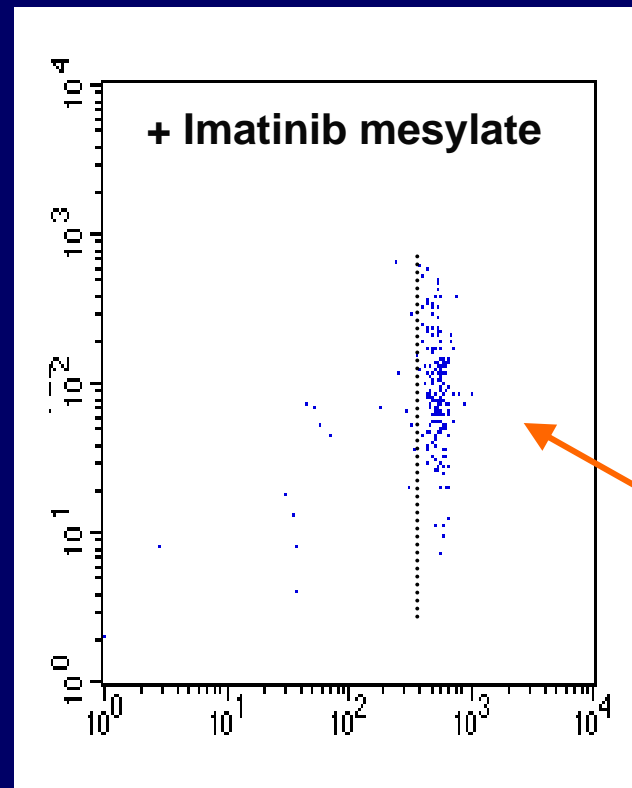
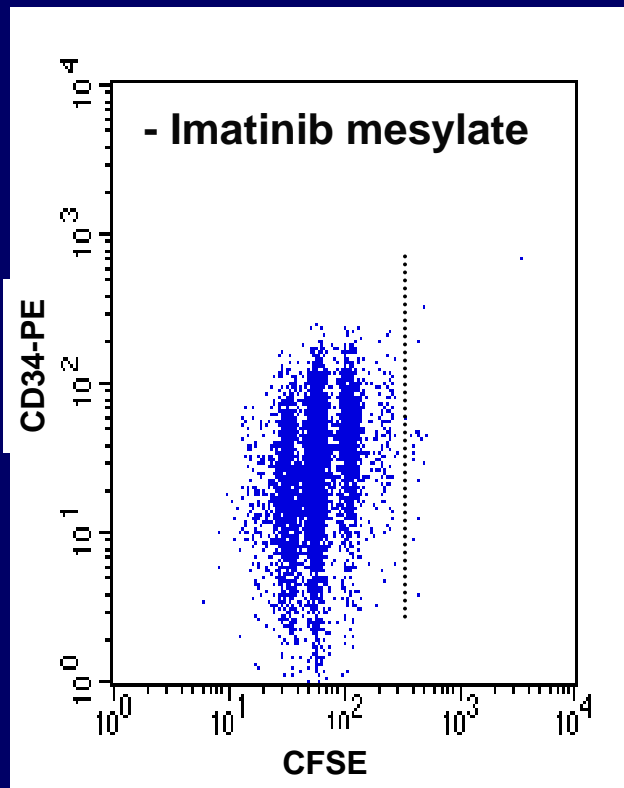
Karyotype: 48,XY,+8,t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11)



# Molecular Persistence

- The majority of chronic phase patients achieve major molecular remission (ratio BCR-ABL <0.1 %)
- A minority are PCR negative
- About 50% of PCR negative patients have relapsed if imatinib was discontinued

# CML Stem Cells Are Resistant to Imatinib



Resistant  
(quiescent)  
CML  
stem cells

Fluorescence