European LeukemiaNet

Monitoring CML patients by RQ-PCR

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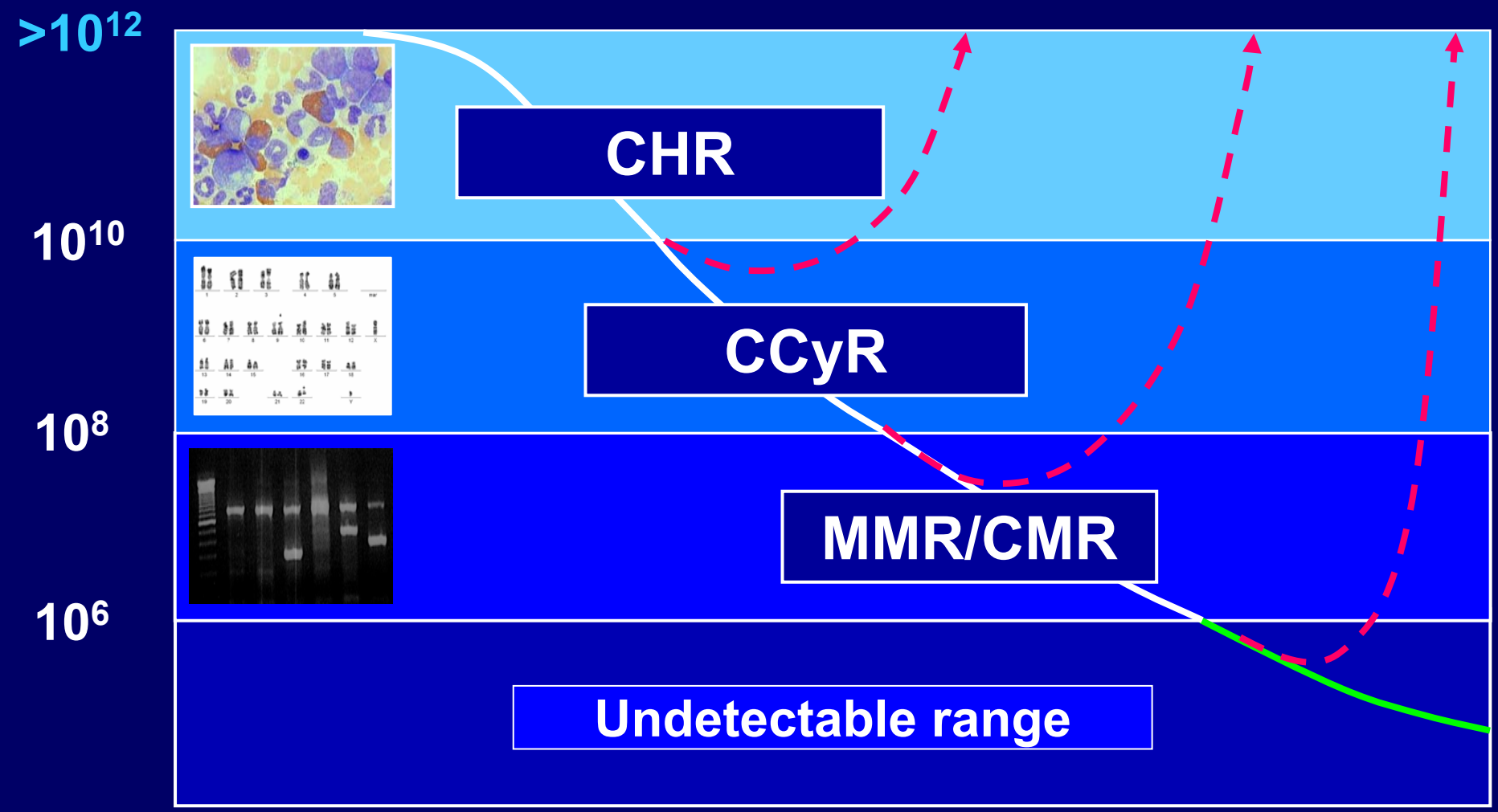
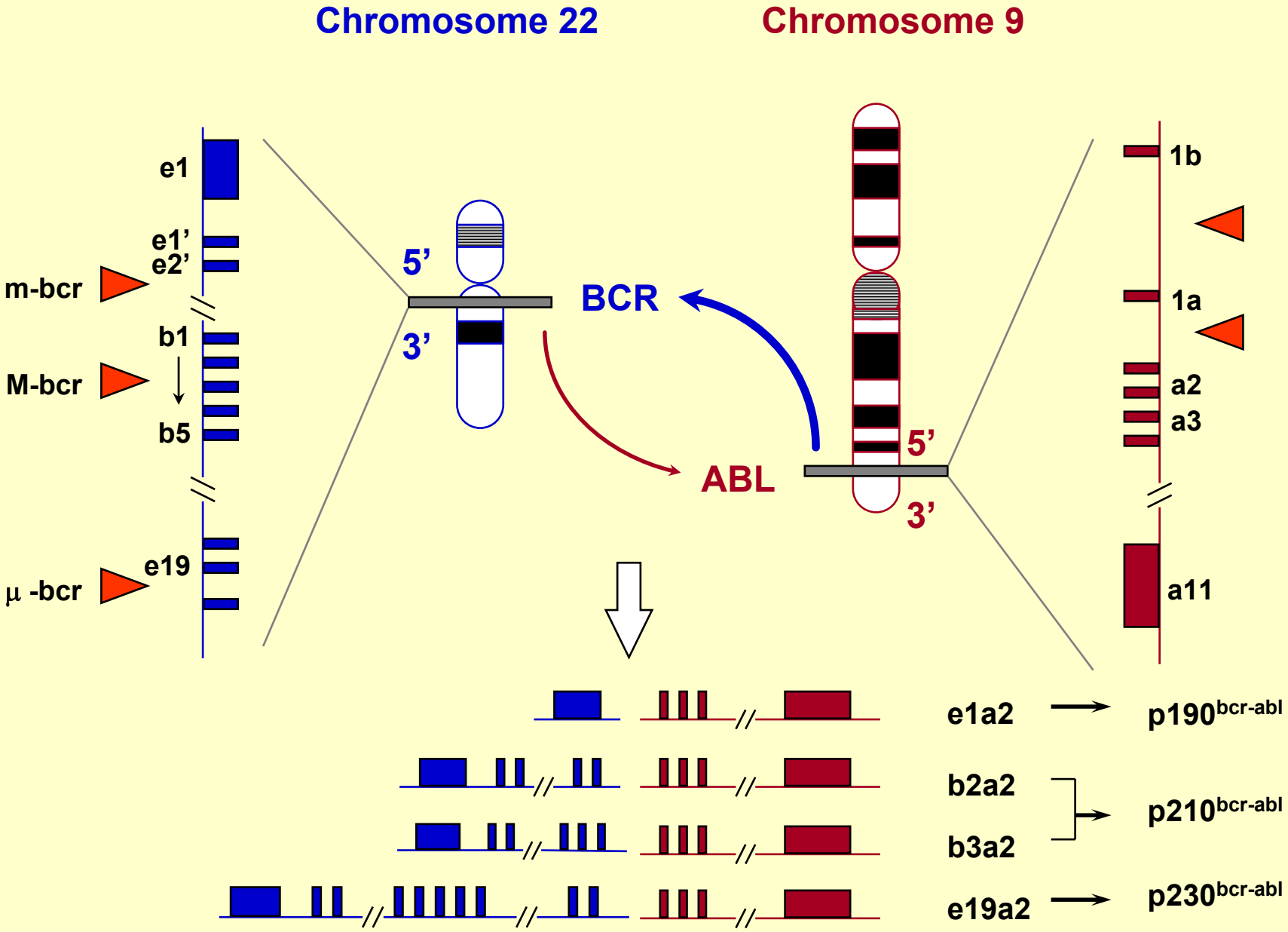
Goals of CML Therapy

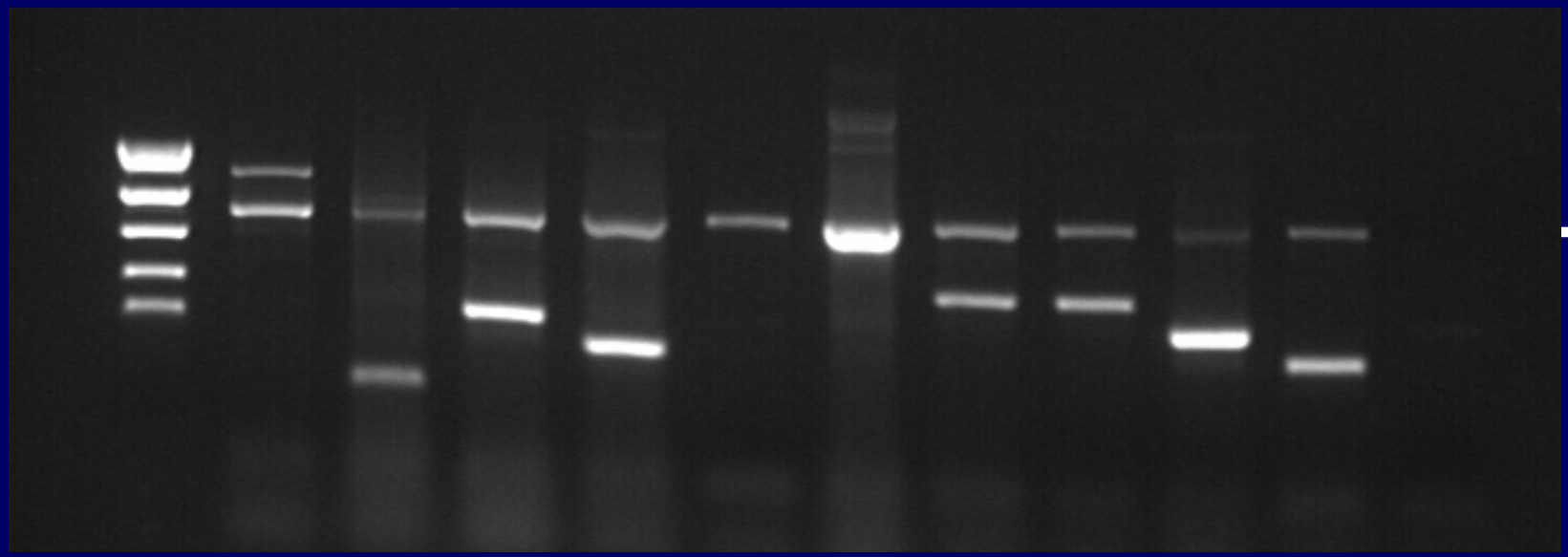


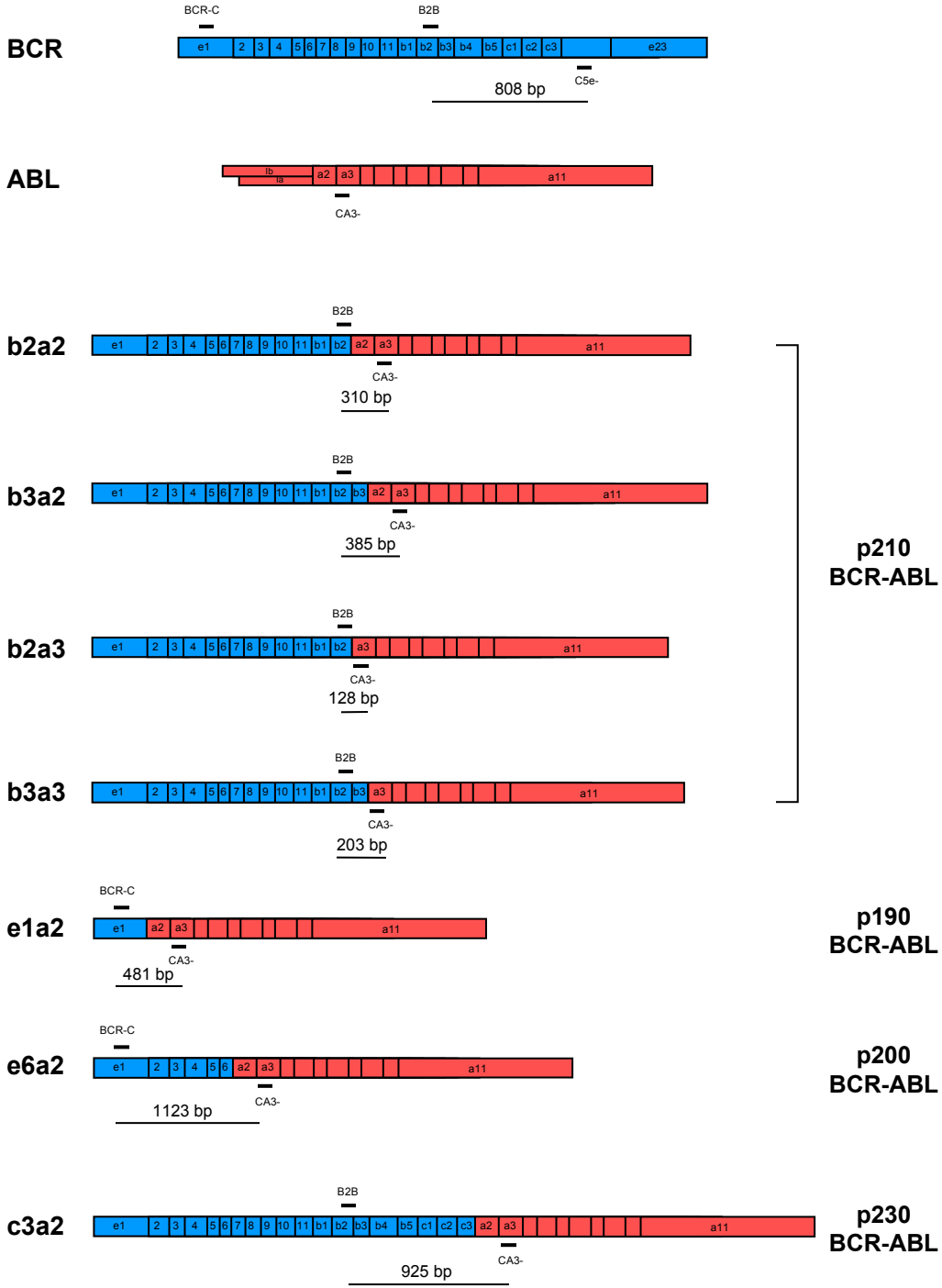

Chromosome 22
Chromosome 9
e1
e1'
e2'
m-bcr
b1
M-bcr
b5
e19
μ -bcr
5'
3'
BCR
ABL
5'
3'
1b
1a
a2
a3
a11
e1a2
p190bcr-abl
b2a2
b3a2
p210bcr-abl
e19a2
p230bcr-abl

The PCR technique, although considered a valid clinical testing procedure, should be used cautiously as a laboratory test until sufficient data are available to show that it meets acceptable criteria of sensitivity, specificity, and positive and negative predictive values. How new PCR technologies such as "real-time" PCR quantification will solve these concerns and become a reliable tool for the clinician merits further investigation. Until then, clinicians should exercise caution in basing clinical decision making on such studies, given the significant morbidity and mortality associated with aggressive therapeutic interventions aimed at molecular disease eradication in patients who might just do as well without.

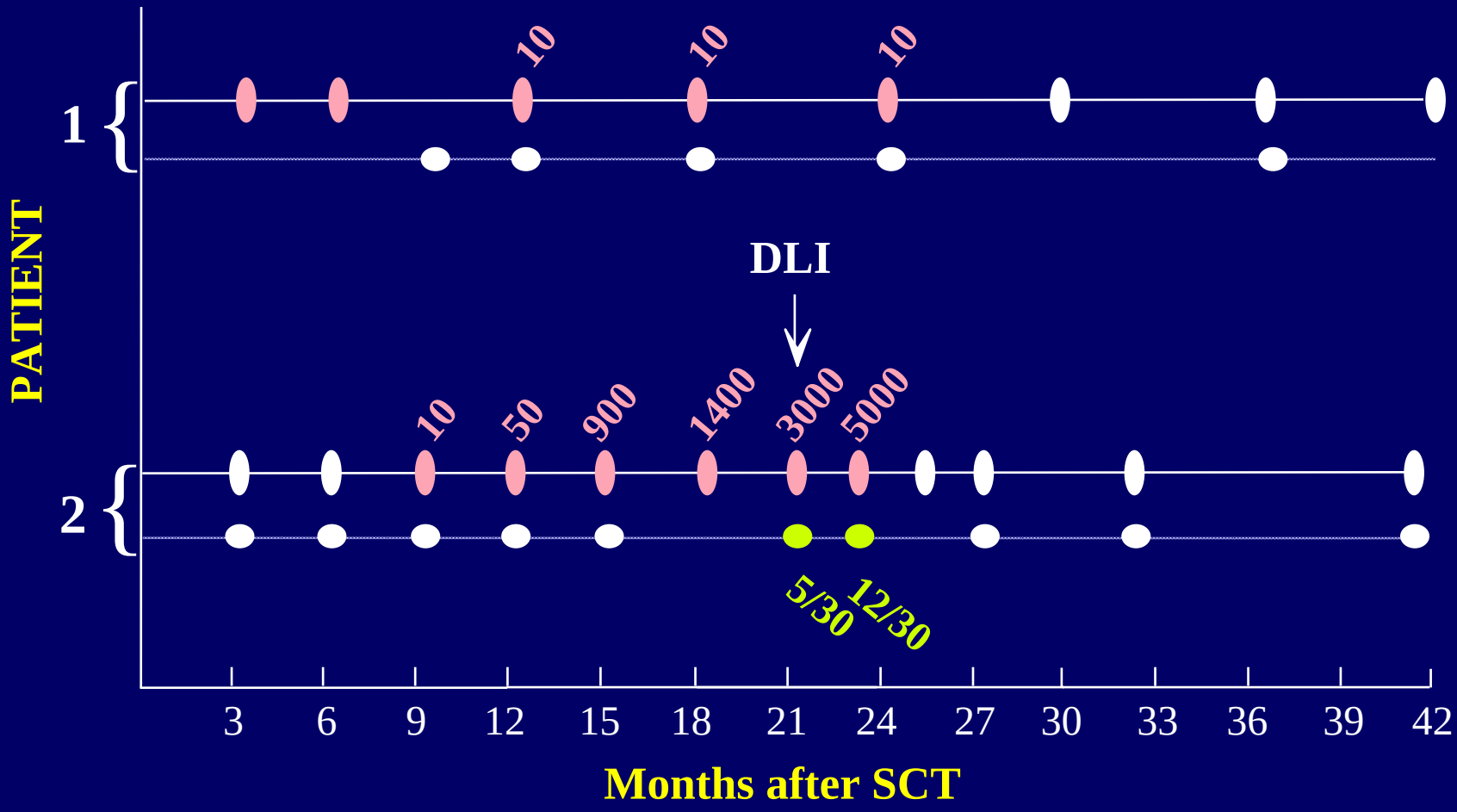
Stefan Faderl et al. (MDACC Houston), Blood 1999

Multiplex-PCR for BCR-ABL transcripts

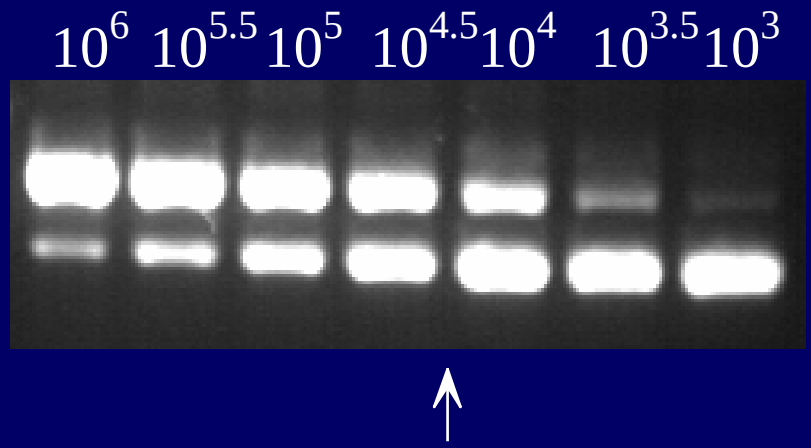



BCR
BCR-C
B2B
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 b3 b4 b5 c1 c2 c3 e23
C5e-
808 bp
ABL
Ib
Ia
a2 a3
a11
CA3-
b2a2
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 a2 a3 a11
310 bp
b3a2
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 b3 a2 a3 a11
385 bp
b2a3
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 a3 a11
128 bp
b3a3
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 b3 a3 a11
203 bp
p210
BCR-ABL
e1a2
e1 a2 a3 a11
481 bp
p190
BCR-ABL
e6a2
e1 2 3 4 5 6 a2 a3 a11
1123 bp
p200
BCR-ABL
c3a2
e1 2 3 4 5 6 7 8 9 10 11 b1 b2 b3 b4 b5 c1 c2 c3 a2 a3 a11
925 bp
p230
BCR-ABL

Early detection of relapse after SCT

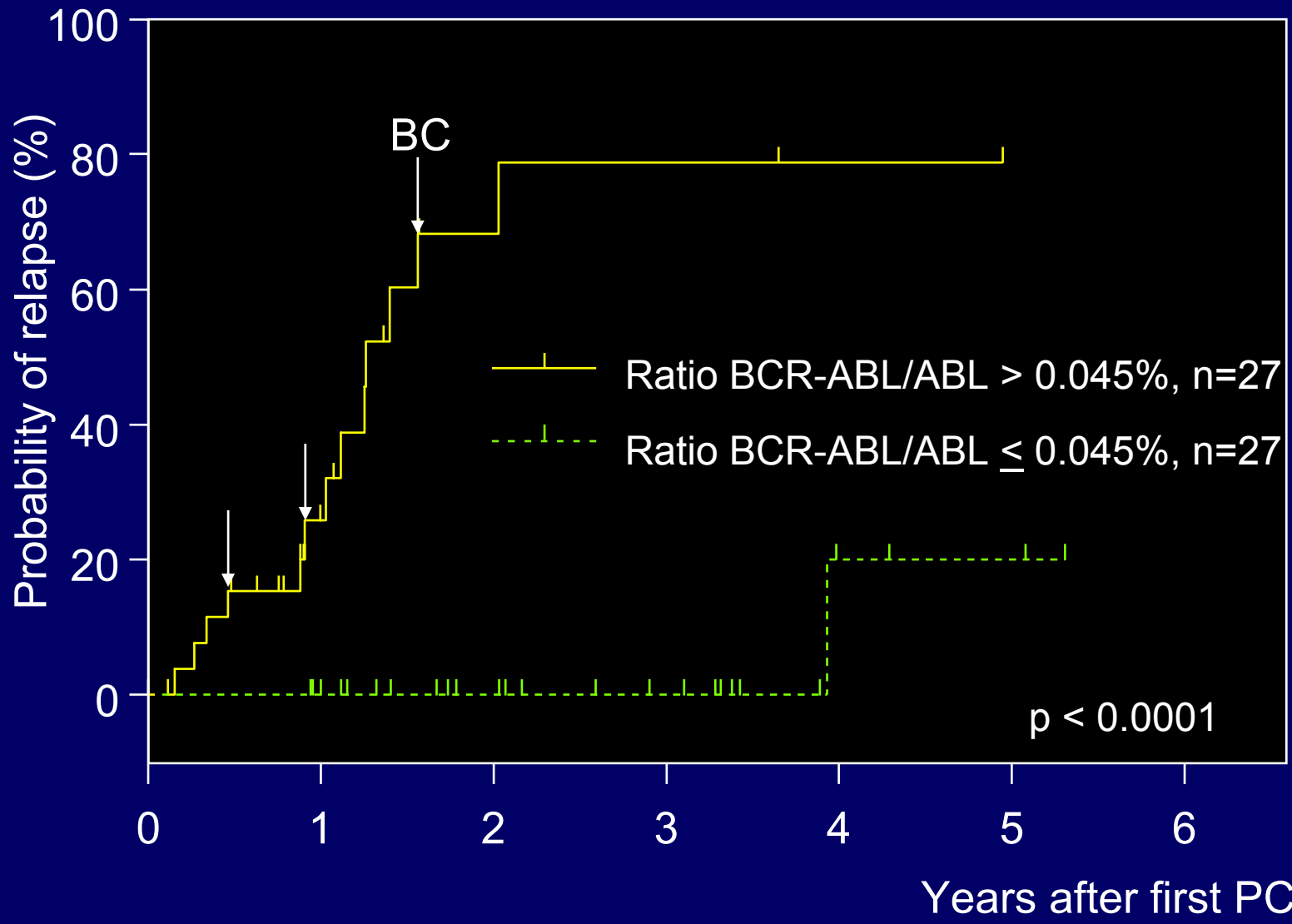


1993: Competitive RT-PCR



Cross et al *Blood 1993*

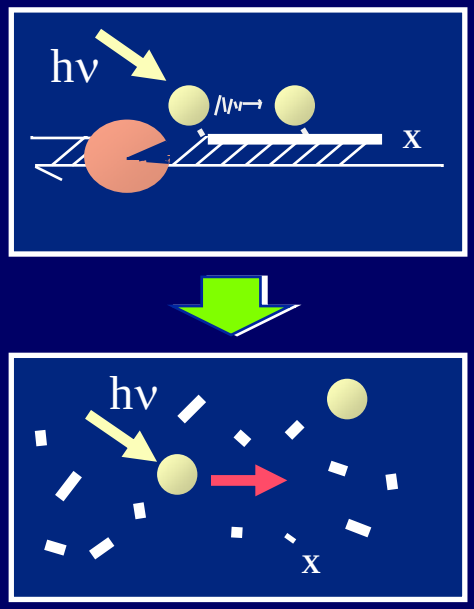
Relapse free survival in complete cytogenetic responders after IFN therapy according to residual BCR-ABL transcript levels



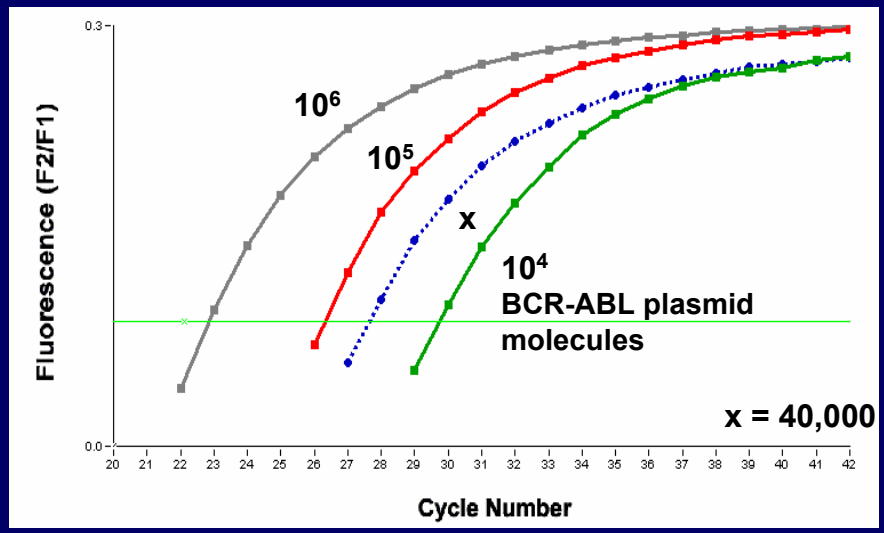
Real time quantitative RT-PCR

I. Hydrolysis Probes

Release from quenching by hydrolysis

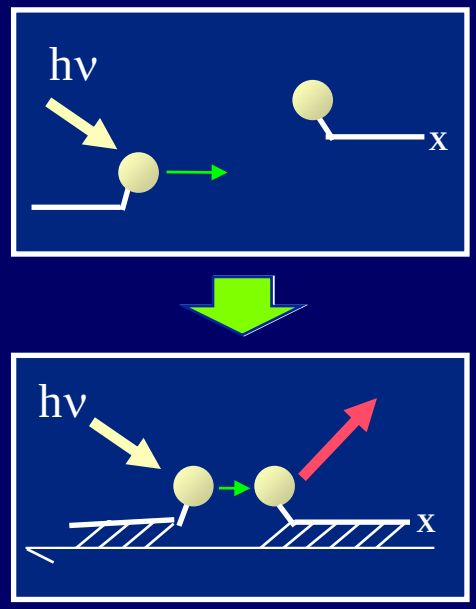


TaqMan™



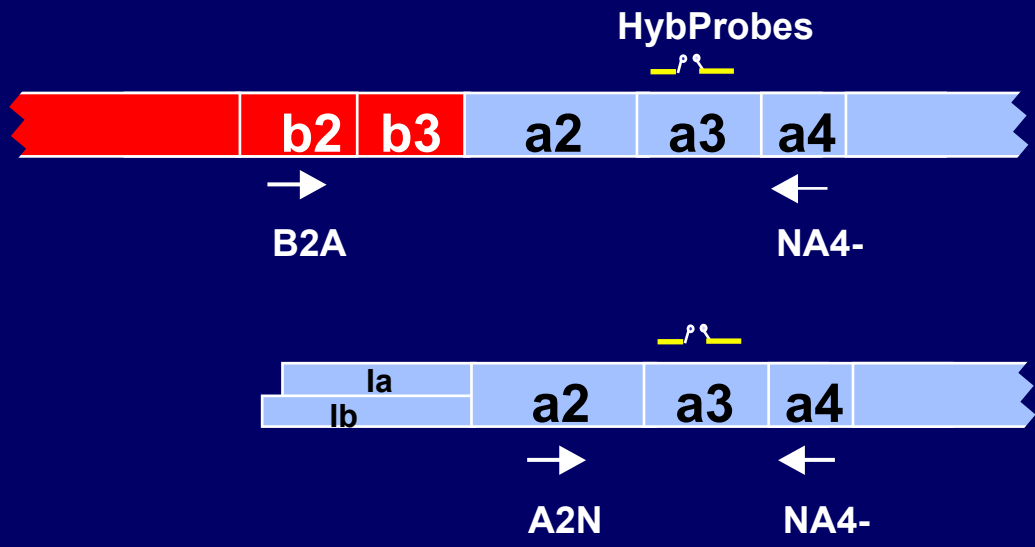
II. Hybridization Probes

Increased resonance energy transfer by hybridization



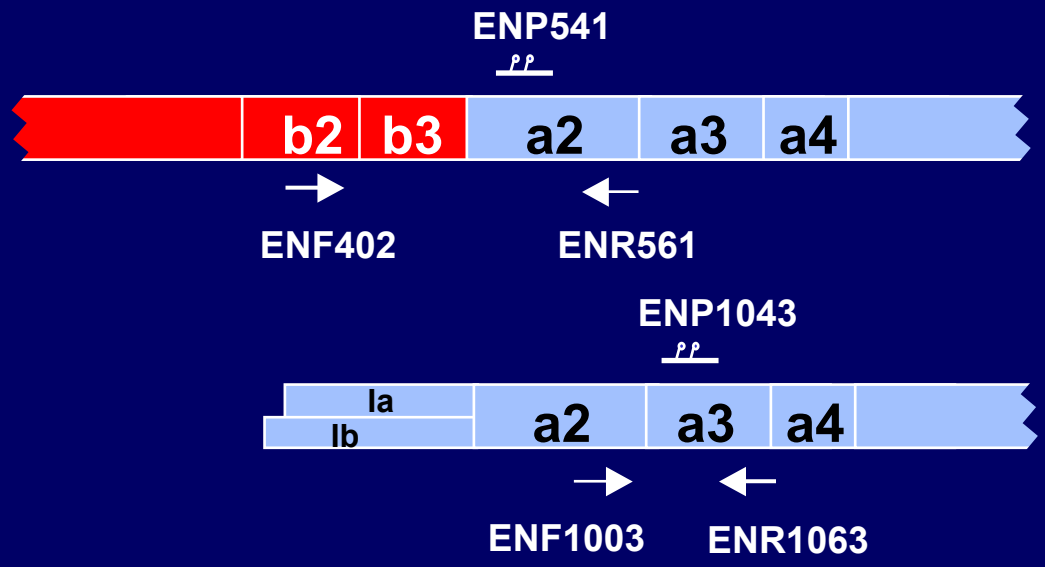
LightCycler™

LightCycler

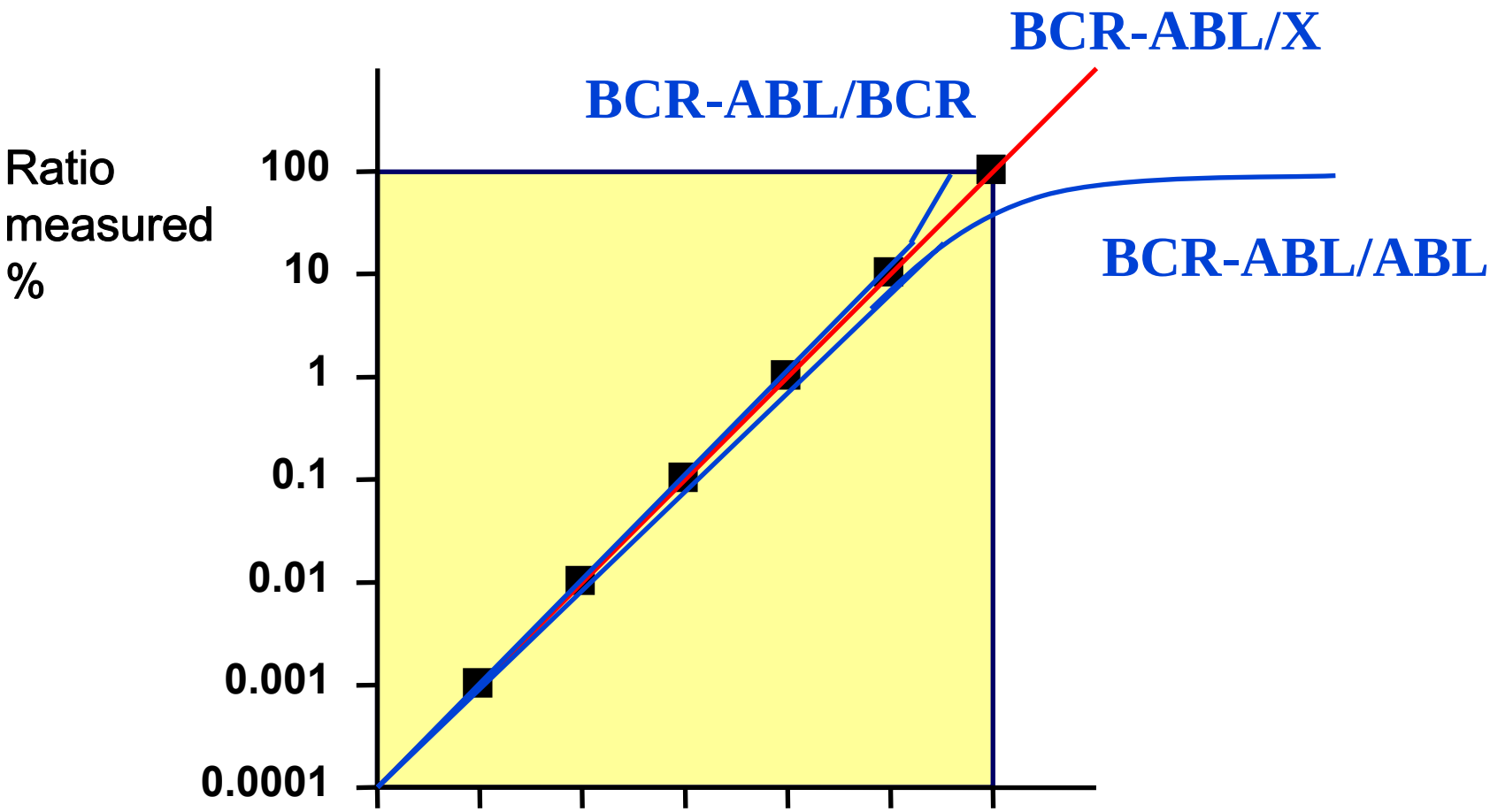


Emig et al. *Leukemia.* 1999.

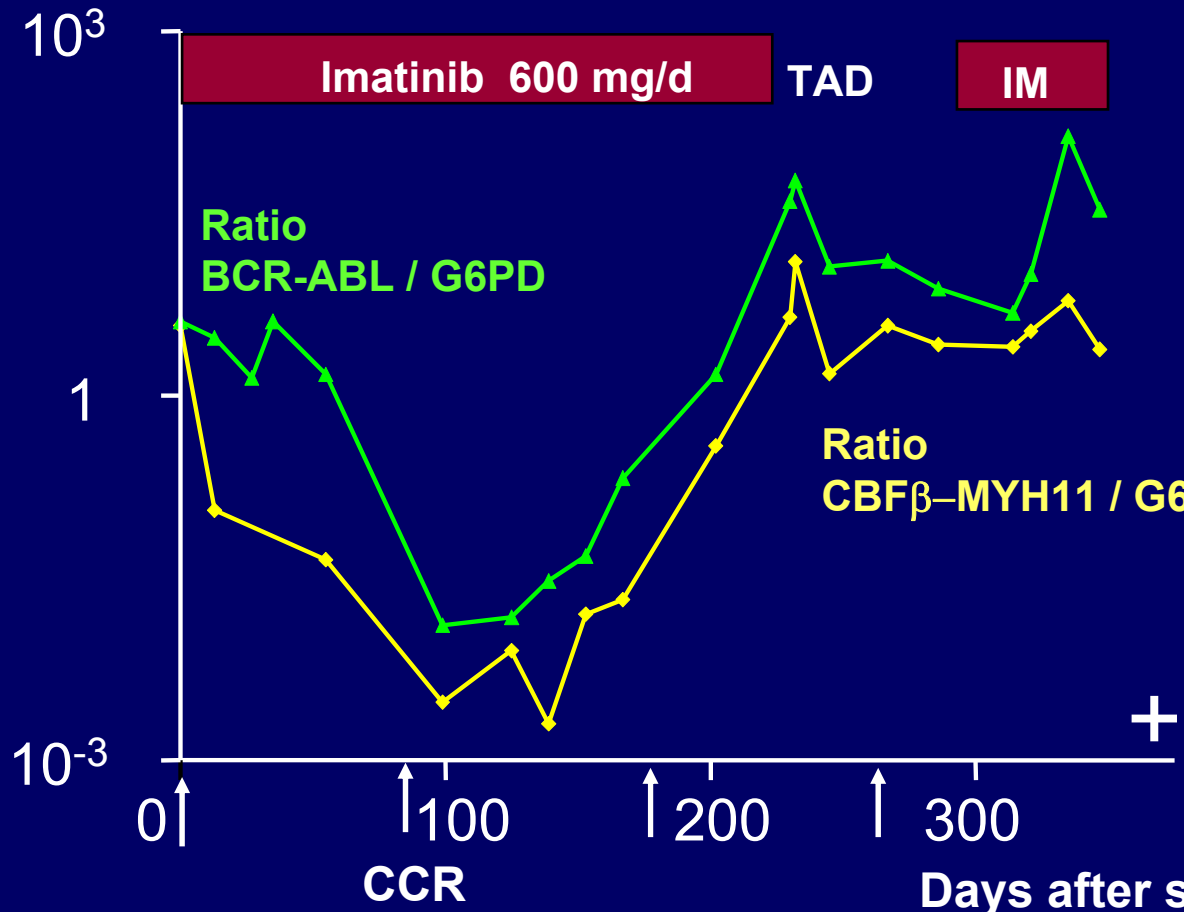
TaqMan



Gabert et al. *Leukemia. 2003*.


BCR-ABL/X
BCR-ABL/BCR
BCR-ABL/ABL
Ratio
measured
%
100
10
1
0.1
0.01
0.001
0.0001

Relapse of myeloid blast crisis after CCR



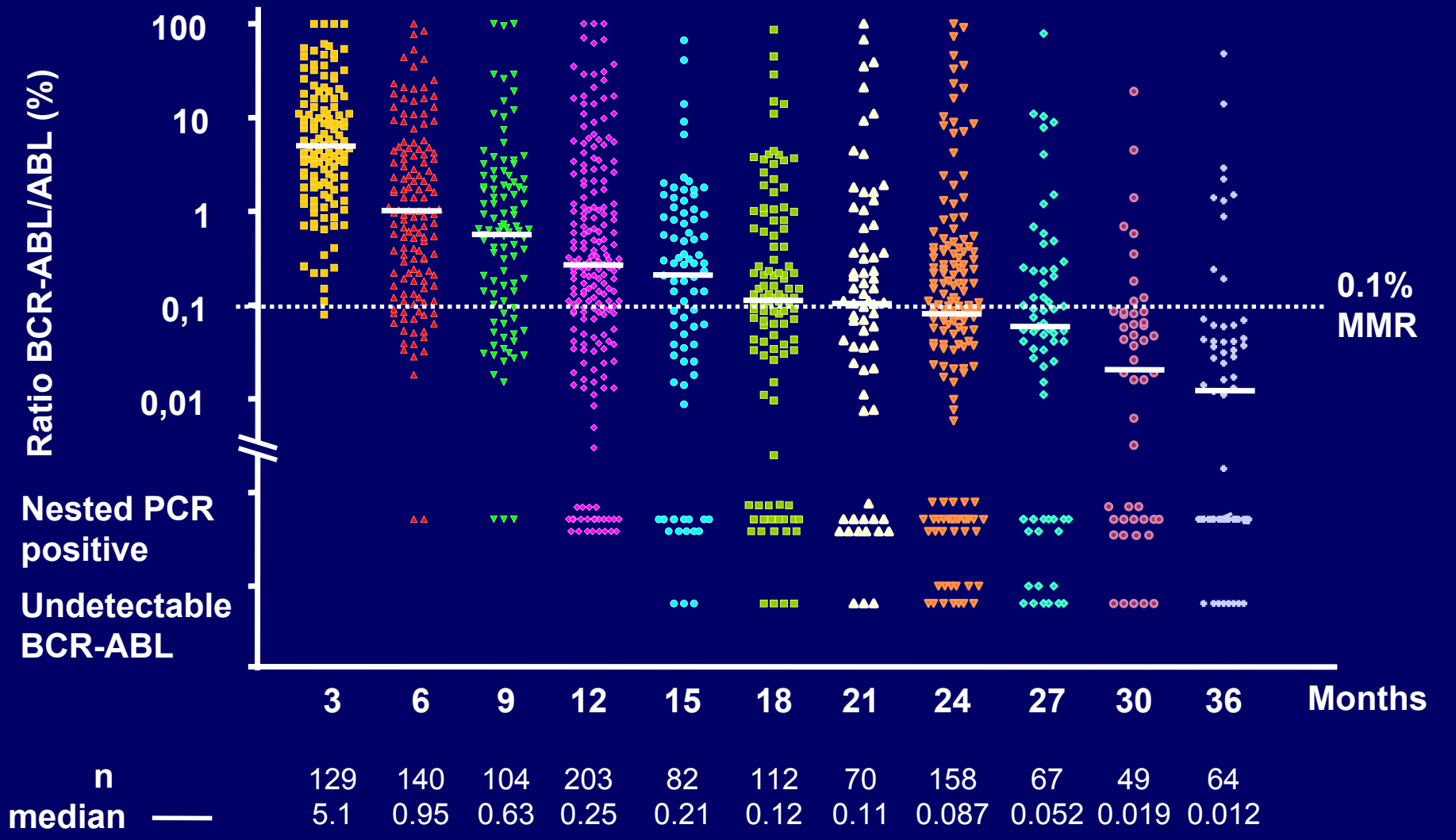
46, XY, Ph	[18]		[6]	[13]
46, XY, Ph, inv(16)	[7]		[2]	[7]
46, XY		[25]	[8]	[5]

Molecular response precedes cytogenetic remission in CP patients

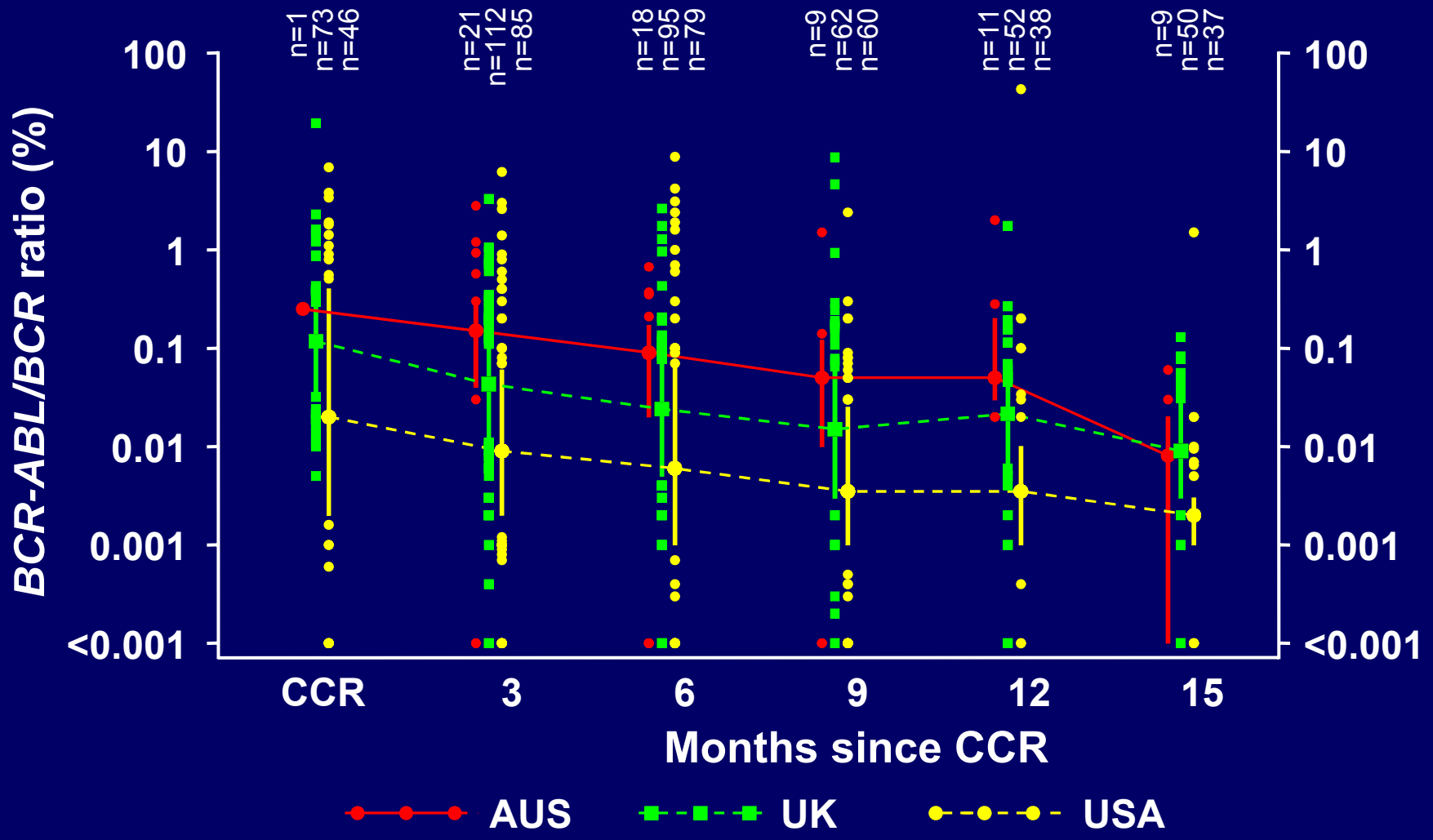
Cytogenetic response at month 6	CR	PR	MR	NR	
n	16	18	12	14	
Ratio BCR-ABL / ABL (%): Month 1	29.0	49.0	50.0	52.0	n.s.
Month 2	**4.7**	31.0	40.0	74.0	p = 0.027
Month 3	**2.5**	18.0	40.0	38.0	p < 0.0001

Merx et al., Leukemia 2002

Molecular Monitoring, German CML Study IV

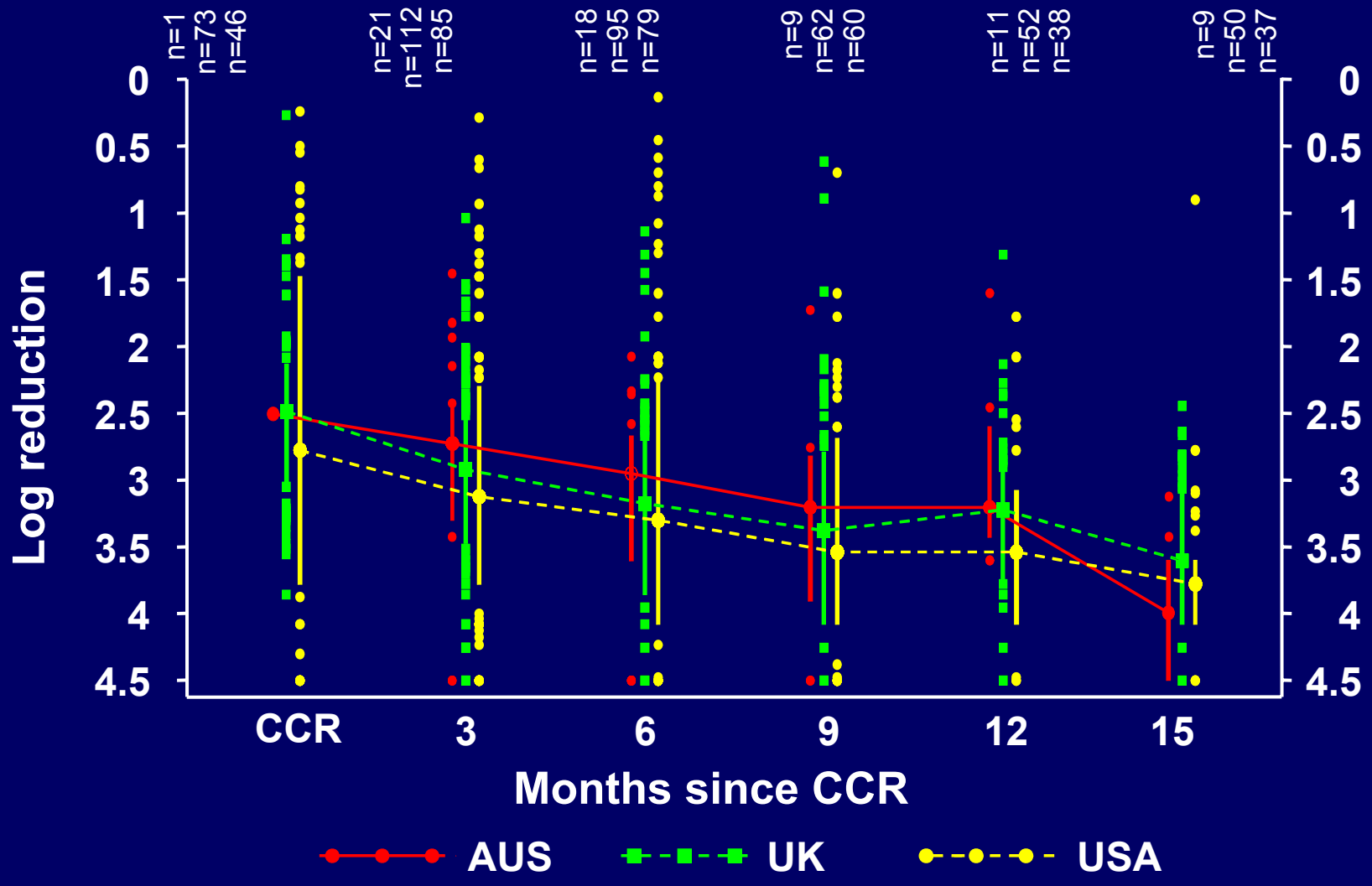


IRIS Study: Ratio BCR-ABL/BCR - by Laboratory



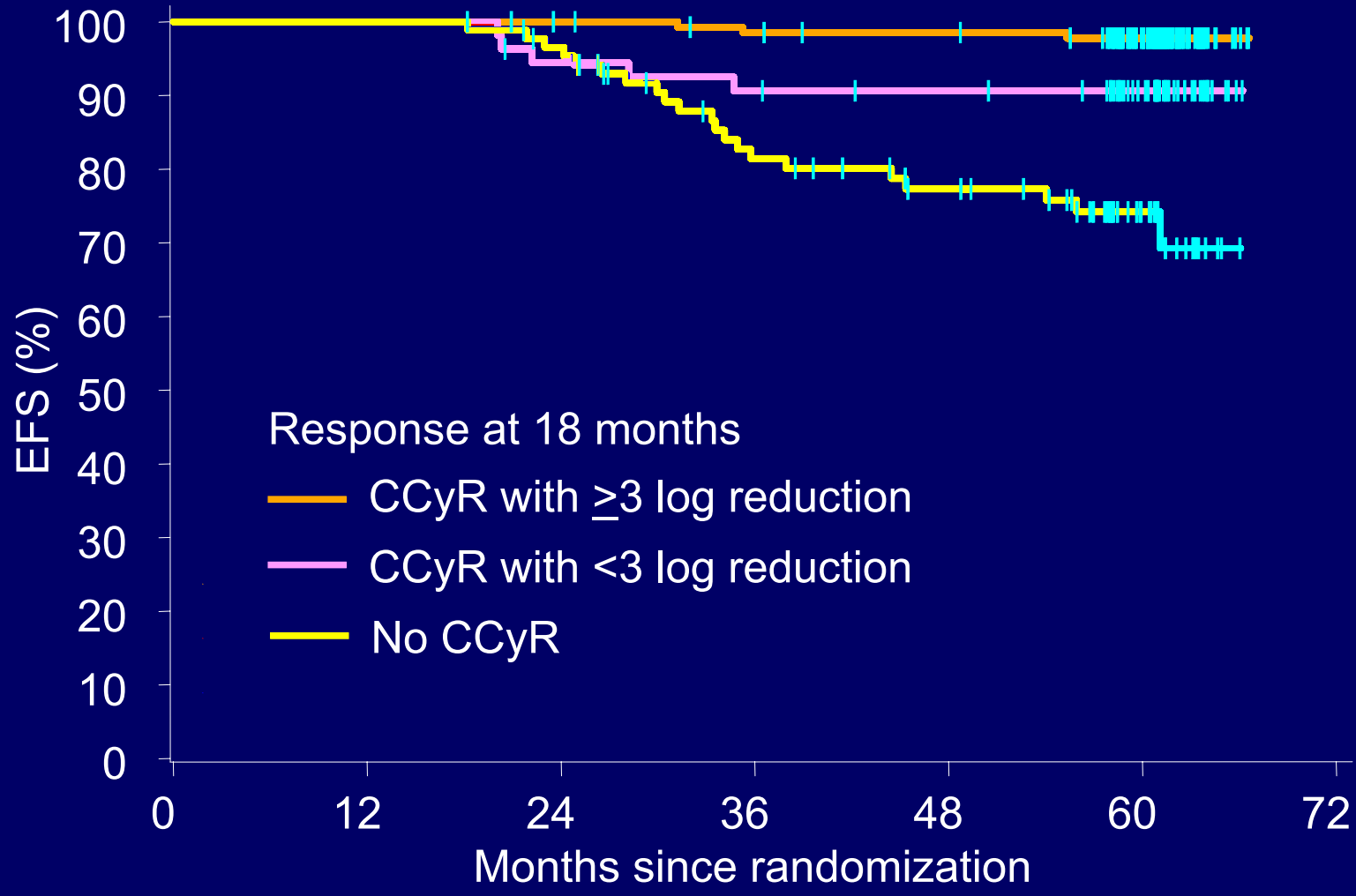
Hughes et al. ASH, 2002.

Standardized Log Reduction - by Laboratory



Hughes et al. ASH, 2002.

IRIS Study: Event-Free Survival by Molecular Response at 18 Months



Baccarani et al. ASH 2006.

The UnOfficial Gleevec (STI571) Site

YAHOO! Groups

Yvonne Heimann here, today I received my 7/9/02 test results. 20 normal cells, Fish NEGATIVE PCR .6
Sounds like Gleevec did it again. Not sure if this qualifies me for the Zero
club but pretty close.

Hi,
I don't think I'm on your list and would like to join the others.
My name is E.F. I was a member of the phase 1 group at UCLA. I was diagnosed 3/98 in Chronic Phase. I began Gleevec (300mg) 1/99 (The first person to take 300mg). Reached CCR 6/99. Still not sure if I'm in MR.
UCLA kept changing PCR tests so my results were never very reliable although others like L.B. have been neg on all the different tests. Last year was neg on 2 successive highly sensitive quantitative tests but relapsed (?) in June...
E.F.

Hi George,

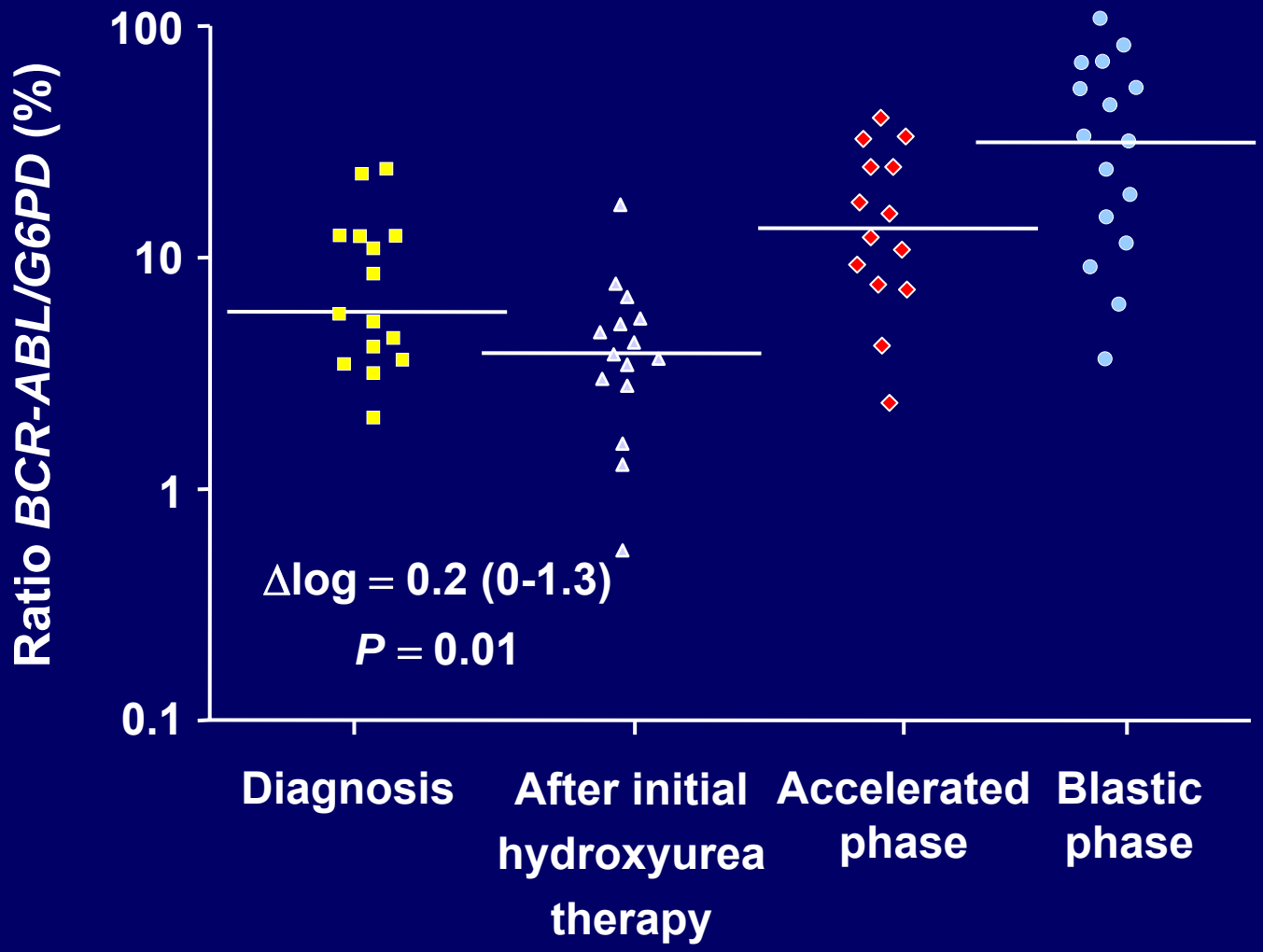
Molecular remission is when they look at a large number of cells in your blood, eg. 1 million. If no bcr/abl is found in 1 million cells, then you are in molecular remission. This can be done only through quantitative pcr testing. 0.08% means you are getting there but still not there. Different institutions have different ways of pcr testing. Some look at 10,000 cells, some look at 1 million. The bigger the sample size, more sensitive is the test. There is no universal standard. The trick is to go on testing at one particular machine and achieve their zero and mantain it. You must ask your doctor what number they consider pcr negative.

Rgds, Anjana

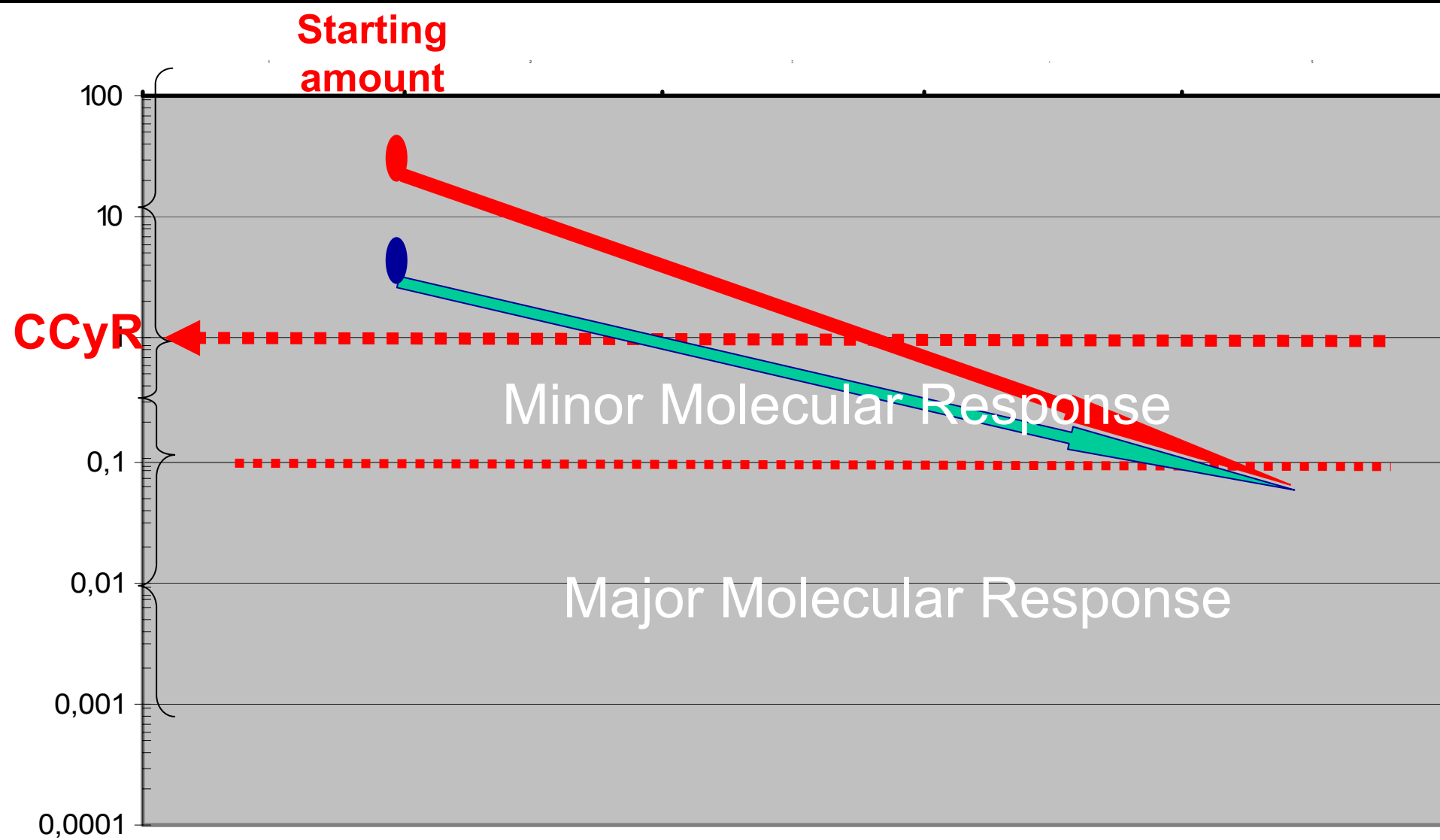
Methods of Expression of Molecular Response

- **Ratios target/standard gene**
  After standardization of methods and rigorous control rounds, eg, ratio BCR-ABL/ABL (%)
- **Lab-specific reference point:**
  Pooled diagnostic samples,
  eg, Δ log ratio BCR-ABL/BCR (%)
- **Individual calculation of relative molecular response:**
  MRD level after therapy/pretherapeutic level,
  eg, individual Δ log ratio BCR-ABL/GUS (%)

Variability of the Individual "Pretherapeutic *BCR-ABL* Level"



The evidence obtained with the IRIS study is that the absolute and not the relative amount is important!

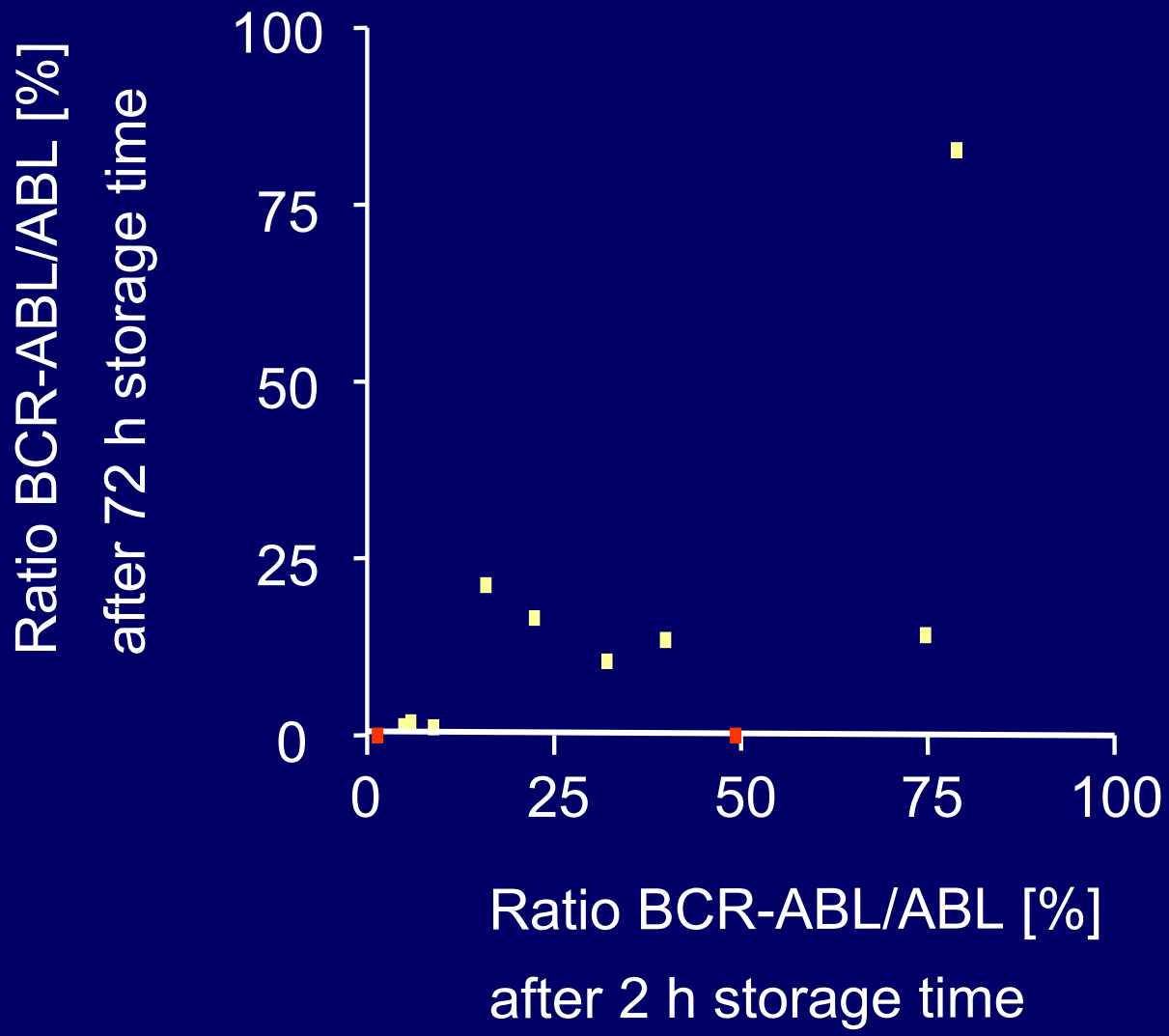


European LeukemiaNet

Recommendations for Standardization

- Use of 10 mL peripheral blood (~5×10^7 WBC) processed within 36 hours
- Bedside RNA stabilization for multicenter trials
- Standardized EAC (TaqMan) and LightCycler PCR protocols
- Single plasmid dilution series for target and housekeeping genes
- Housekeeping gene(s) for routine use: total *ABL, BCR* or *beta-GUS*

Unstabilized PB after 2 hrs vs. 72 hrs

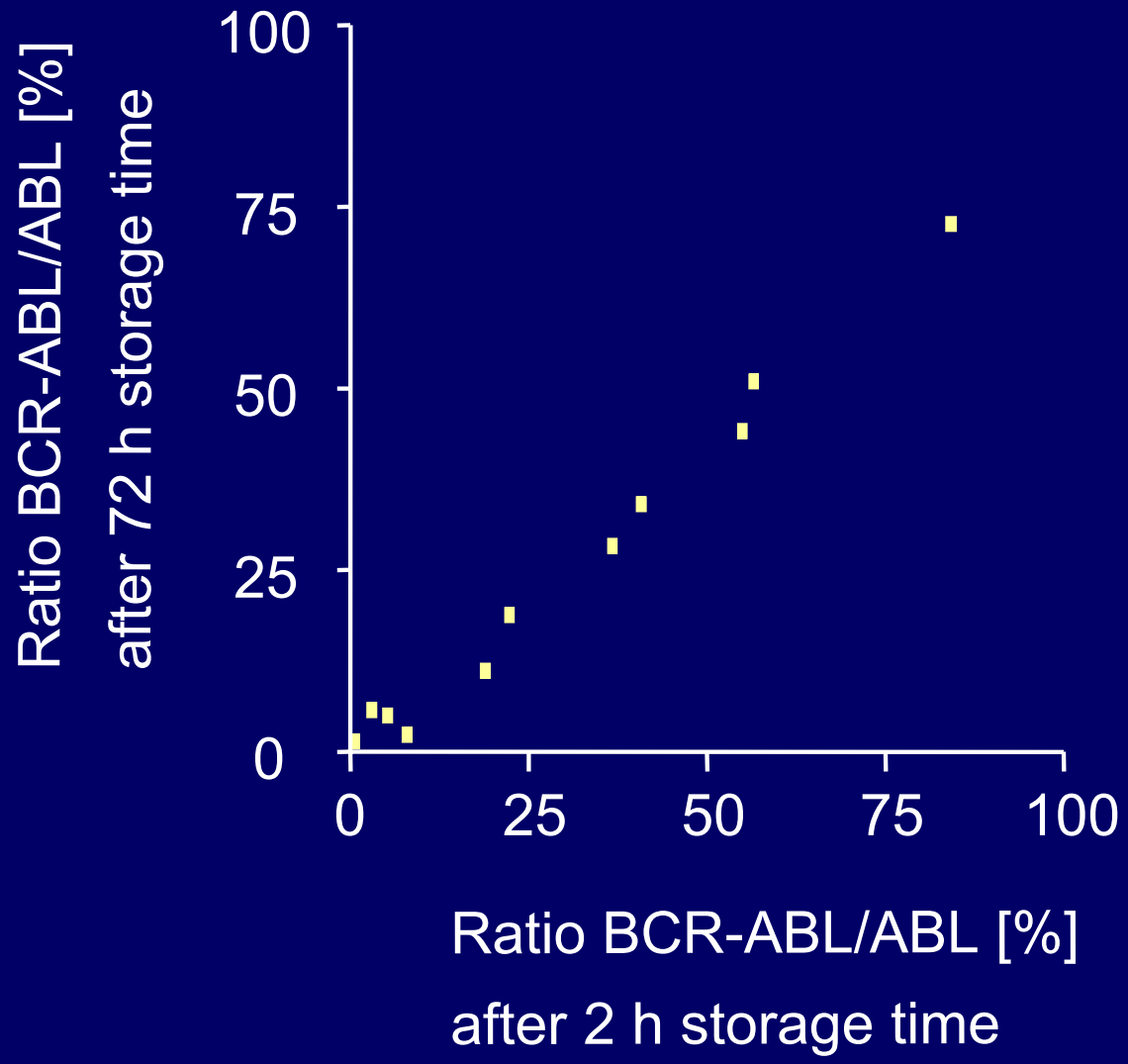


r=0.65

p=0.03

Mueller et al., Leukemia 2002

PAXgene Stabilized PB after 2 hrs vs. 72 hrs

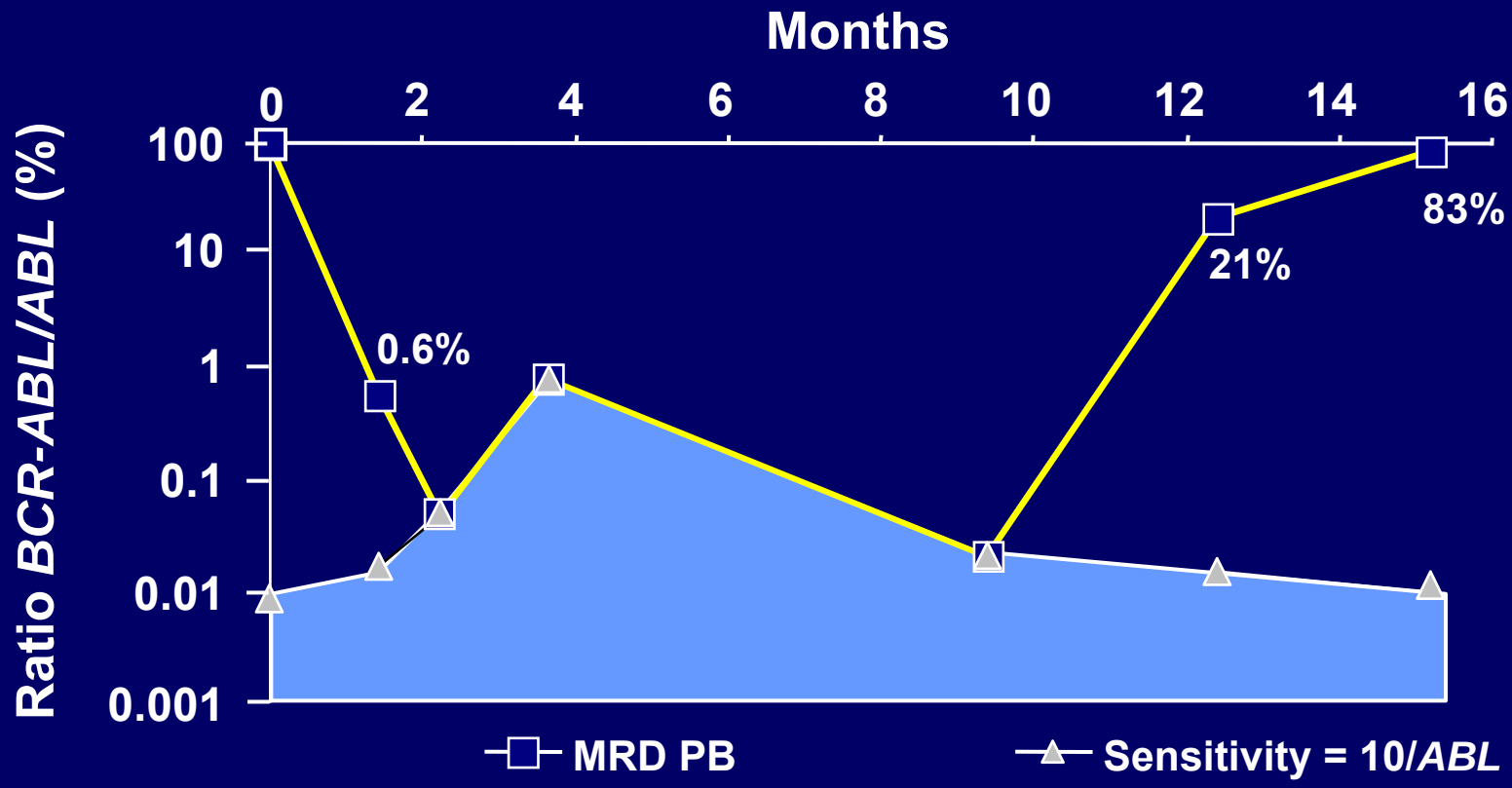


r=0.99

p<0.0001

Mueller et al., Leukemia 2002

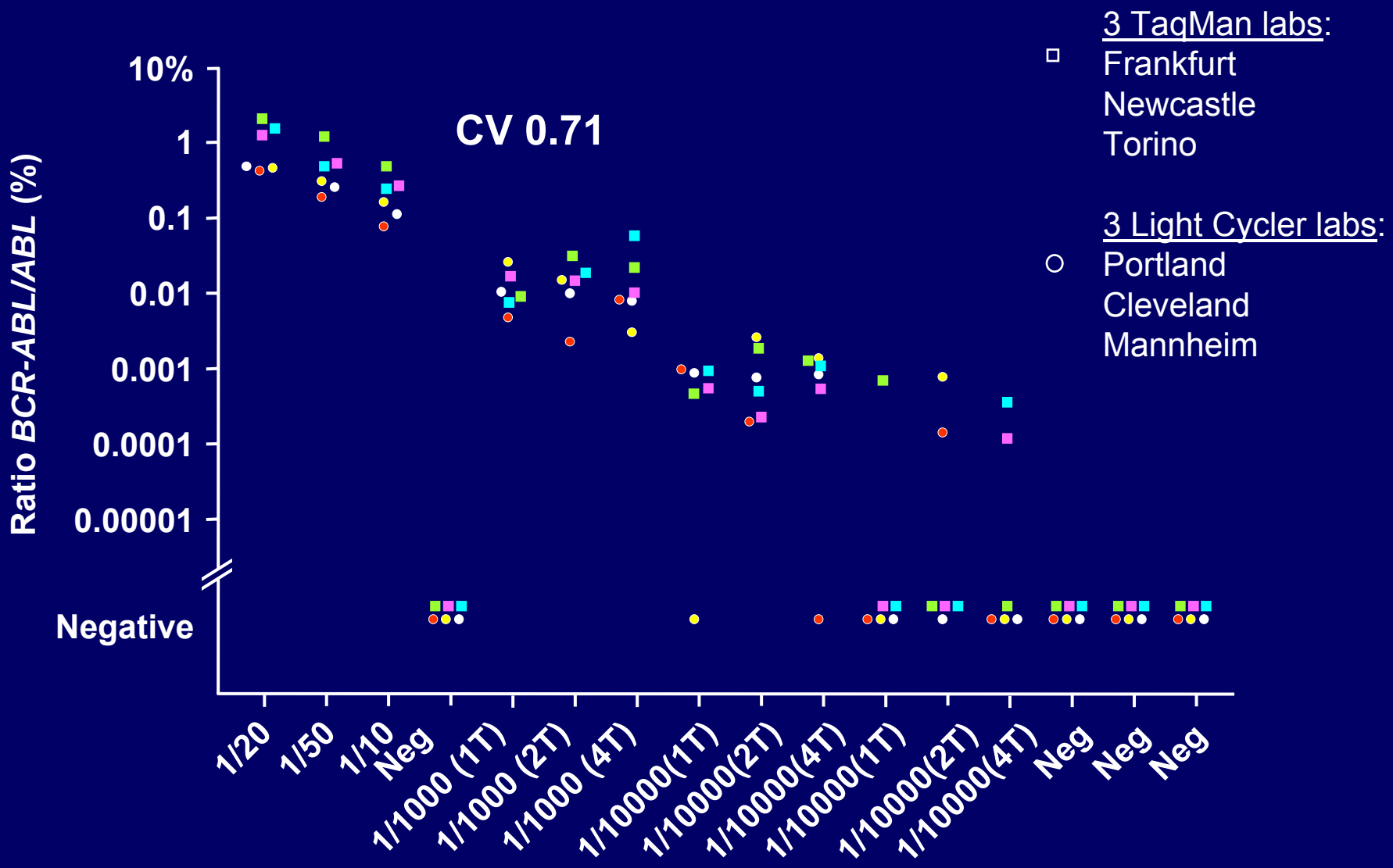
Sensitivity Depends on the Quality of the Individual Sample



Disease detectable:	Disease undetectable:
BCR-ABL/ABL (%)	Negative / [no. of *ABL* transcripts]

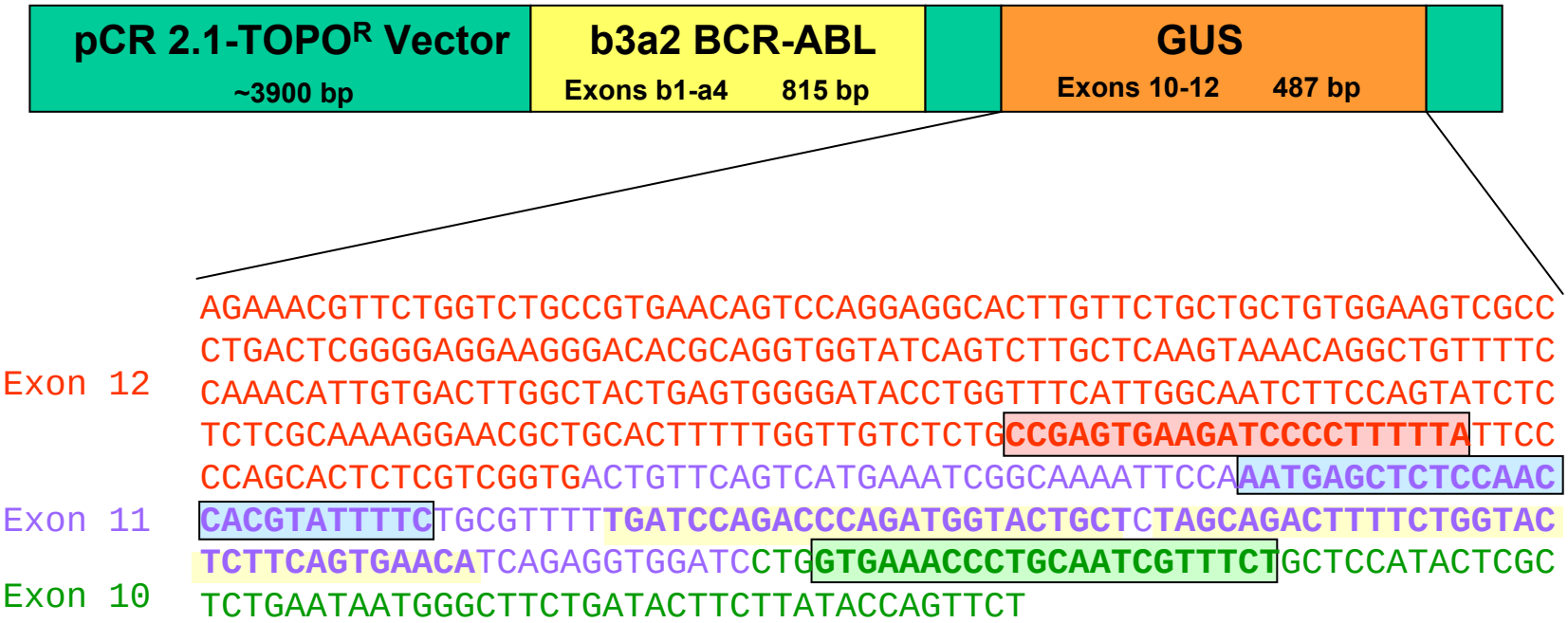
Beillard et al. *Leukemia*. 2003.

International Control Round (n=6)



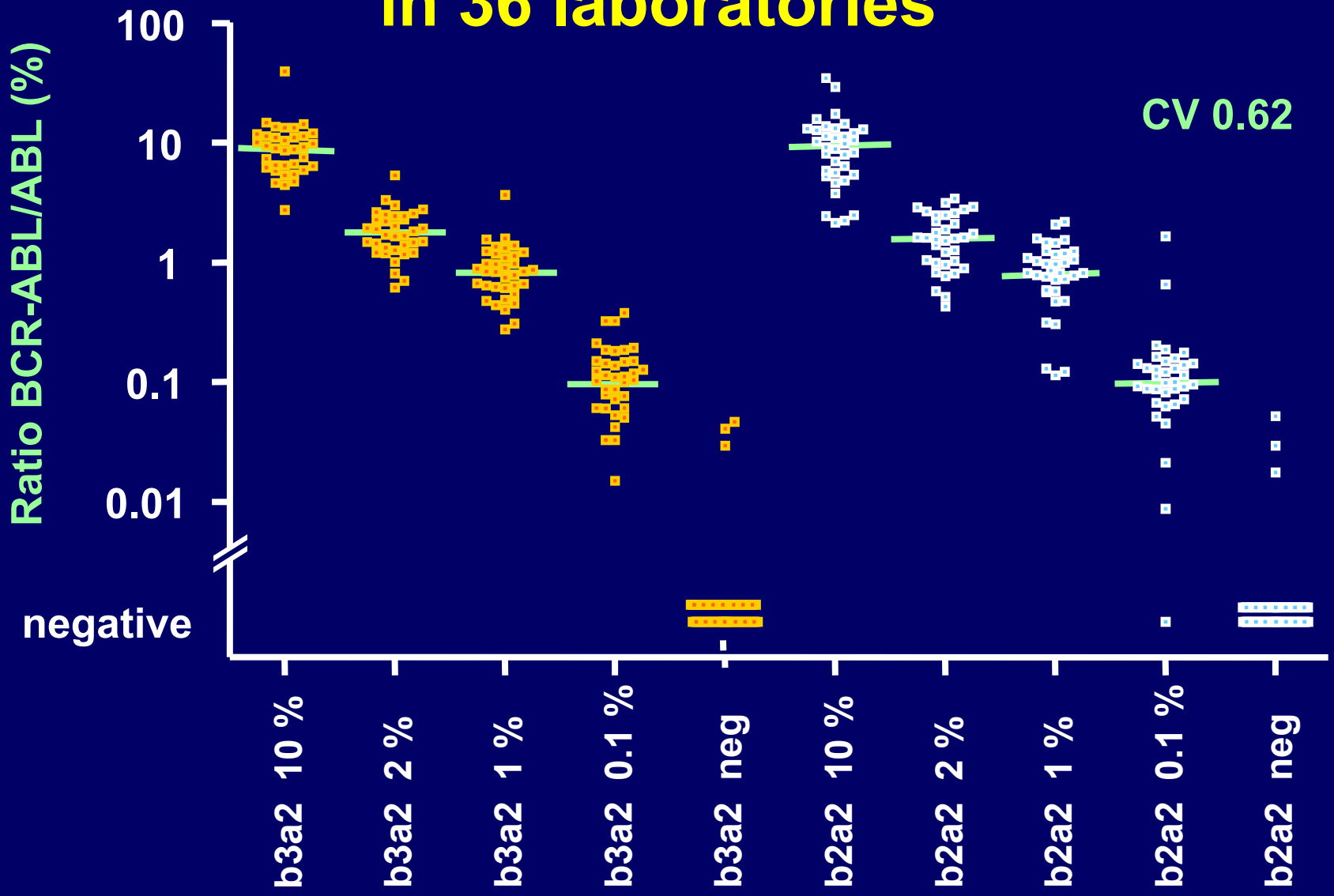
Müller et al. ASH, 2003.

European study to harmonize methodologies to quantify BCR-ABL mRNA transcripts



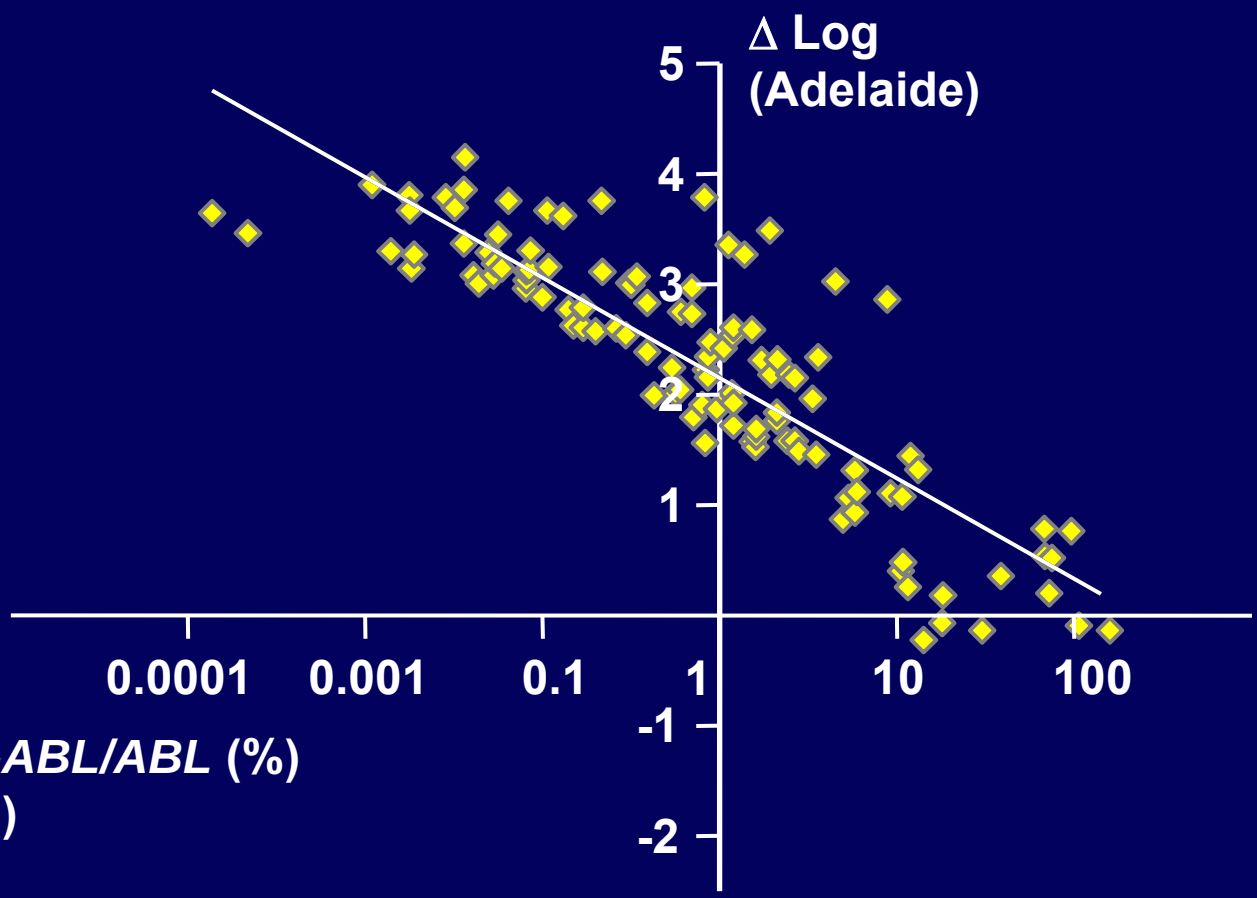
	ENR1162	ENF1102	
TaqMan	CCGAGTGAAGATCCCCTTTTTA	AATGAGCTCTCCAACCACGTATTTTC	101 bp
	ENR1162	GUS10-LC	
Lightcycler	CCGAGTGAAGATCCCCTTTTTA	GTGAAACCCTGCAATCGTTTCT	205 bp

Variability of Ratios BCR-ABL/ABL (%) in 36 laboratories



Müller et al., *Leukemia* 2007

Log Reduction (IRIS Method) vs Ratio *BCR-ABL/ABL*

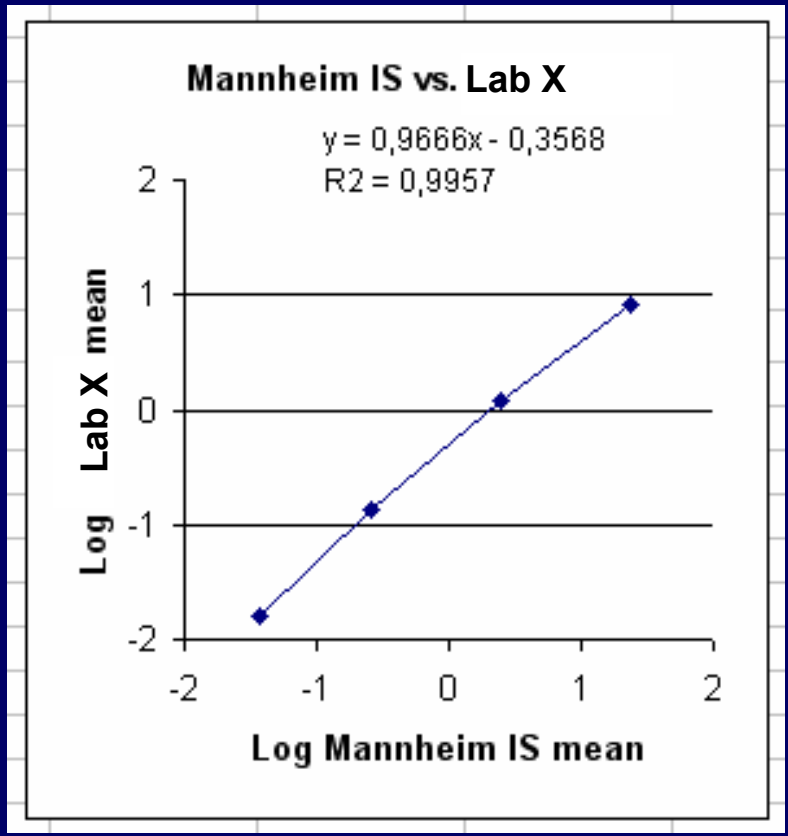


n=134

Log reduction = -0.91 [log (*BCR-ABL/ABL* (%)] + 2.15
3 log = 0.12%

Paschka et al. ASH, 2004.

„Good“ example



Formula: Log y = (slope × (log MMR^{IS})) + intercept

CF = MMR^{IS}/ antilog y

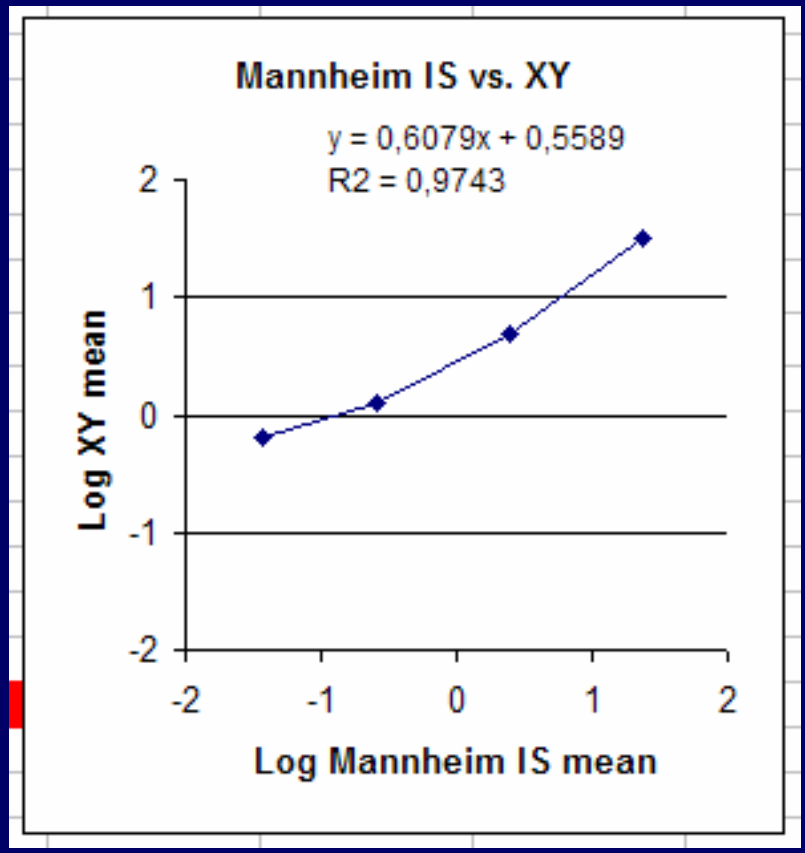
Example: Log y = (0.9666 × (-1)) – 0.3568

CF = 0.1/0.04749 = **2.1057**

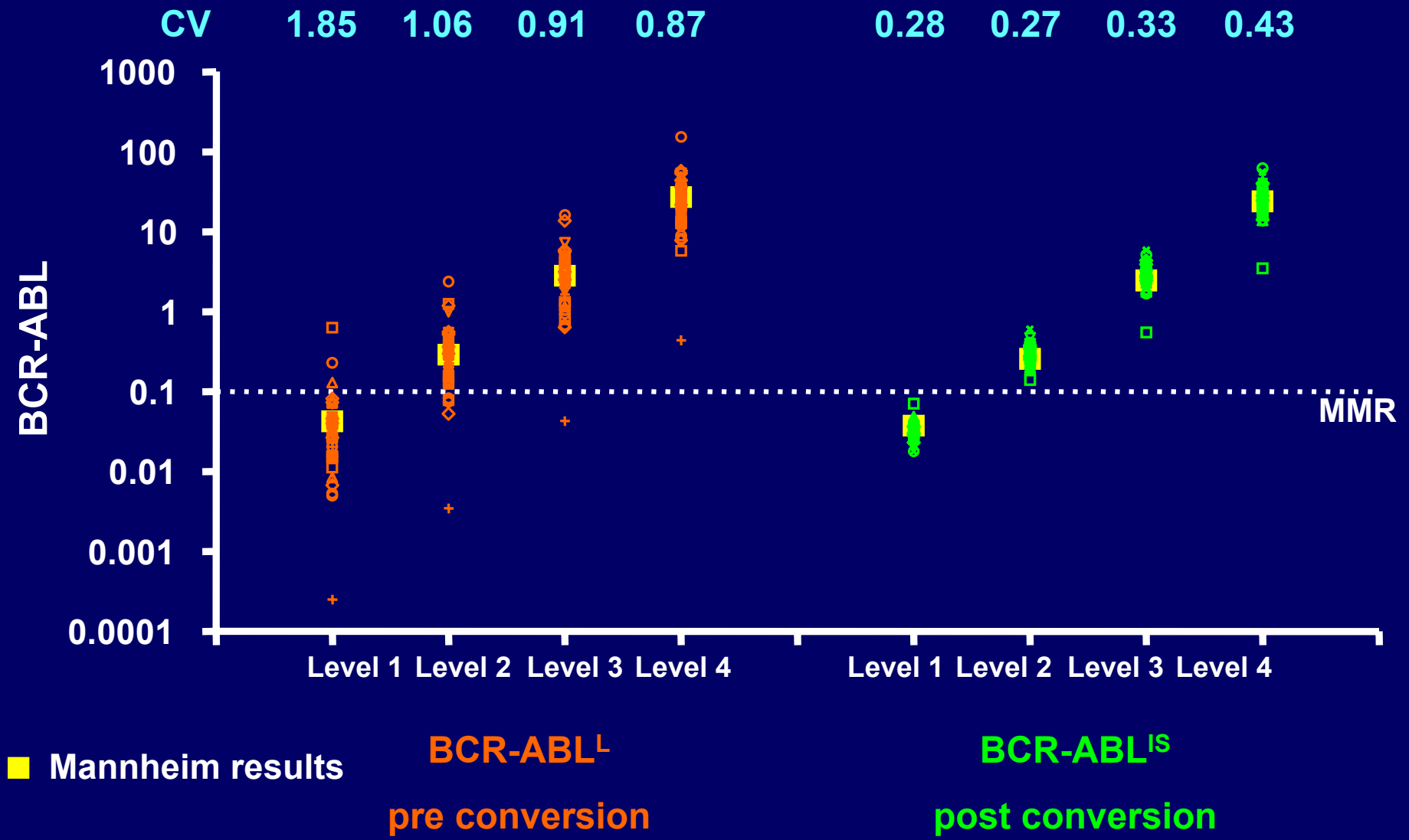
Mean values after conversion to the international scale (IS)		
	CF 0.878	**CF 2.1057**
	Mannheim mean	**Lab X** mean
Level 1	0,037	0,033
Level 2	0,26	0,29
Level 3	2,45	2,58
Level 4	23,81	17,13

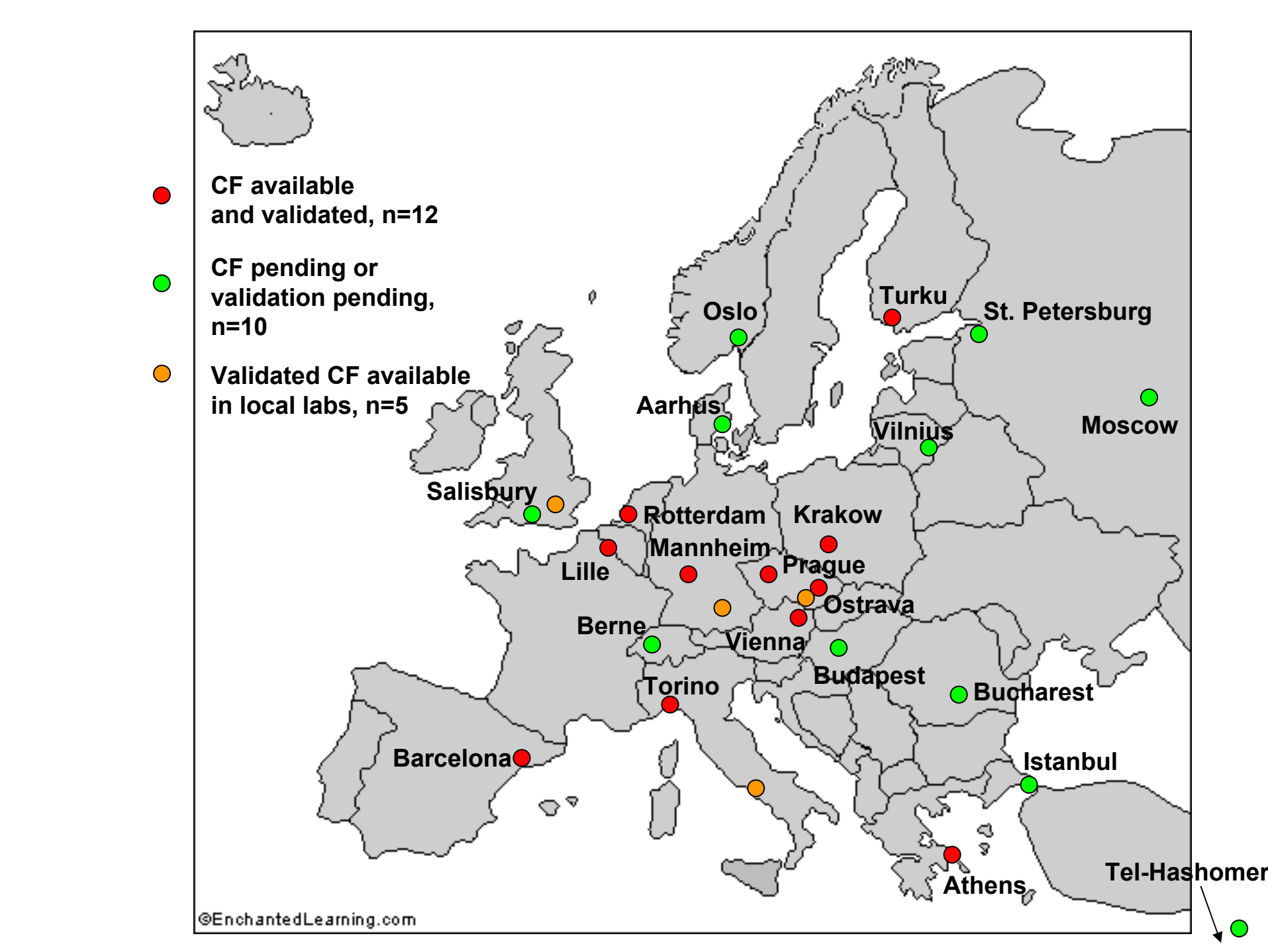
„Bad“ example

One of 4 labs with linearity problem



BCR-ABL levels in 37 labs pre and post conversion




CF available
and validated, n=12
CF pending or
validation pending,
n=10
Validated CF available
in local labs, n=5
Oslo
Turku
St. Petersburg
Moscow
Aarhus
Vilnius
Salisbury
Rotterdam
Krakow
Mannheim
Prague
Lille
Ostrava
Berne
Vienna
Budapest
Torino
Bucharest
Barcelona
Istanbul
Athens
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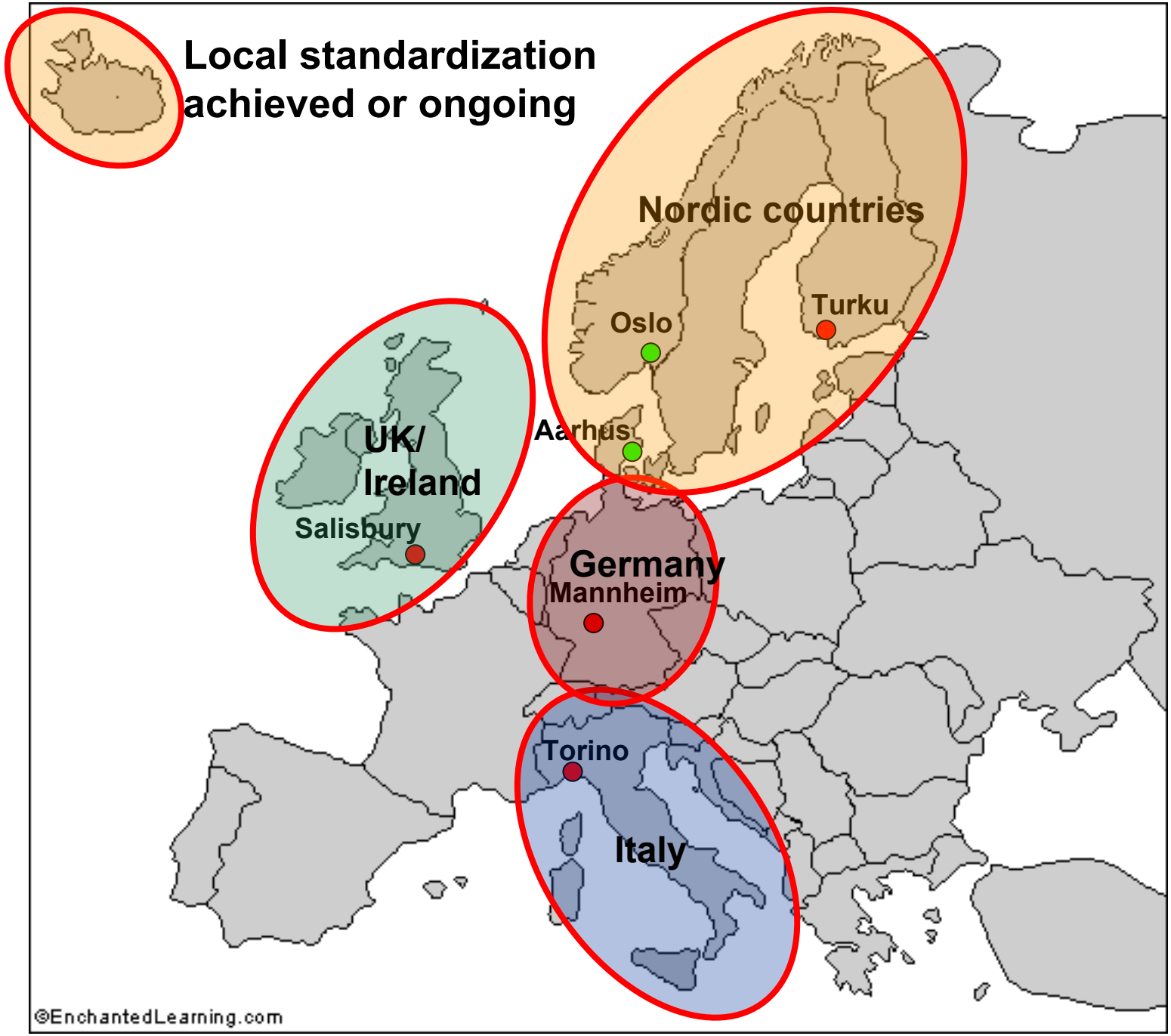
Local standardization
achieved or ongoing
Nordic countries
Oslo
Turku
Aarhus
UK/
Ireland
Salisbury
Germany
Mannheim
Torino
Italy
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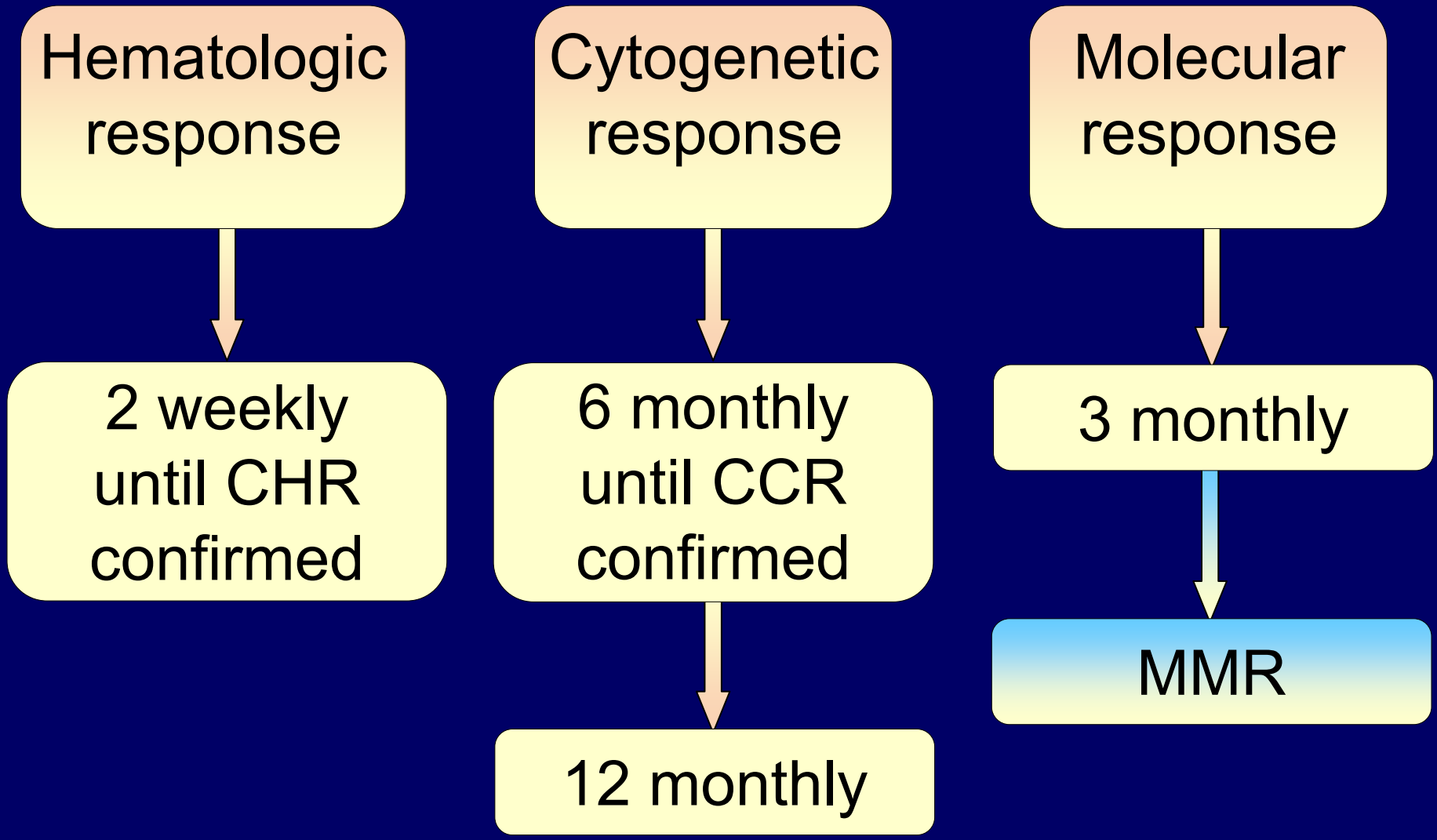
Recommendations by an Expert Panel



Baccarani et al, Blood;2006;108;1809

Value of Q-PCR as a Screen for Mutations

214 patients with serial Q-PCR tests

	>2-Fold Rise in *BCR-ABL*	
	YES	**NO**
	56 (26%)	**158 (74%)**
***BCR-ABL* mutations**	34/56 (61%)	1/158 (0.6%)
Acquired resistance		
Mutations	31/34 (91%)	1/1
No mutations	13/22 (59%)	1/158 (0.6%)

Branford et al. *Blood*. 2004.

God Bless

America

Français
Italiano
Deutsch
Español

BMS-354825 Discussion

"Caution" All Gleevec Patients, Please Read This!

"Click Here" for new Physician Interviews about CML and Gleevec (STI571)
Check Back Often...New Physician Interviews Are Added Regularly

You Can Help Win The "

Home
Jerry's Diary
AMN Discussion
BMS Discussion
CML Discussion
Childhood CML
GIST Discussion
Gleevec FAQ
Gleevec History
Thanks Dr. "D"

PCR

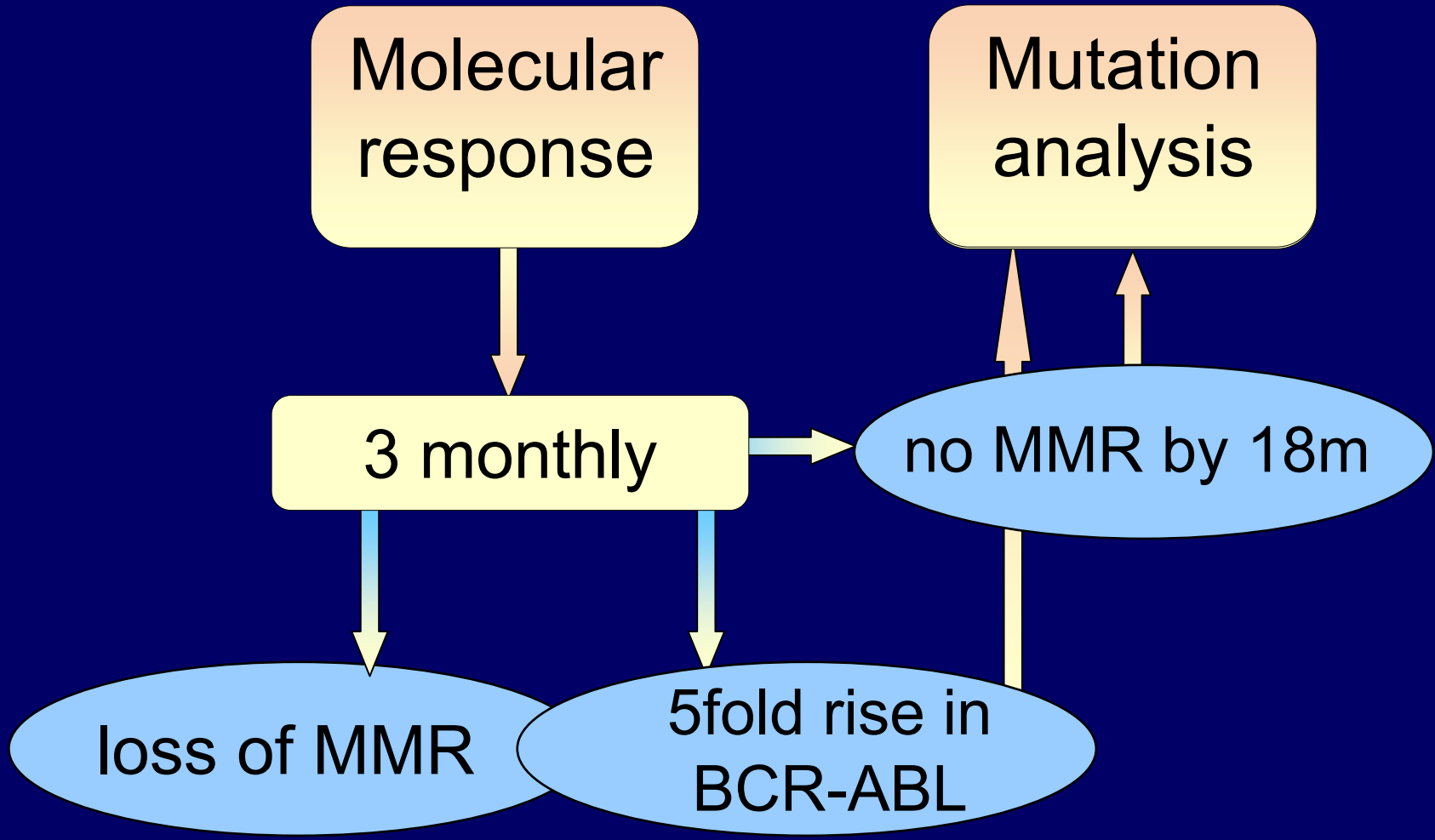
by Sharon T

three months i had my first PCR quantative test and the results were 0.001%. Today it was 0.027% My Dr. says it is a very sensitive test and that I should not be worried. I will have another in three months. Is 0.027% a bad number?
Help?
Sharon T

Name:

Email:

Recommendations by an Expert Panel



Baccarani et al, Blood;2006;108;1809

European LeukemiaNet

Mutation testing:
The impact of 2nd generation TK inhibitors

Andreas Hochhaus

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Ruprecht-Karls-Universität Heidelberg
Mannheim, Germany

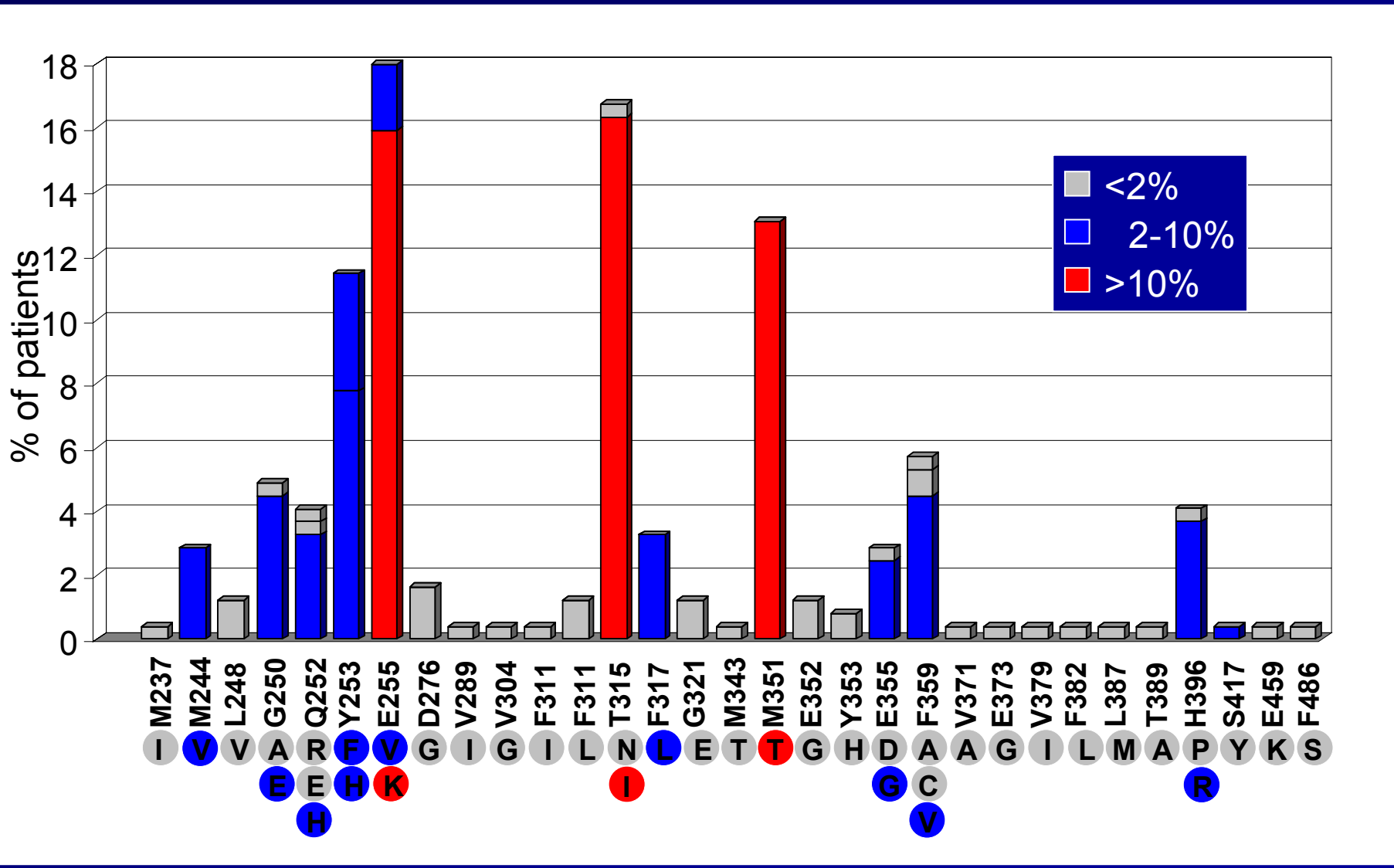
Molecular - Cytogenetic Causes of Resistance

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

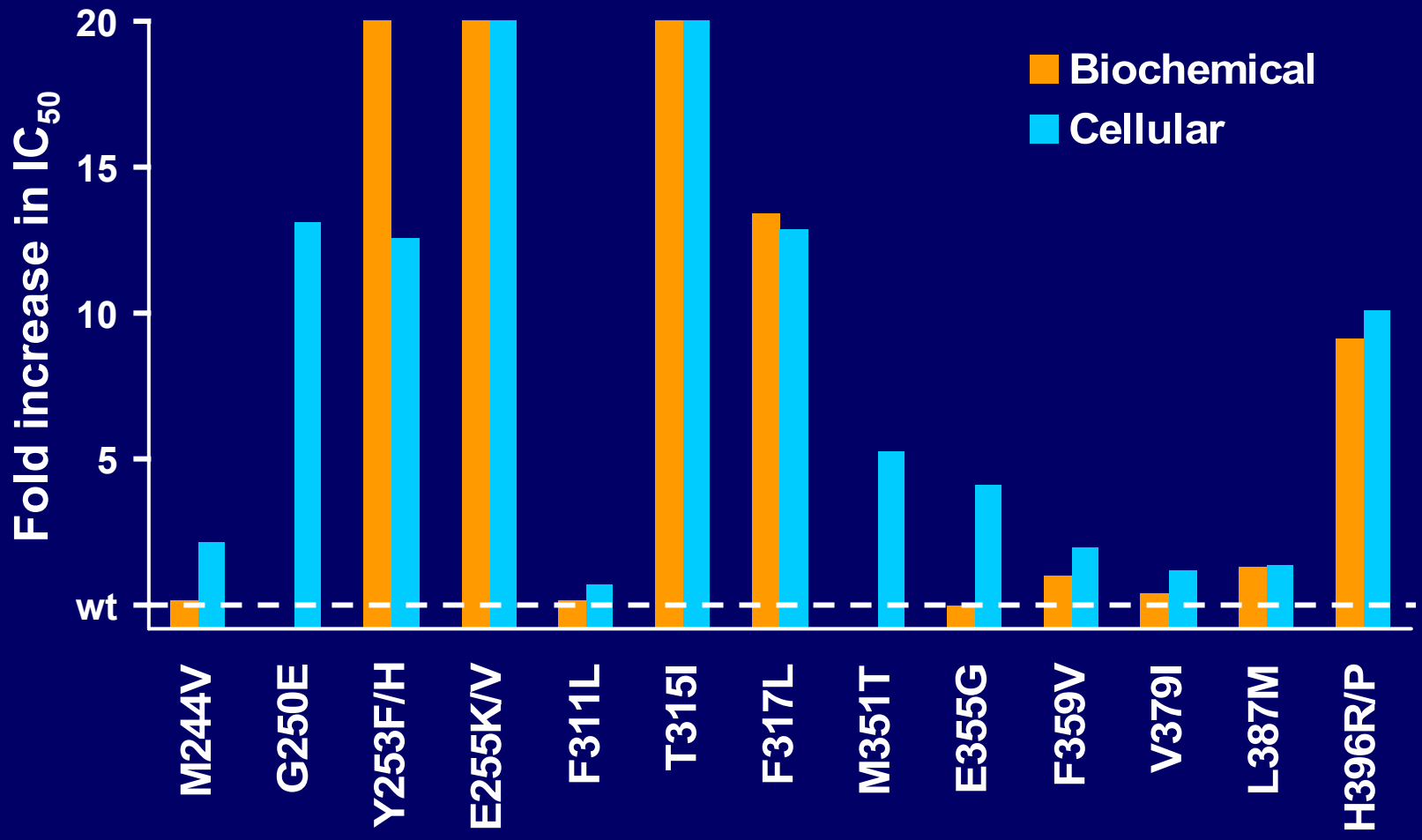
Lahaye et al. *Cancer*. 2005

Detection of BCR-ABL mutations

Kinase domain mutations associated with imatinib resistance



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



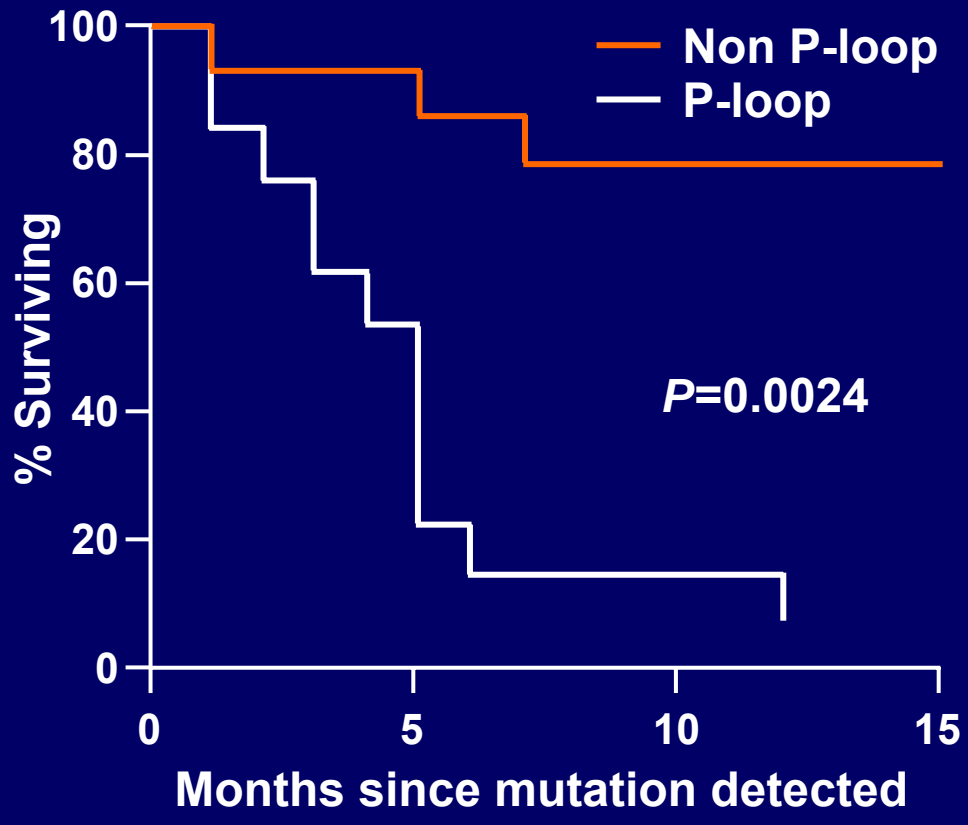
Corbin et al. *Blood.* 2003.

Survival After Imatinib Resistance

Initial Australian experience, n=27

P-Loop: Highly conserved area

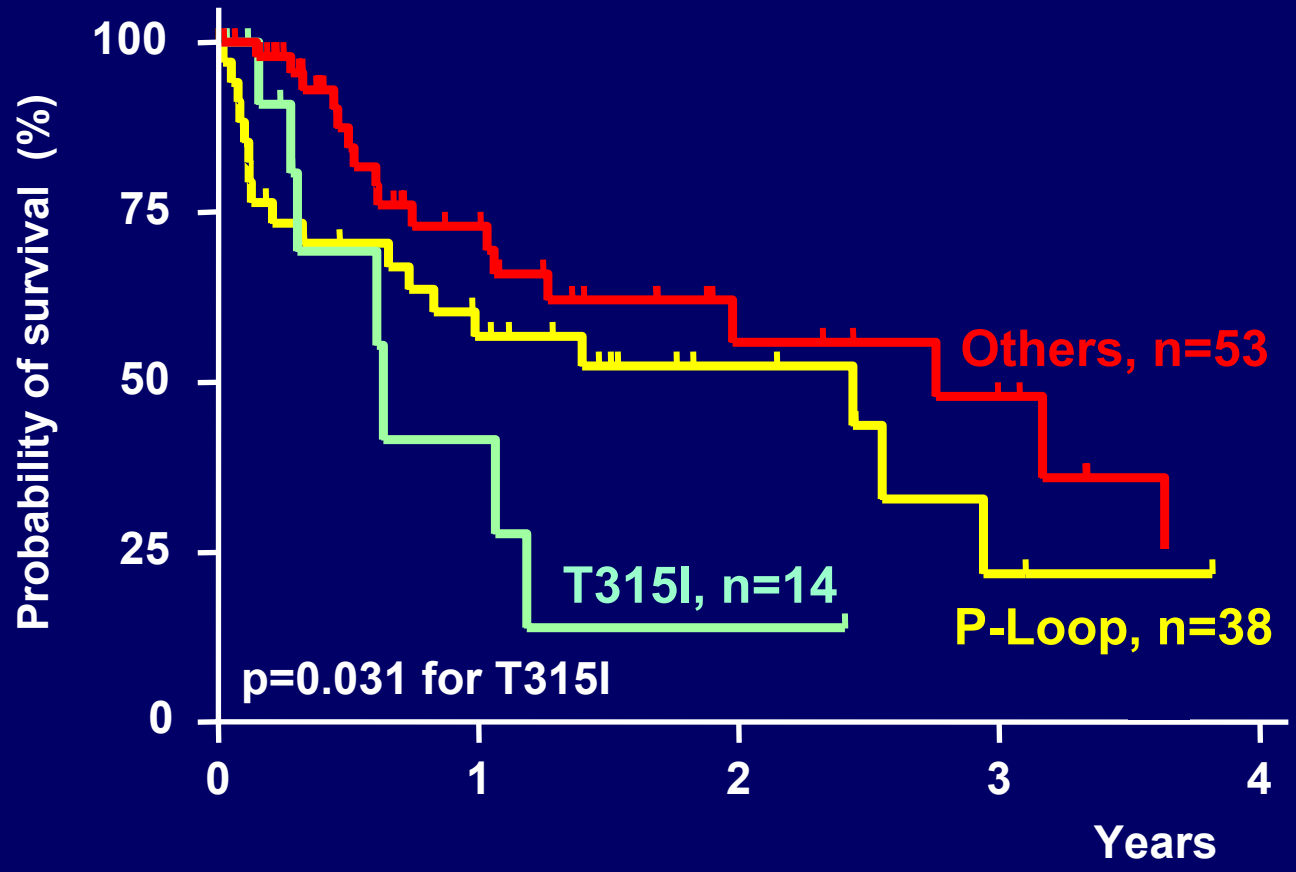
250		252	253		255		
G	G	G	Q	Y	G	E	V
	E		H	F		K	
	R			H		V	



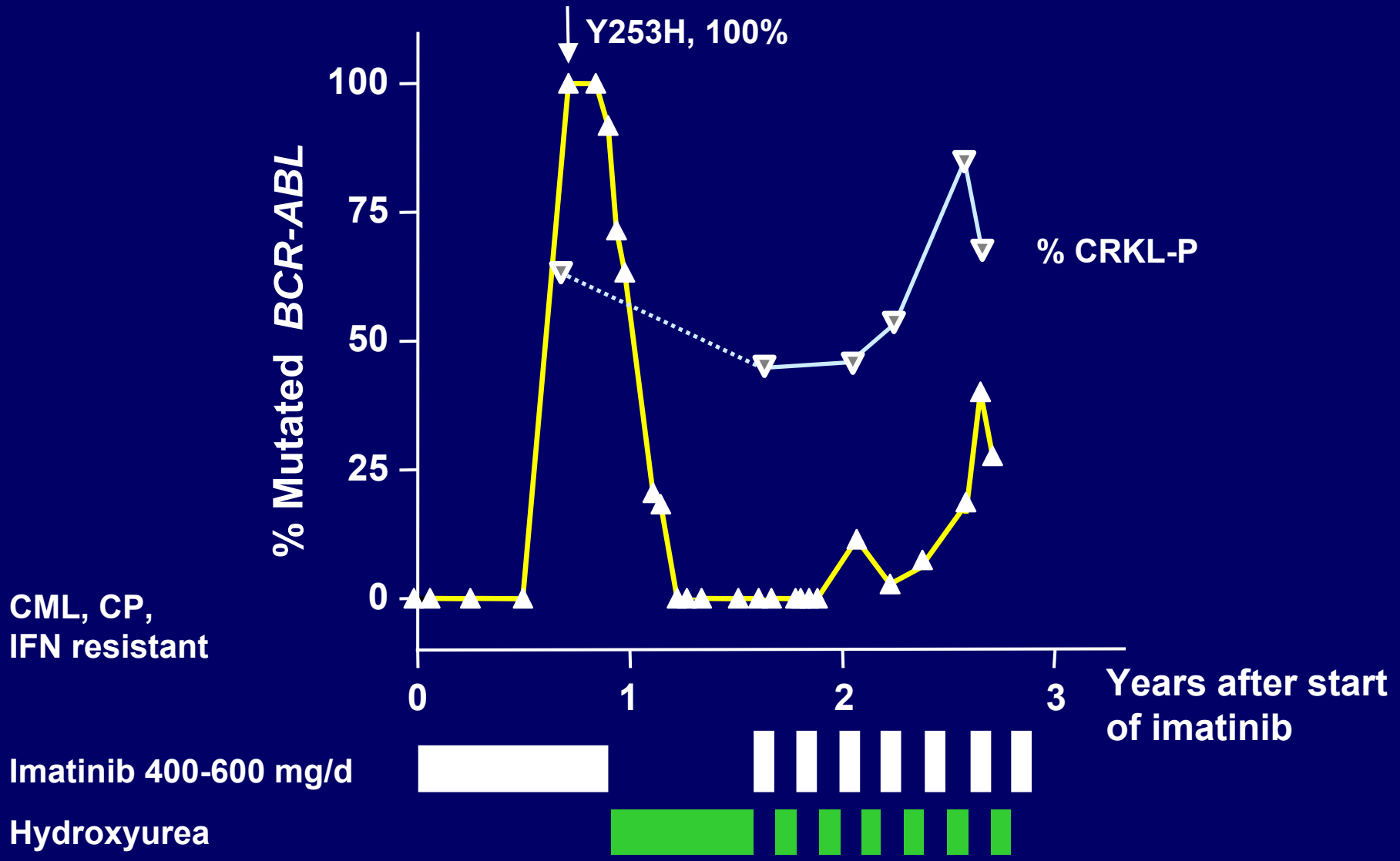
Branford et al. *Blood.* 2003.

Survival After Imatinib Failure

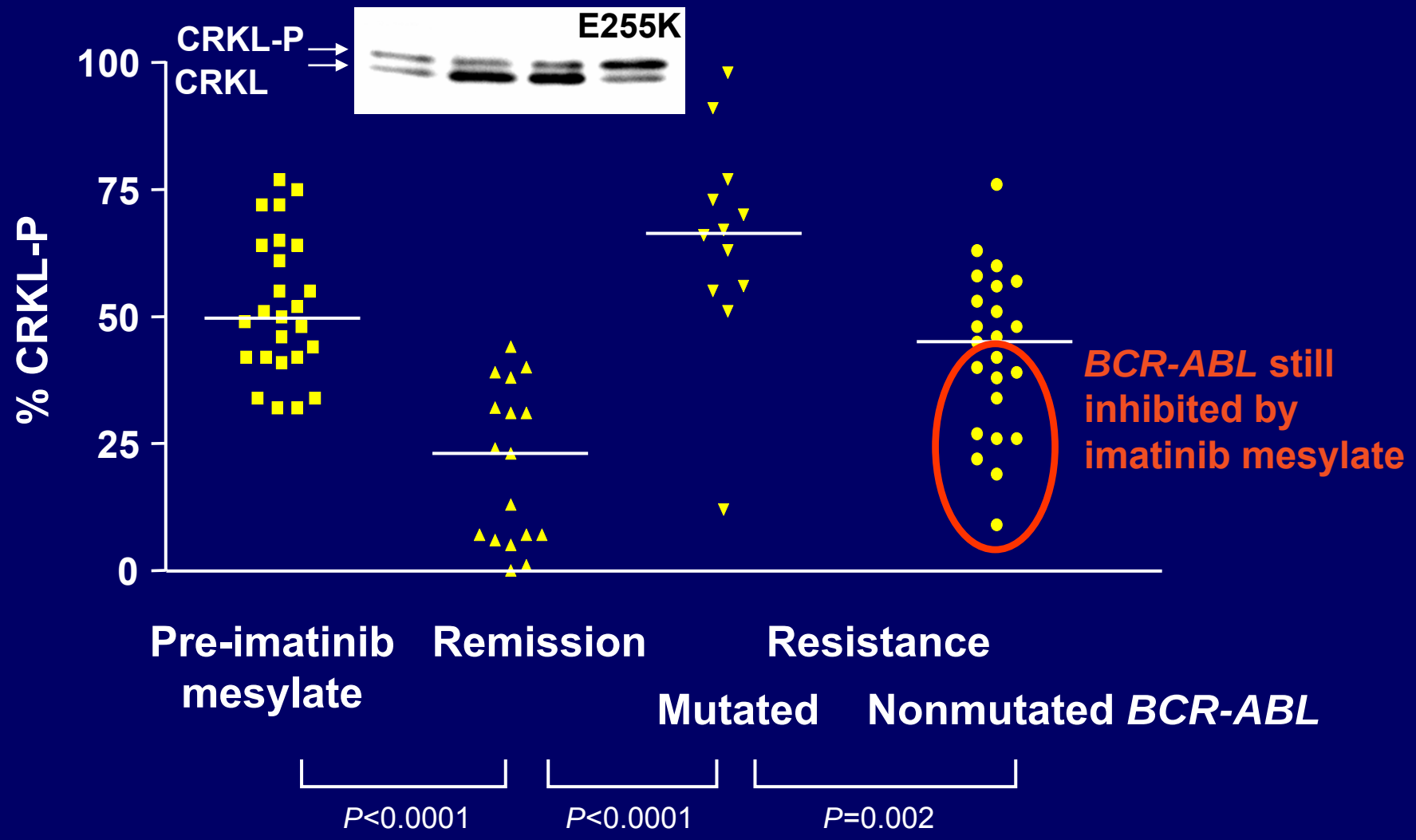
Current Experience, n=105



Dynamics of resistance clones

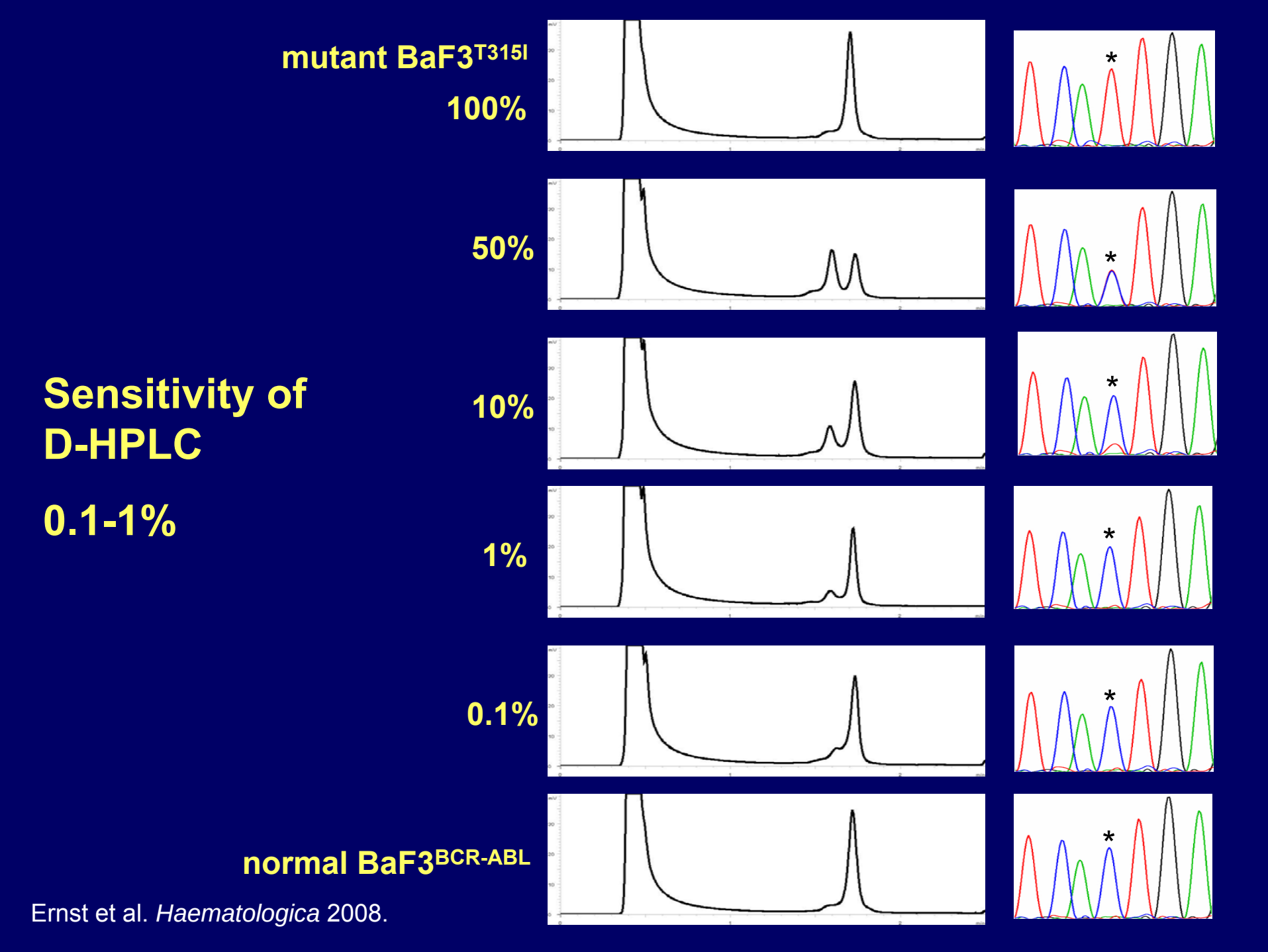


CRKL-P in Resistant Patients

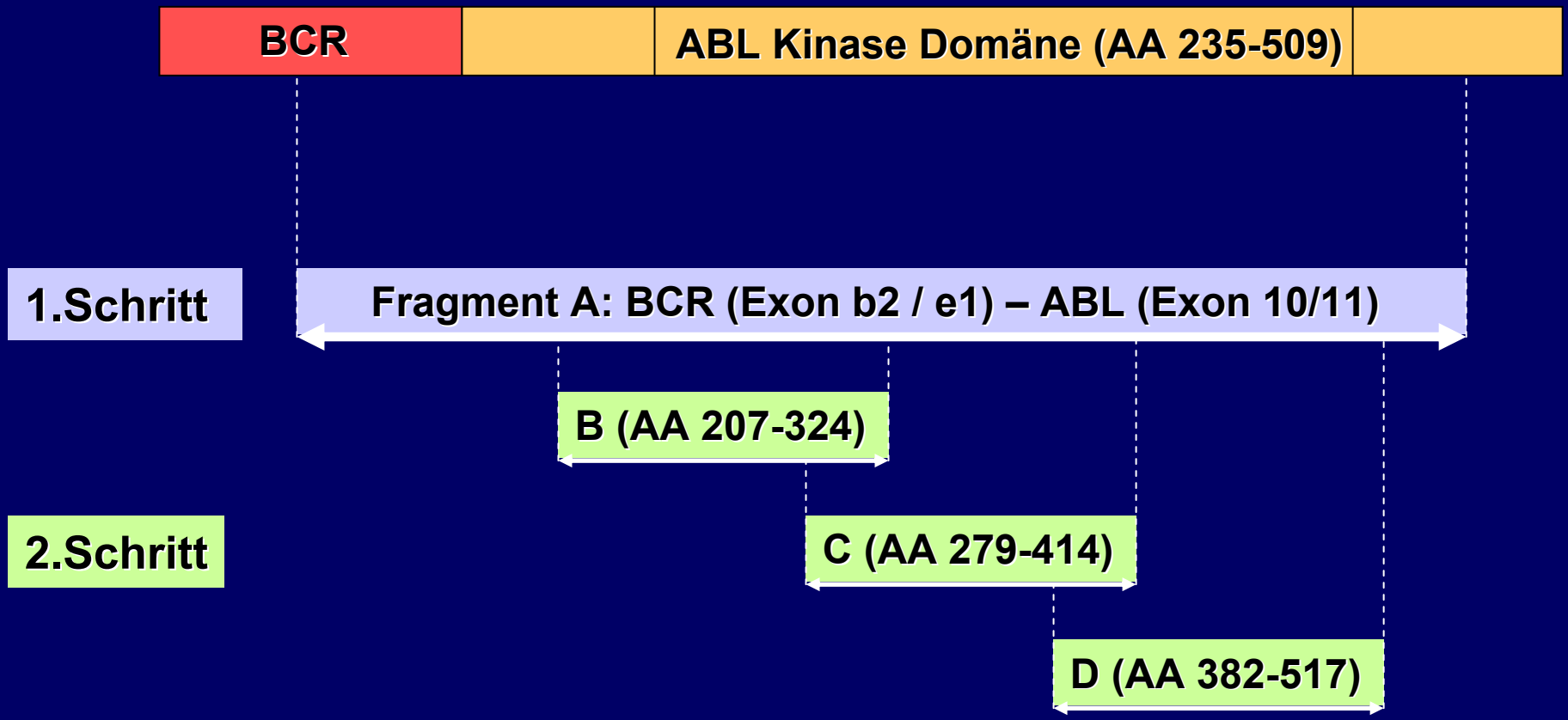


Methods to detect and quantify BCR-ABL mutations

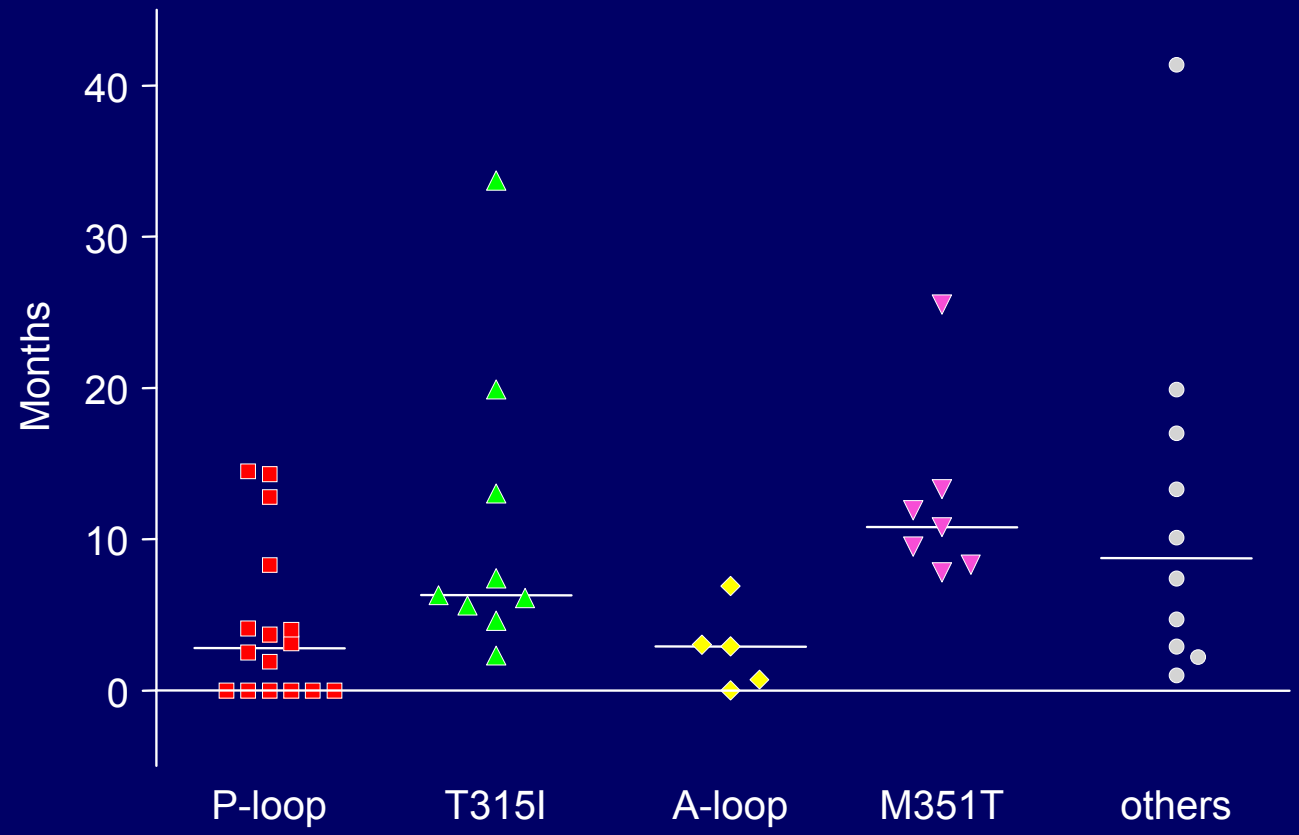
	Specificity	Sensitivity
Sequencing	non specific	~10%
Restriction digest analysis	specific	~2-5%
D-HPLC	non specific	0.1-1%
Allele specific PCR	specific	0.01%
Sequencing of clones	non specific	1-5%


mutant BaF3T315I
100%
50%
10%
1%
0.1%
normal BaF3BCR-ABL
Sensitivity of D-HPLC
0.1-1%
*
Ernst et al. Haematologica 2008.

D-HPLC: Nested-PCR



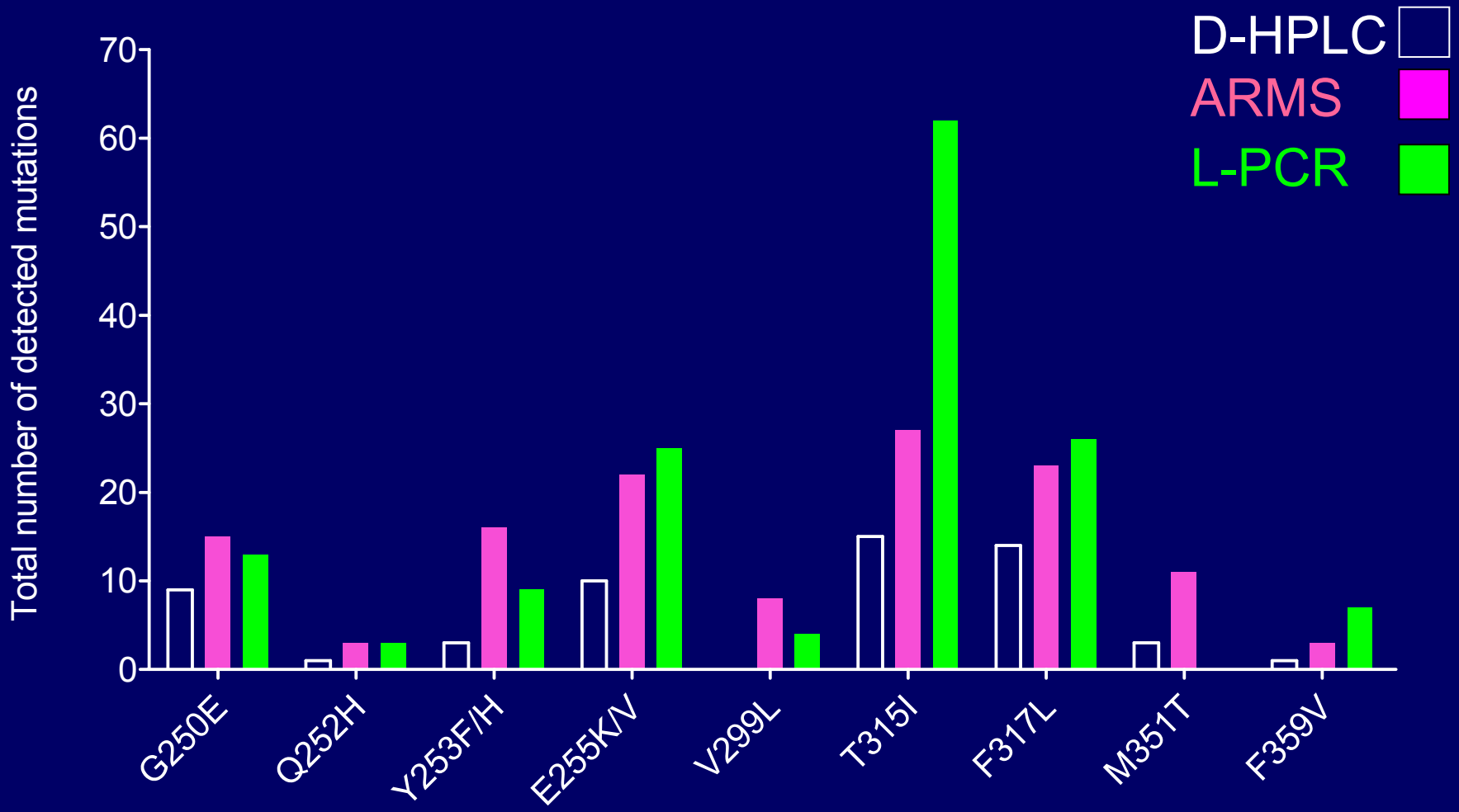
Interval of D-HPLC positivity to hematological relapse

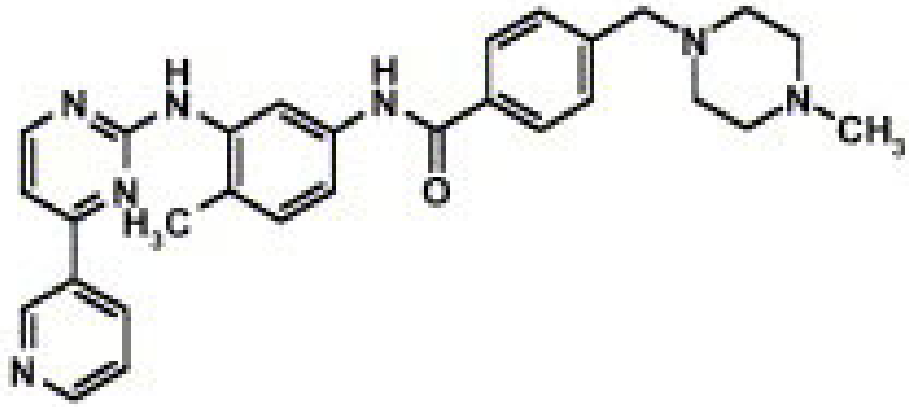


	P-loop	T315I	A-loop	M351T	others
Median (months)	**2.8**	**6.3**	**2.9**	**10.8**	**8.7**

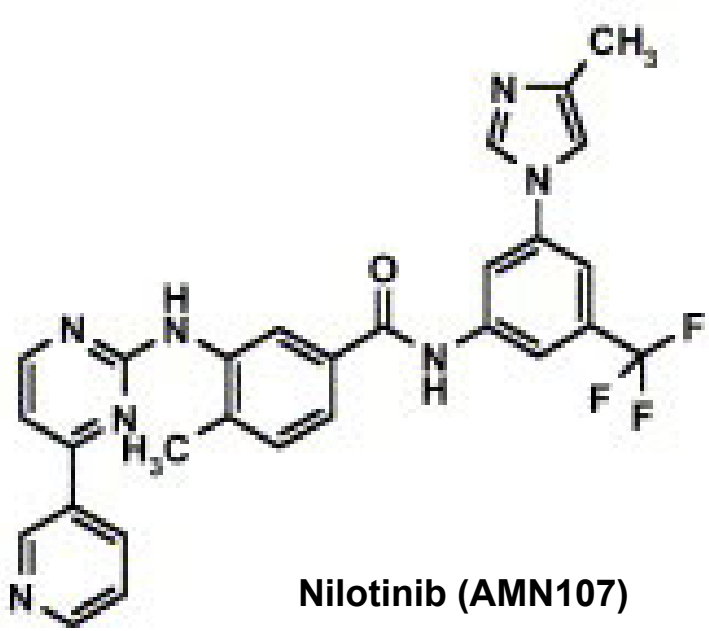
Ernst et al. *Haematologica* 2008.

Comparison of D-HPLC vs. allele-specific PCRs for 11 key mutations

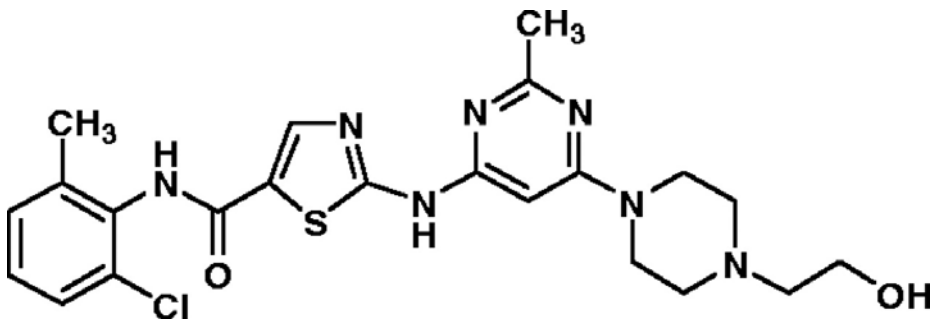




Imatinib (STI-571)



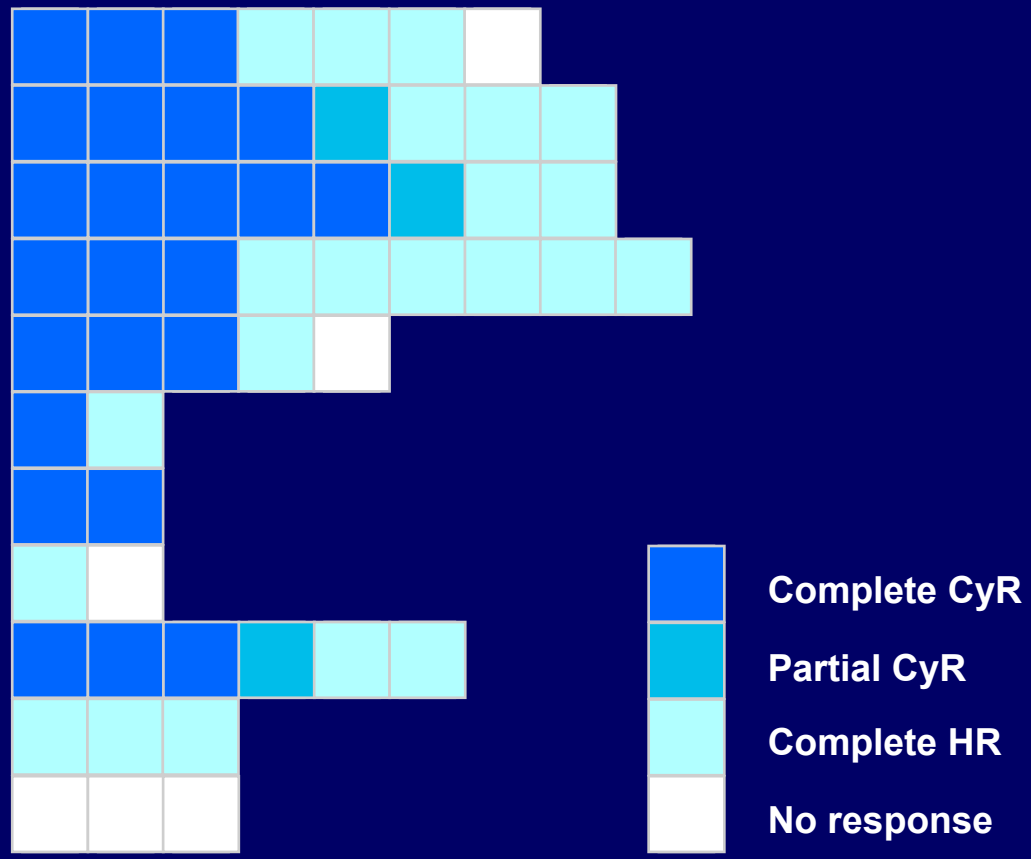
Nilotinib (AMN107)



Dasatinib (BMS-354825)

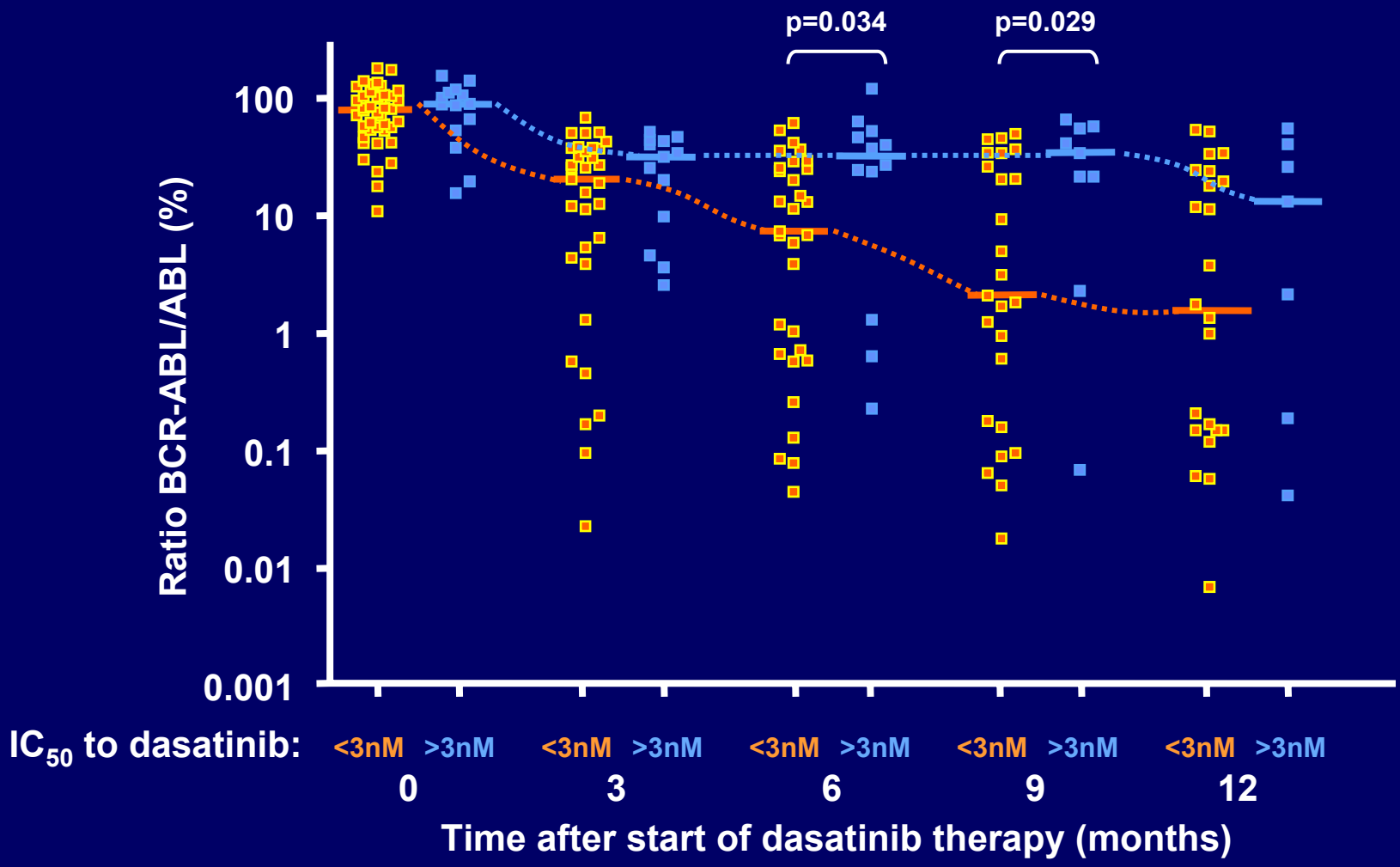
Response to dasatinib is associated with cellular IC_{50} of post-imatinib mutation (CP-CML)

	Cellular IC_{50}		
	Dasatinib nM	Imatinib nM	n
H396P/R	0.6-1.3	850-4200	7
M351T	1.1	930	8
M244V	1.3	2000	8
G250E	1.8	1350-3900	9
Y253F/H	1.3-10	>10000	5
L387M	2	1000	2
F359V	2.2	1200	2
Q252H	3.4	1300-3900	2
E255K/V	5.6-13	4400-8400	6
F317L	7.4-18	810-1500	3
T315I	>1000	>10000	3



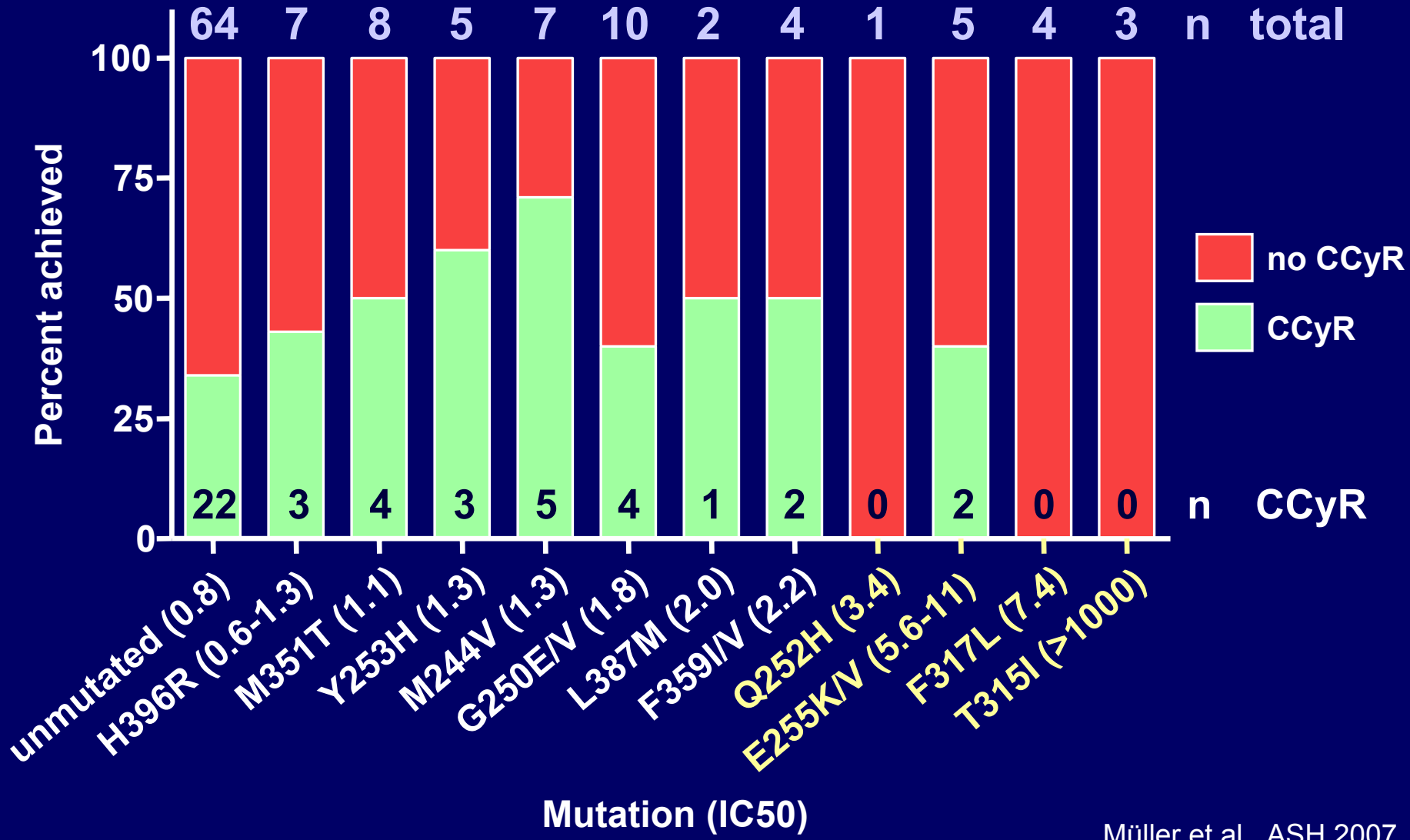
Müller et al., ASH 2006

Differential kinetics of response to dasatinib depending on cellular IC_{50} of pretreatment mutation



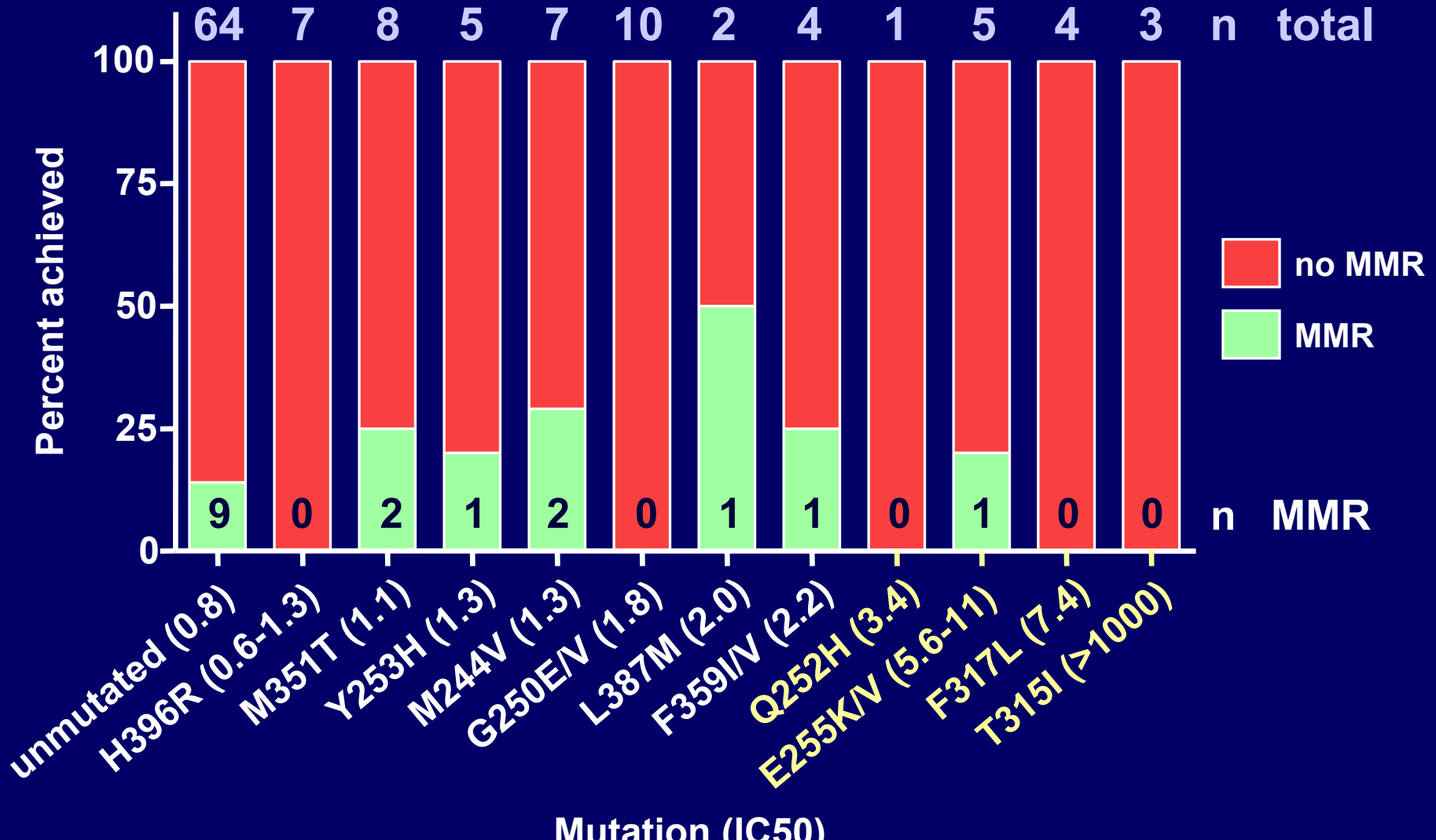
Müller et al., ASH 2006

Achievement of CCyR by dasatinib according to baseline mutations



Müller et al., ASH 2007

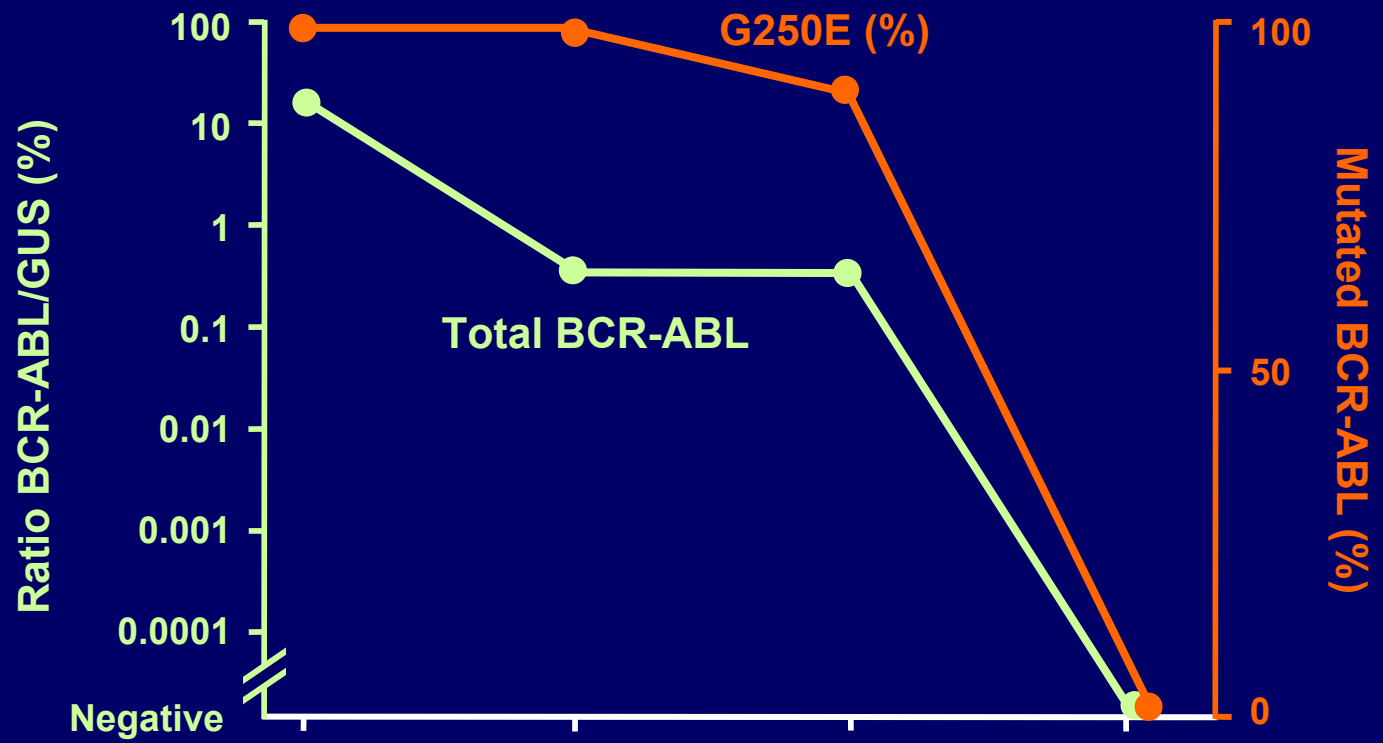
Achievement of MMR (<0.1% BCR-ABL^{IS}) after 24 months by dasatinib according to baseline mutations



Müller et al., ASH 2007

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

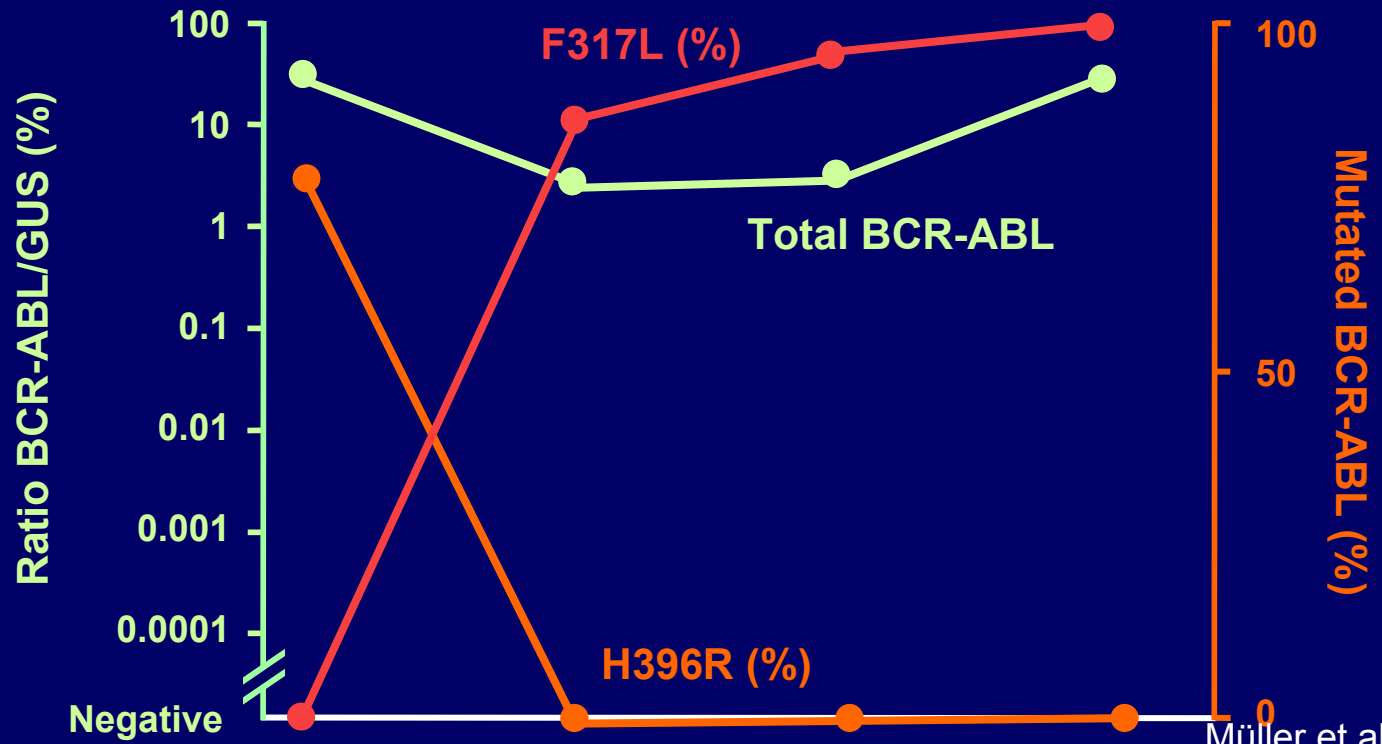
- **Pattern 1: n=30**
  - ⇨ **Baseline mutation disappeared AND BCR-ABL decreased**



Müller et al., ASH 2007

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

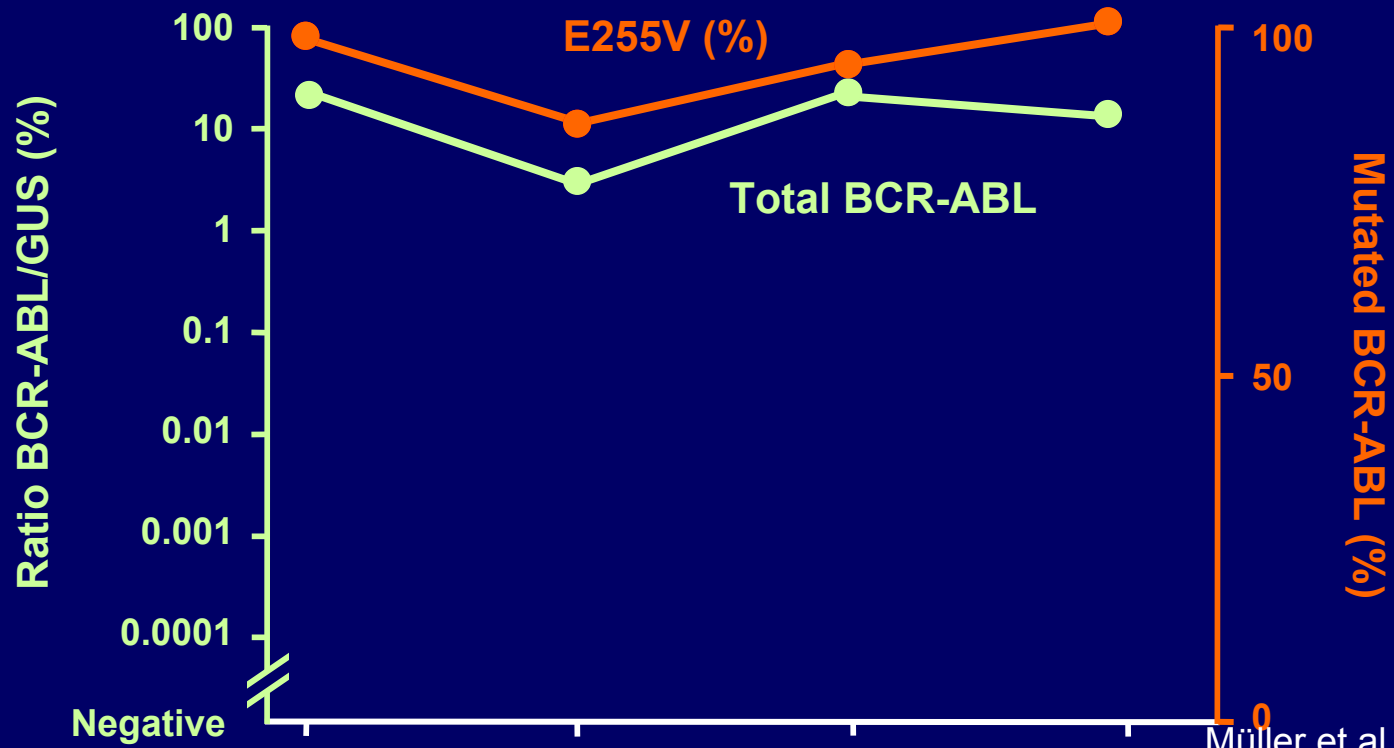
- **Pattern 2: n=23**
  - ⇨ **Baseline mutation disappeared AND BCR-ABL persisted**
    - **– new mutations evolved in n=12 patients**
      (T315I n=3; F317L n=7; M351T n=2; Y320C n=1)



Müller et al., ASH 2007

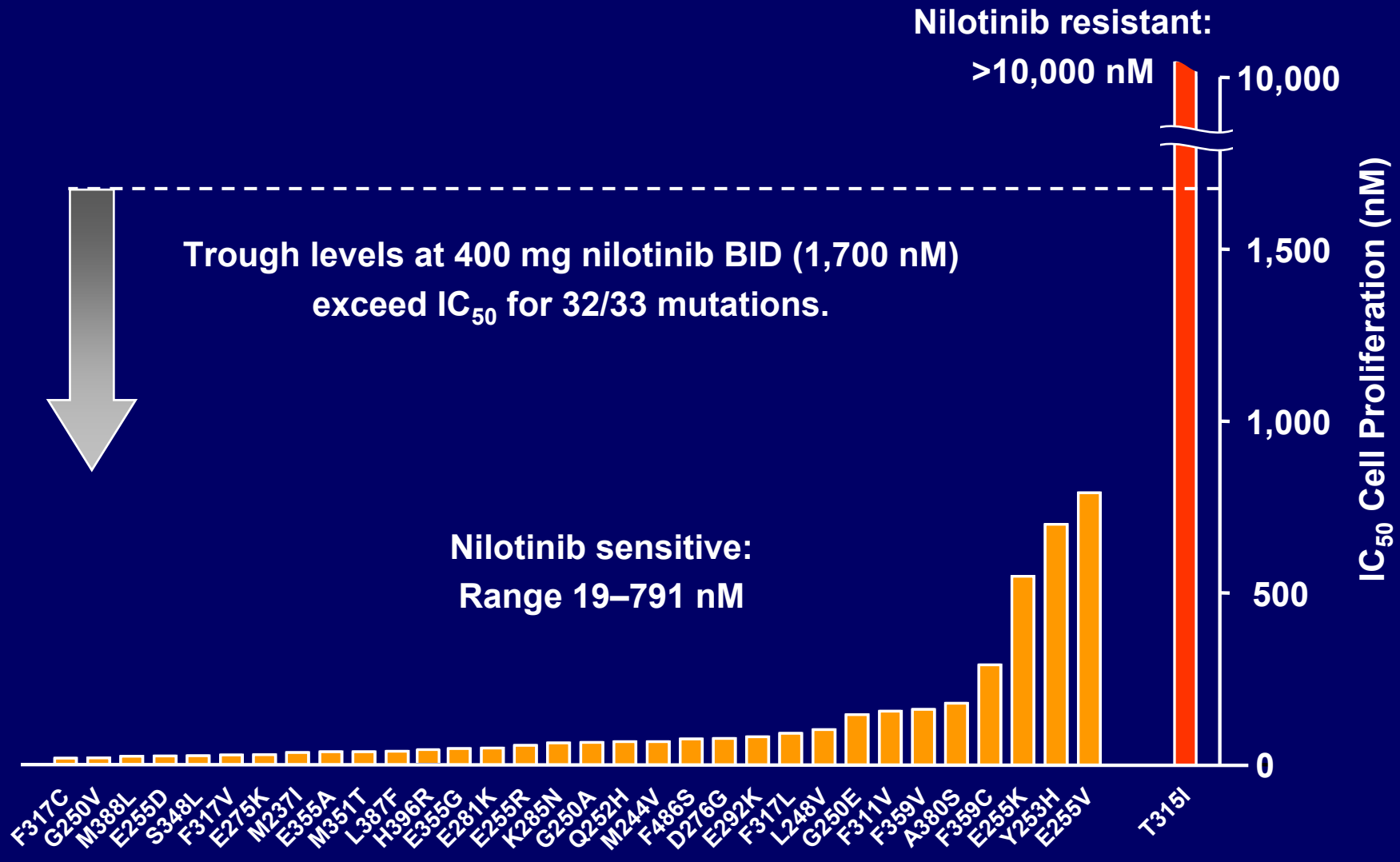
Course of mutated and unmutated BCR-ABL – 3 patterns –

- **Pattern 3: n=18**
  - ⇨ **Baseline mutation persisted AND BCR-ABL persisted**
    - – **additional mutations evolved in n=3 patients (T315A n=1; V299L n=2)**



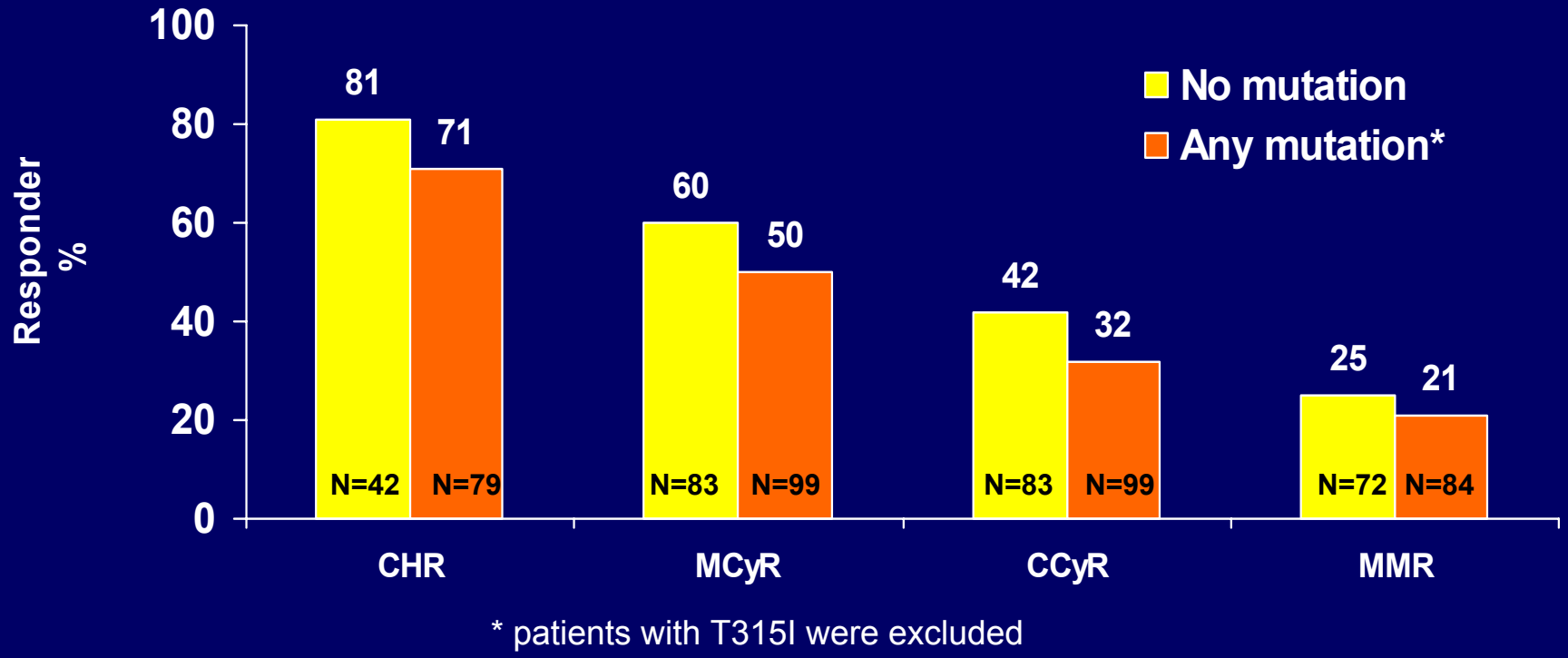
Müller et al., ASH 2007

Nilotinib sensitivity of cells expressing imatinib-resistant BCR-ABL with point mutations



Manley PW, et al. *Proc Am Assoc Cancer Res*. 2005

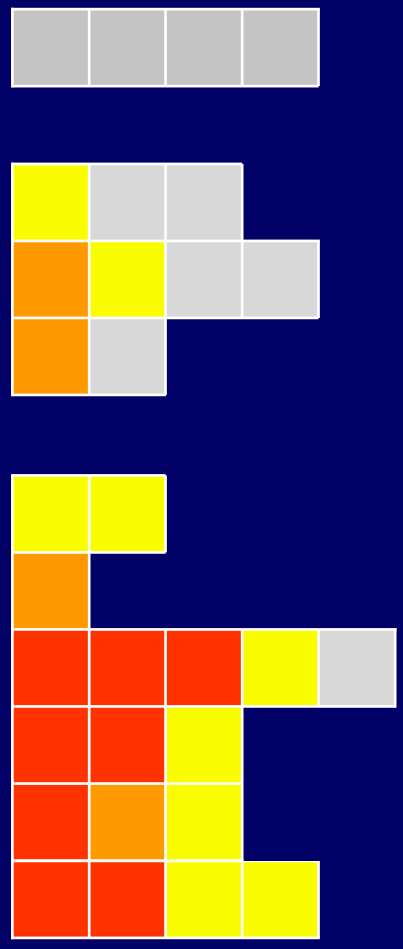
Best Responses to Nilotinib in Imatinib-Resistant Patients with and without Baseline Mutations by 12 Months

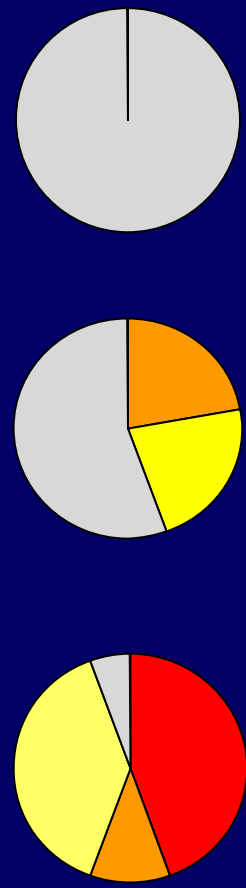


- Clinical efficacy of nilotinib demonstrated in both mutant and non-mutant group

CML-CP: Best response within 6 months by cellular IC_{50} to nilotinib

Baseline Mutation	Cellular IC_{50} (nM)
T315I	>10,000
Y253H	700
E255K	548
F359C	161
F317L	91
D276G	77
M244V	67
E355G	47
H396R	41
M351T	38





IC_{50} >10,000 nM:
4 no response

IC_{50} >100 nM:
2 PCyR
2 CHR
5 no response

IC_{50} <100 nM:
8 CCyR
2 PCyR
7 CHR
1 no response

ASH 2006

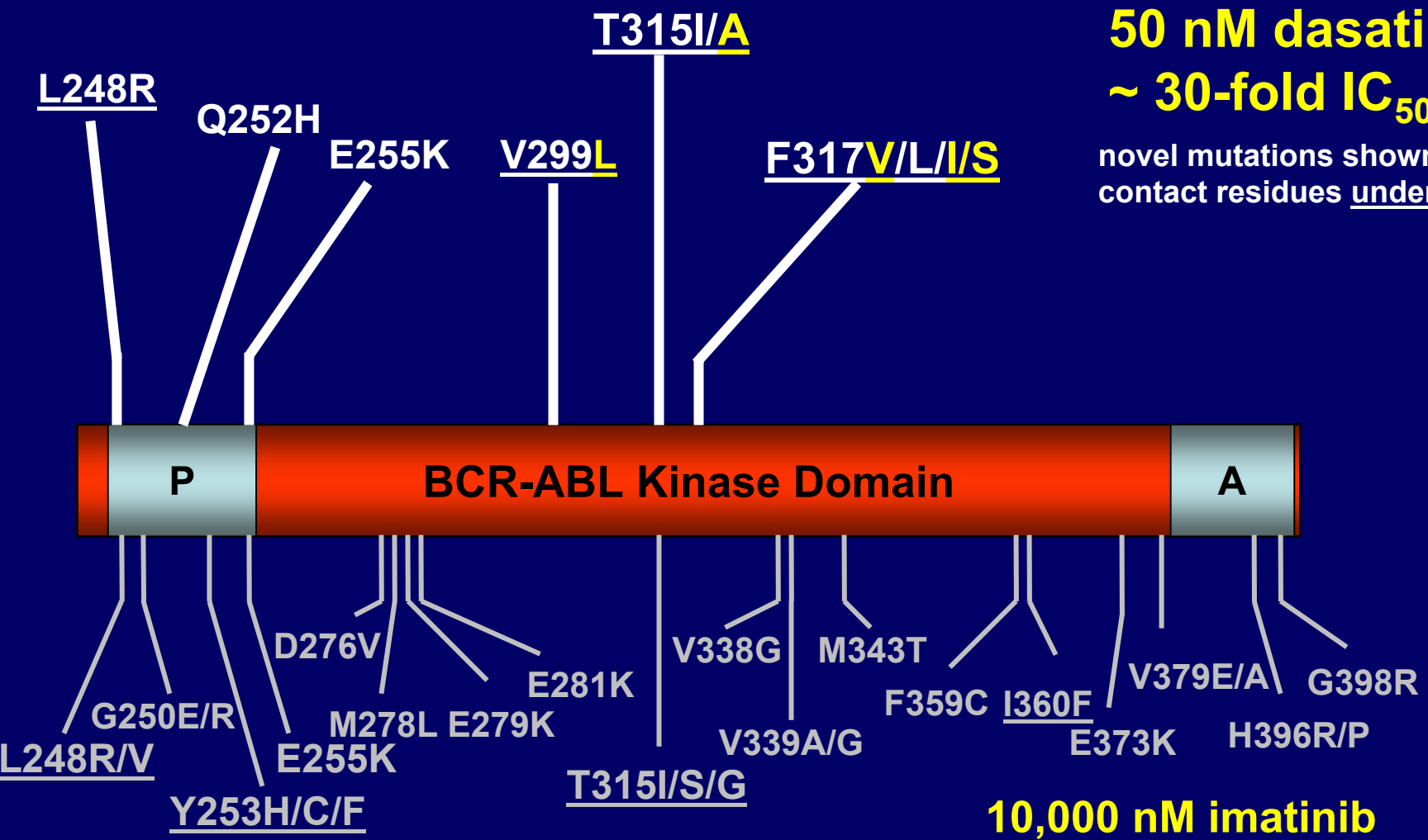
Best Responses to Nilotinib by 12 Months in Imatinib-Resistant Patients with Different Mutant Types

Mutation	IC_{50} (nM)	CHR n/N (%)	MCyR n/N (%)	CCyR n/N (%)	MMR n/N (%)
No Mutation		34/42 (81)	50/83 (60)	35/83 (42)	18/72 (25)
IC50 ≤150 nM		31/38 (82)	28/49 (57)	18/49 (37)	12/44 (27)
IC50 >150 nM					
Y253H	700	0/6 (0)	1/8 (13)	0/8 (0)	0/7 (0)
E255K/V	548/791	5/8 (63)	3/8 (38)	0/8 (0)	1/7 (14)
F359C/V	258/161	2/9 (22)	1/10 (10)	0/10 (0)	0/9 (0)
Others		14/17 (82)	19/38 (50)	14/38 (37)	5/28 (18)

* patients with T315I were excluded

- Patients with mutations of IC_{50} ≤150 nM achieved comparable responses to the non-mutant group
- Less favorable responses seen in patients with Y253H, E255K/V, and F359C/V.

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



50 nM dasatinib
~ 30-fold IC_{50}

novel mutations shown in yellow
contact residues underlined

10,000 nM imatinib
~ 30-fold IC_{50}

adapted from Azam *et al*.

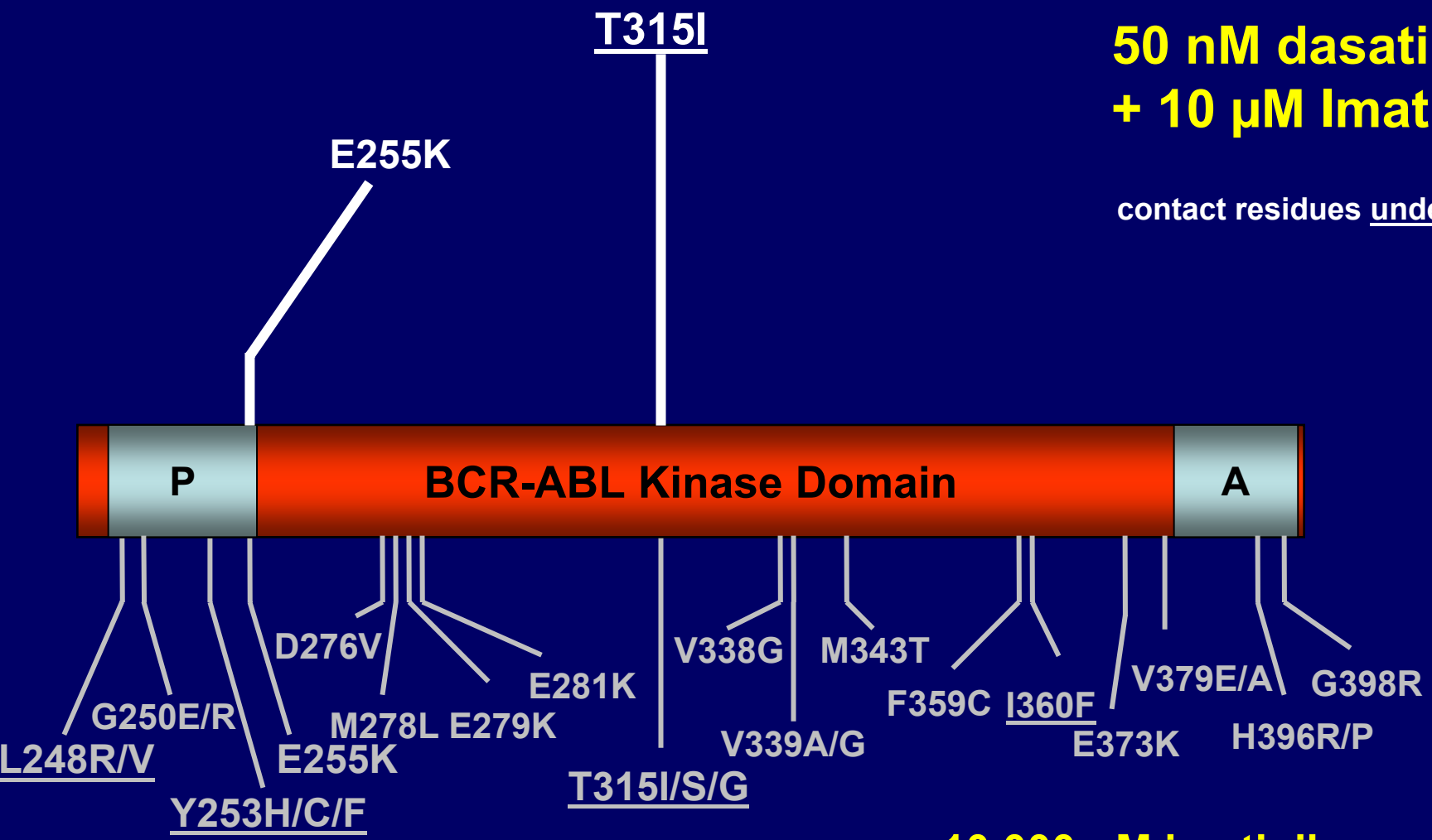
Burgess et al, PNAS, 2005

Distinct mechanisms of resistance to imatinib and dasatinib

BaF3 Clone	IC_{50} for growth (nM)	
	Imatinib	Dasatinib
Unmut. BCR-ABL	<1000	<5
T315I	>10000	>500
T315A	1000	100
F317L	2000	10
F317V	<1000	60
V299L	1000	20
L248R	>10000	20

Burgess et al, ASH 2004

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



50 nM dasatinib
+ 10 µM Imatinib

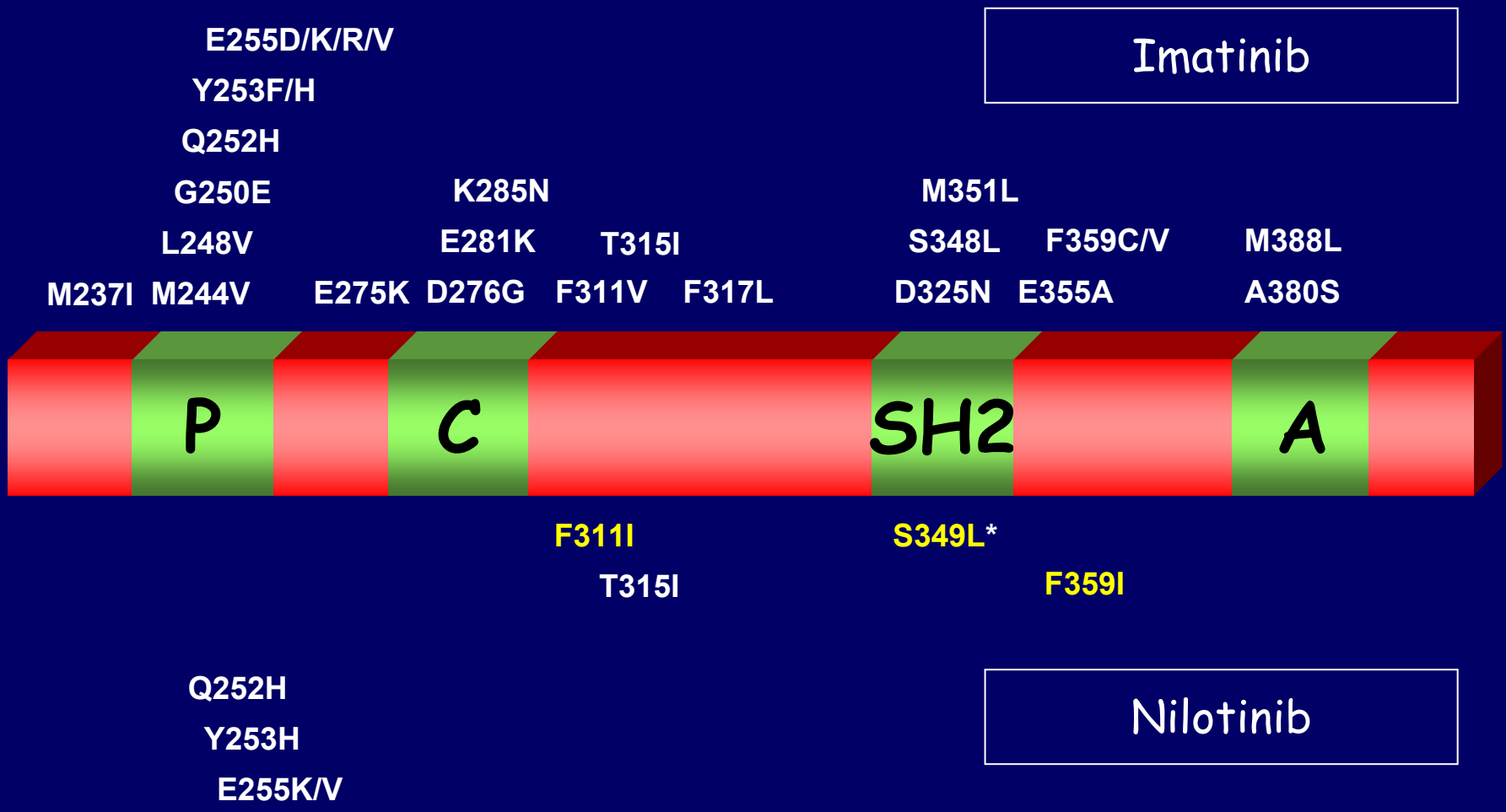
contact residues underlined

10,000 nM imatinib
~ 30-fold IC_{50}

Burgess et al, PNAS, 2005

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

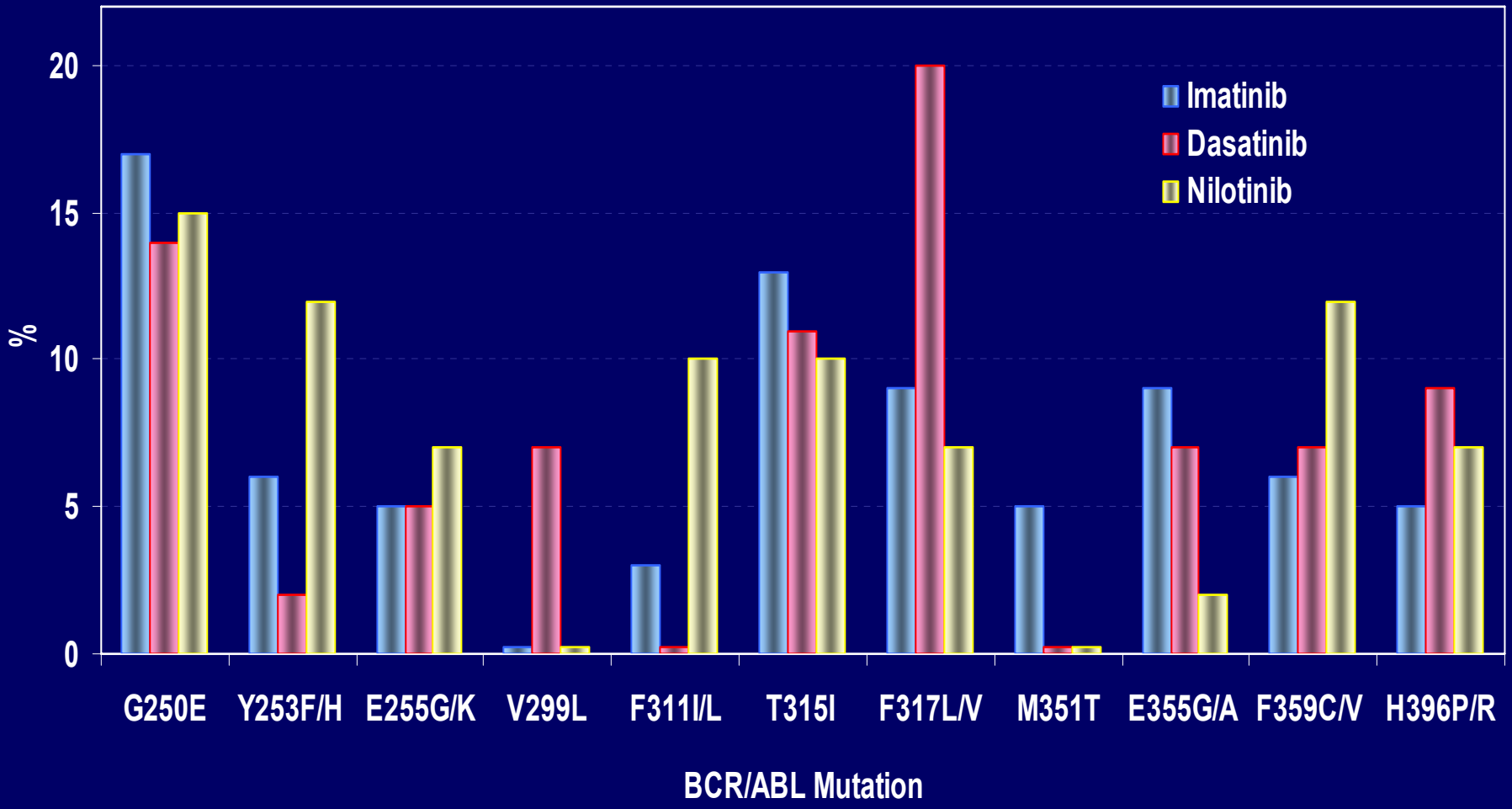
Nilotinib produces a limited number of resistance mutations



* Q252H/S349L Double mutant

von Bubnoff et al. Blood 2006

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



- T315I and F359V recovered after treatment with Bosutinib

Jabbour et al., ASH 2006

Emerging BCR-ABL mutations on Dasatinib

- **New mutations: n=22**
  - **F317L n=7; T315I n=3; V299L n=2; T315A n=1; M351T n=2; Y320C n=1; L248V n=1; G250E n=1; Y232H n=1; K271R n=1; A344V n=1; E507G n=1**
  - **Incidence: 22 cases in 288 yrs pt. exposure in 201 pts ⇨ 7.6%/patient-year**
  - **Time to detection: 11 months (median, range 3–23)**
  - **Baseline mutation: in 17 of 22 (77%) patients**
  - **Post CCyR: 8/22 pts ⇨ risk to develop new mutations after achievement of CCyR 8/55 = 14.5%**
  - **Post MMR: 4/22 pts ⇨ risk to develop new mutations after achievement of MMR 4/30 = 13.3%**

Müller et al., ASH 2007

Newly Detectable Mutations During Nilotinib Therapy

	All		Resistant		Intolerant	
	n/N	(%)	n/N	(%)	n/N	(%)
All Emerging Mutations	43/275	(16)	40/187	(21)	3/88	(3)
With Baseline Mutations	30/113	(27)	29/104	(28)	1/9	(11)
Without Baseline Mutations	13/162	(8)	11/83	(13)	2/79	(3)

- Patients with baseline mutation had higher incidence of emerging mutation compared to patients without baseline mutation
- Of 187 resistant patients, E255K/V was newly detected in 10 (5%) patients; T315I in 9 (5%); G250E in 7 (4%), and Y253H in 5 (3%) patients
- 57% of resistant patients with newly detectable mutation progressed

mutation analysis of BCR-ABL KD

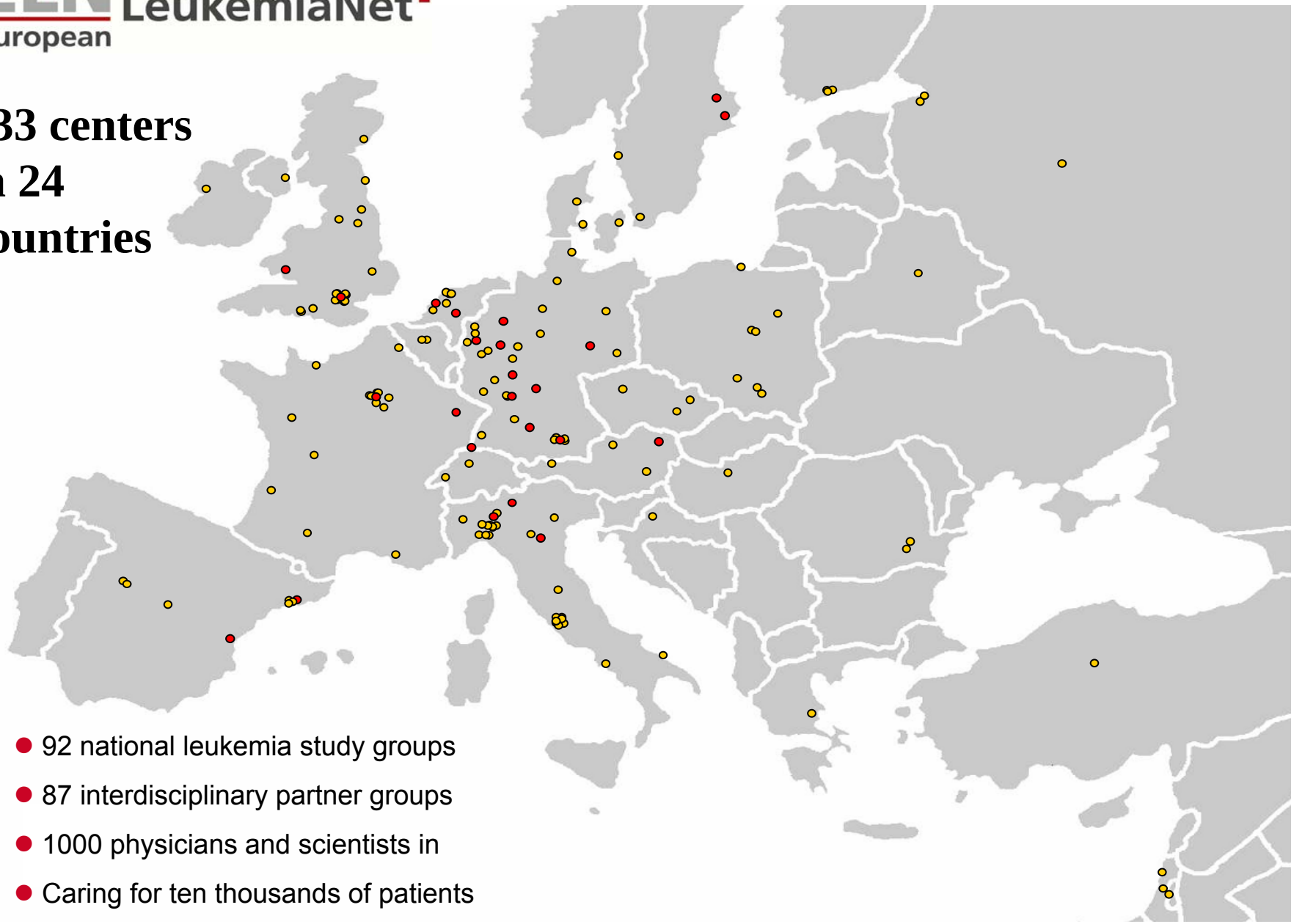
3m - no CHR	loss of CHR	progression
6m - no MCR	loss of CCR	AP
12m - no CCR	loss of MMR	BC
18m - no MMR	ACA in Ph+ cells	rise in BCR-ABL

Baccarani et al, Blood;2006:108,1809


ELN
European
LeukemiaNet

European LeukemiaNet

133 centers in 24 countries



- 92 national leukemia study groups
- 87 interdisciplinary partner groups
- 1000 physicians and scientists in
- Caring for ten thousands of patients