

Role of Molecular Monitoring in Clinical Practice

Andreas Hochhaus, Mannheim

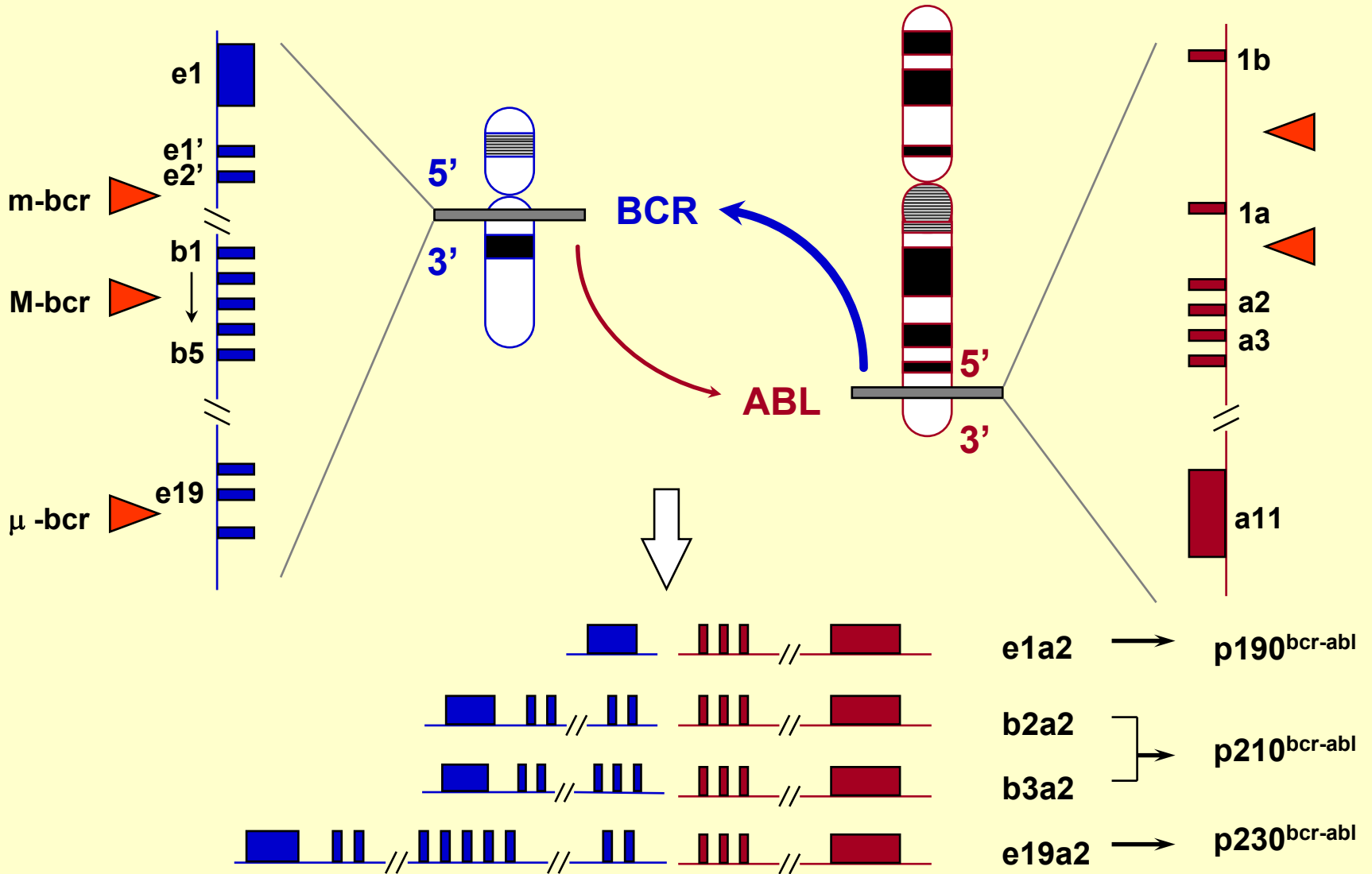
EUTOS for CML



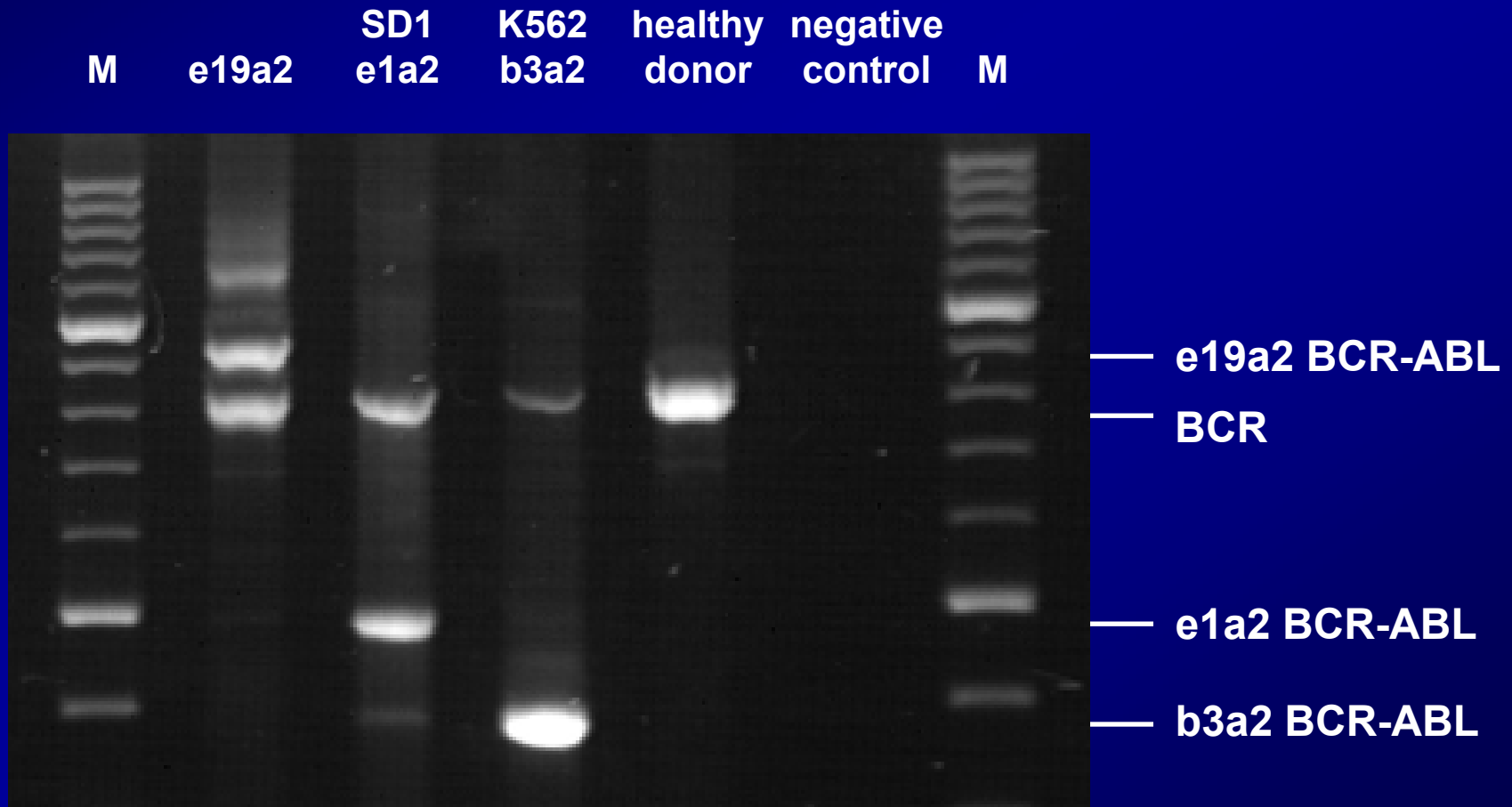
European Treatment and Outcome Study

Chromosome 22

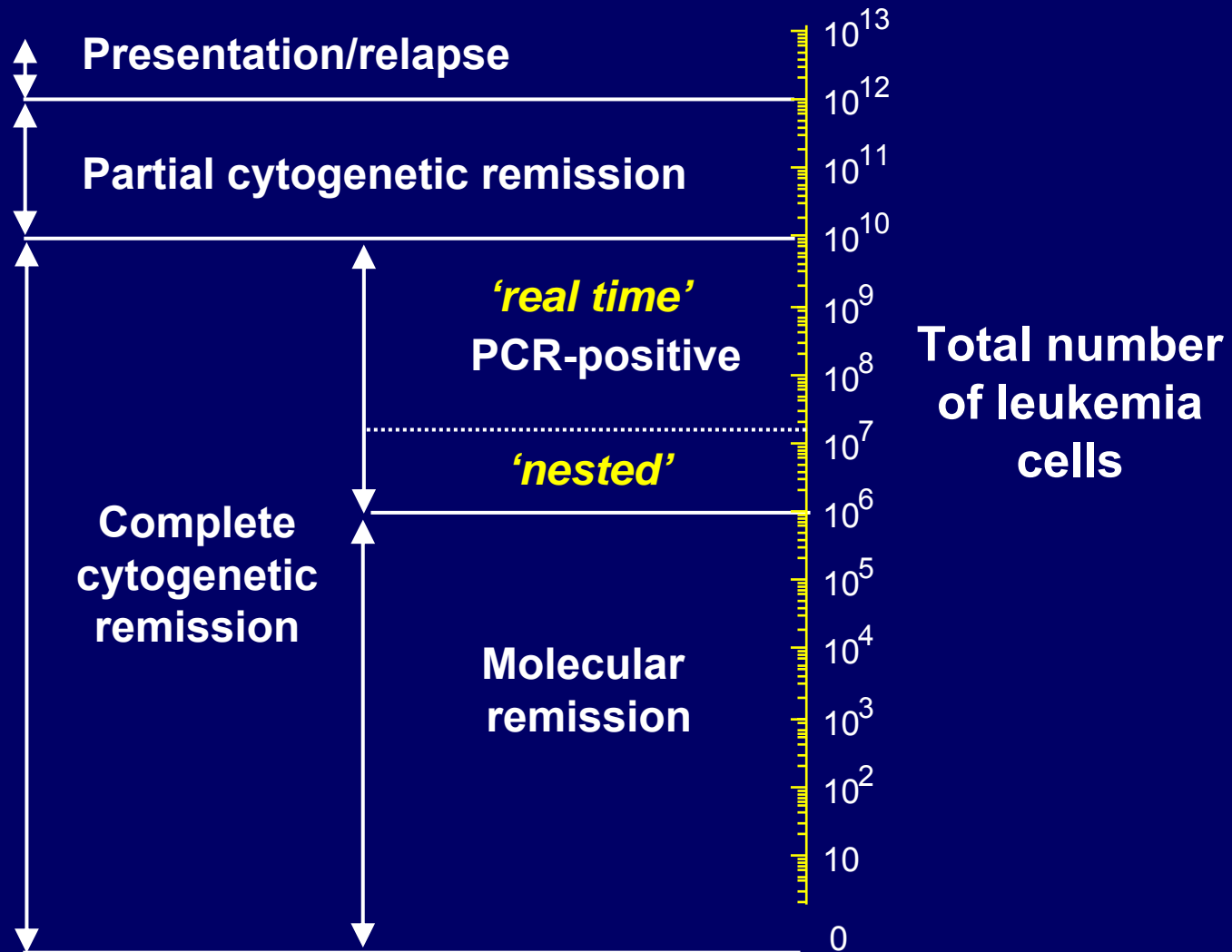
Chromosome 9



Detection of typical and rare BCR-ABL transcripts by multiplex PCR



Leukemia Cells at Presentation, Relapse, and Remission



Why Use Molecular Monitoring in CML?

- **> 3 logs more sensitive than conventional cytogenetics**
- **Peripheral blood can be used**
- **Early detection of relapse after allogeneic SCT.**
- **Prognostic significance in CCR to IFN α .**
- **Relationship of MRD level and PFS after imatinib.**
- **Definition of CMR**

Cross et al. Blood. 1993; 82(6): 1929-36.

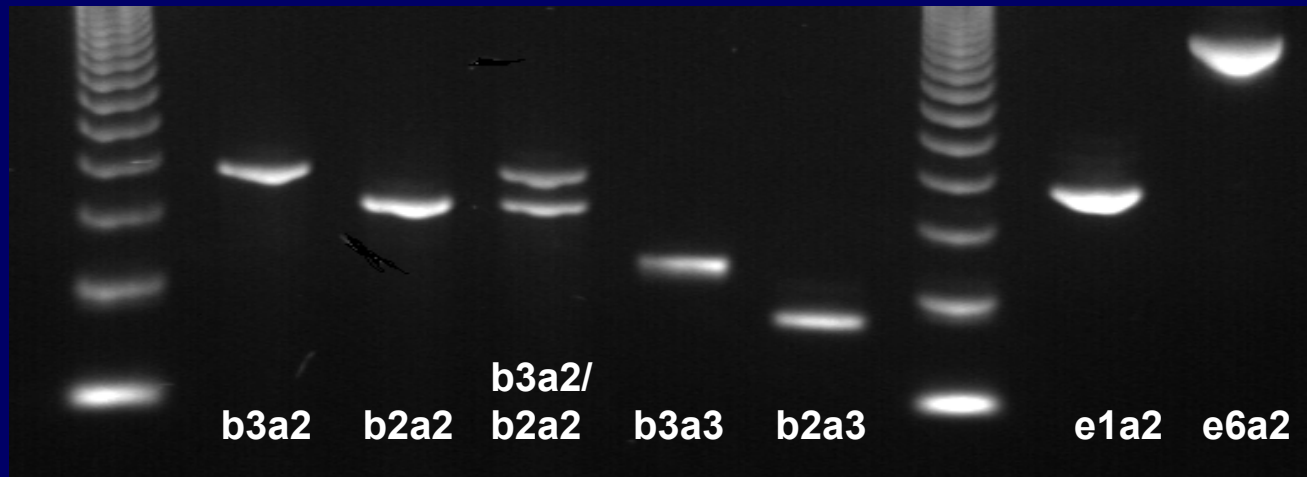
Corsetti et al. Leukemia. 1998; 12(6): 998-9.

Hochhaus et al. Blood. 2000; 95(1): 62-6.

Hughes et al. N Engl J Med. 2003; 349(15):1423-32.

Druker et al. N Engl J Med. 2006; 355(23): 408-17.

Qualitative PCR: Typical and atypical BCR-ABL transcripts



typical
transcripts

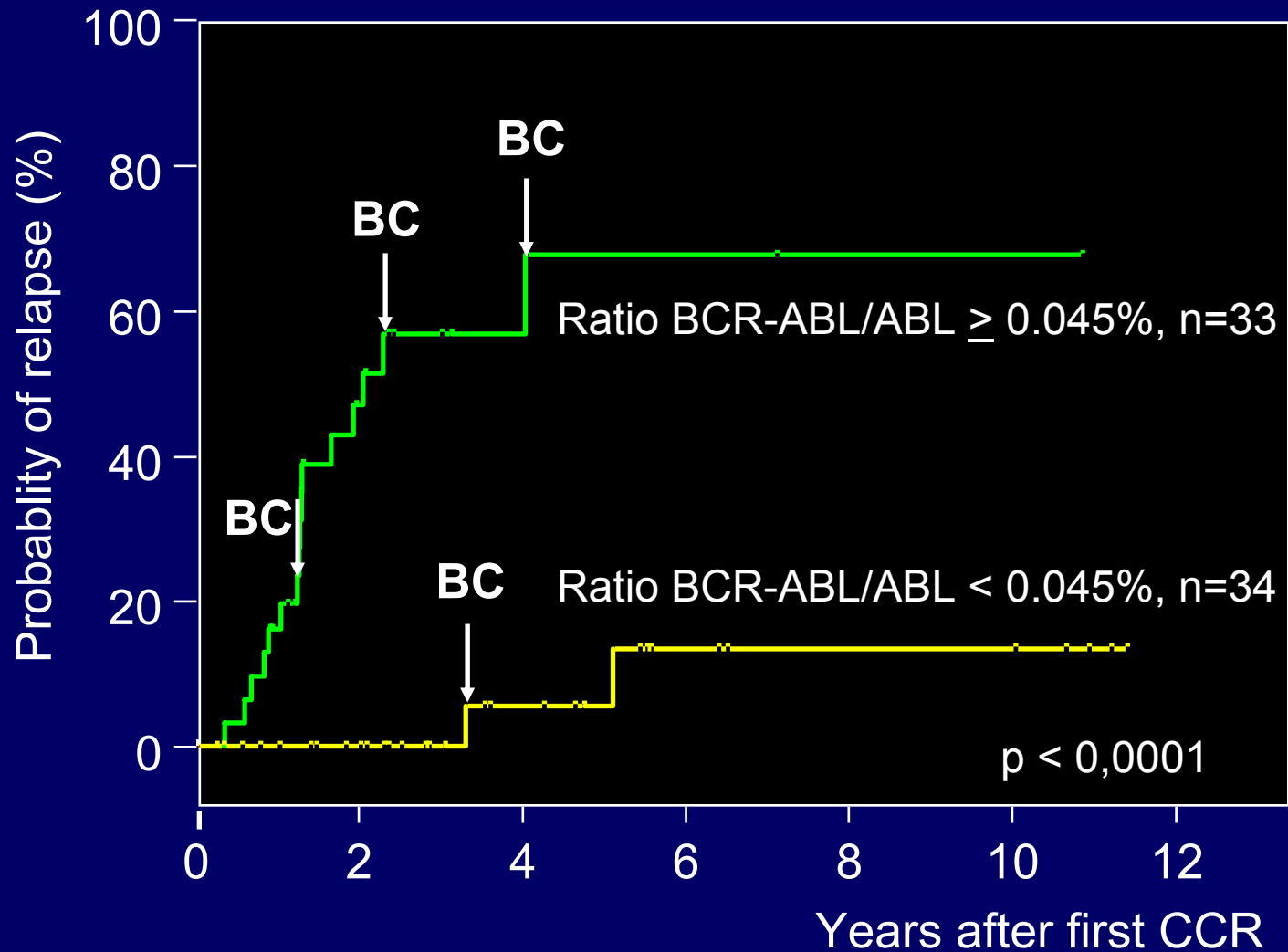


rare



rare,
specific BCR primers
needed

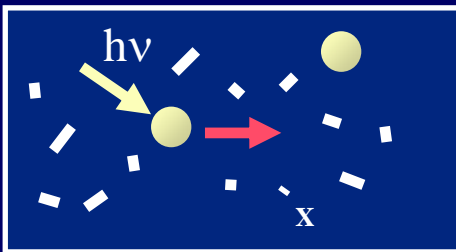
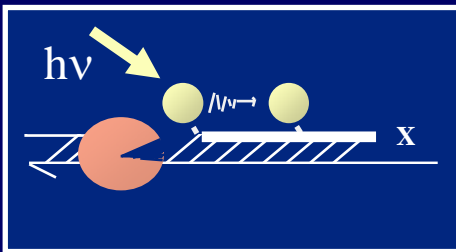
Relapse free survival on patients with CCR after IFN- α therapy



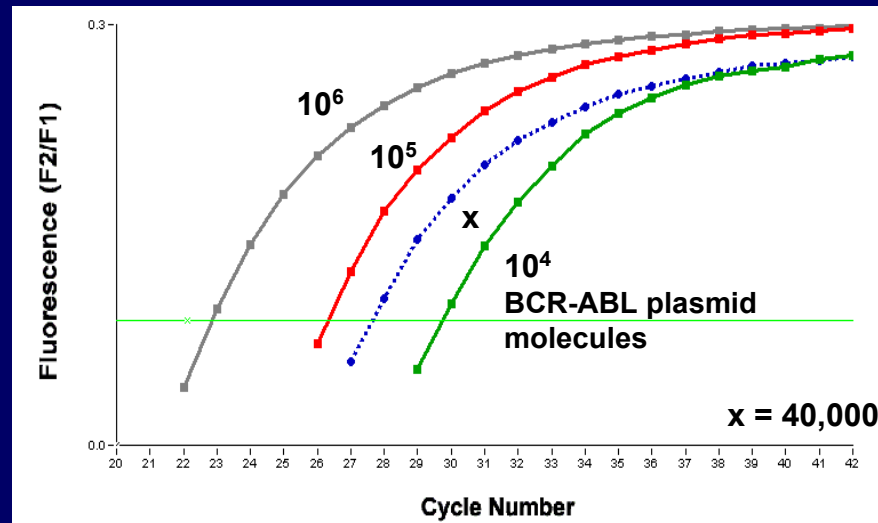
Real time quantitative RT-PCR

I. Hydrolysis Probes

Release from quenching by hydrolysis

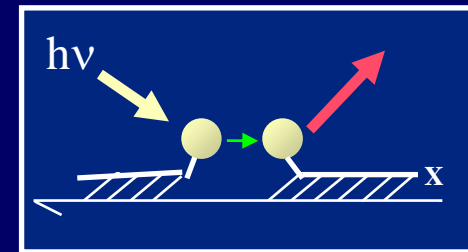
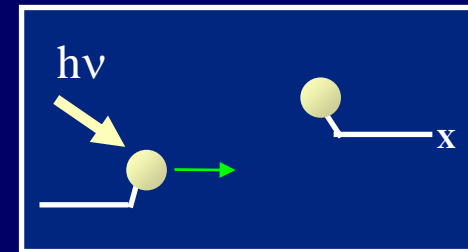


TaqMan™



II. Hybridization Probes

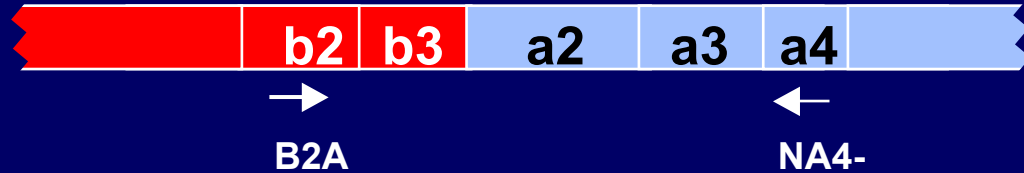
Increased resonance energy transfer by hybridization



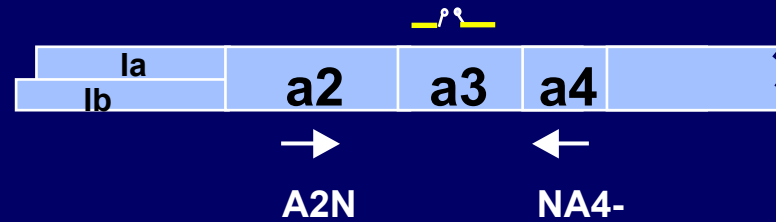
LightCycler™

LightCycler

BCR-ABL



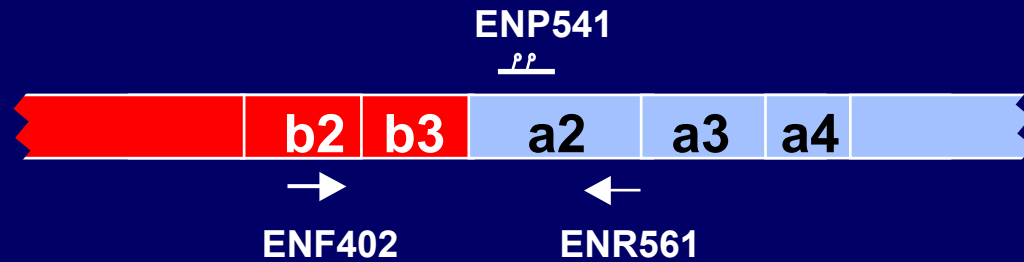
ABL



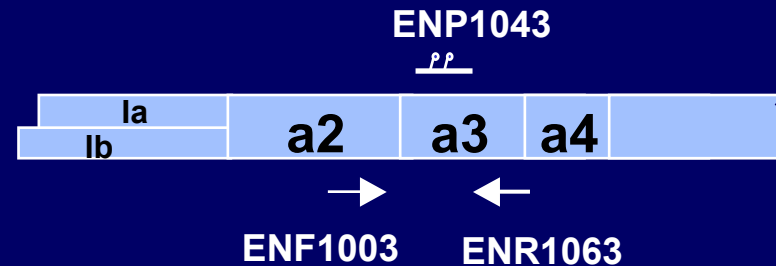
Emig et al. *Leukemia*. 1999.

TaqMan

BCR-ABL



ABL



Gabert et al. *Leukemia*. 2003.

Methods of Expression of Molecular Response

Ratios target/standard gene

After standardization of methods and rigorous control rounds, eg, ratio *BCR-ABL/ABL* (%)

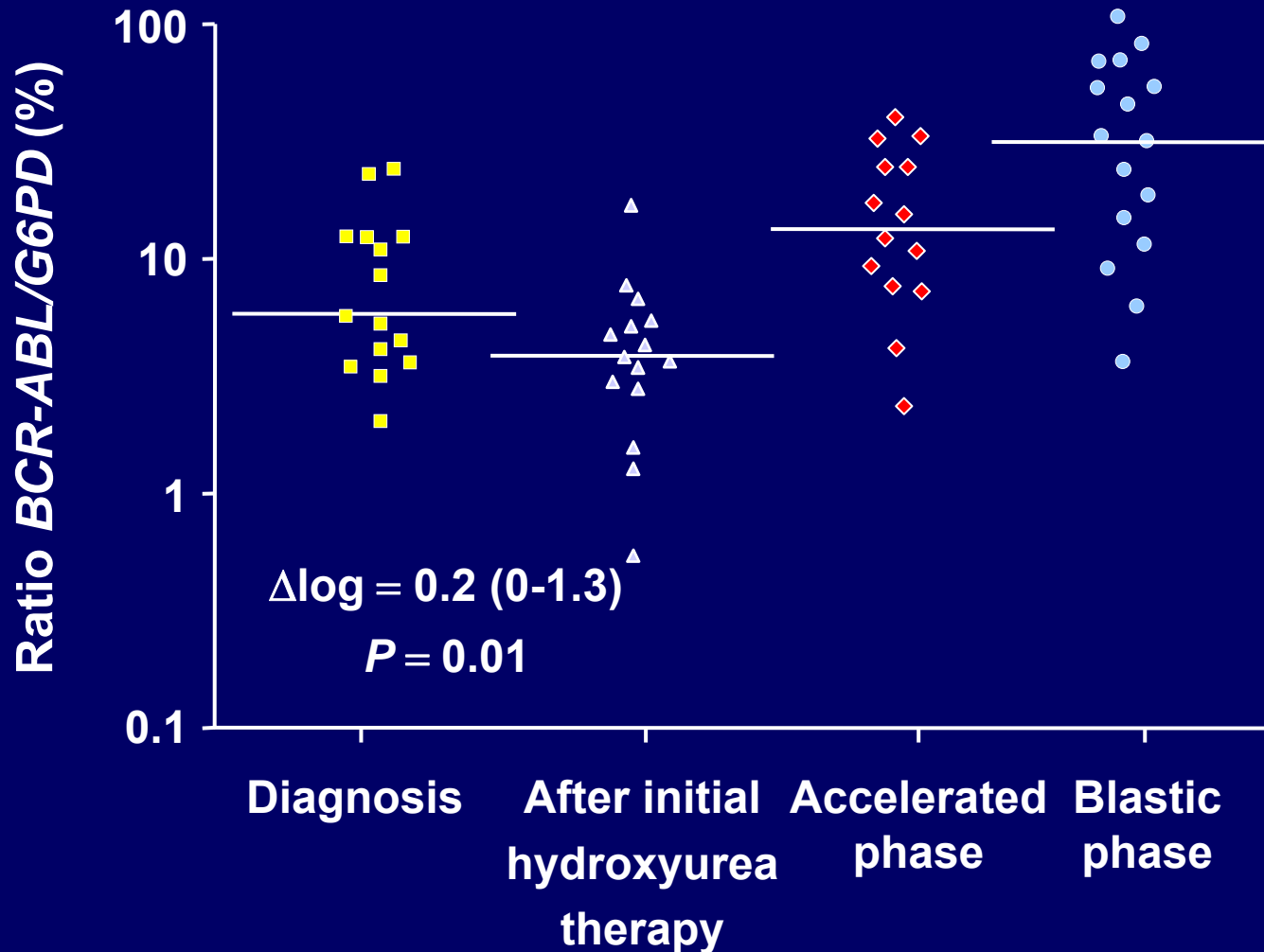
Lab-specific reference point:

Pooled diagnostic samples,
eg, $\Delta \log$ ratio *BCR-ABL/BCR* (%)

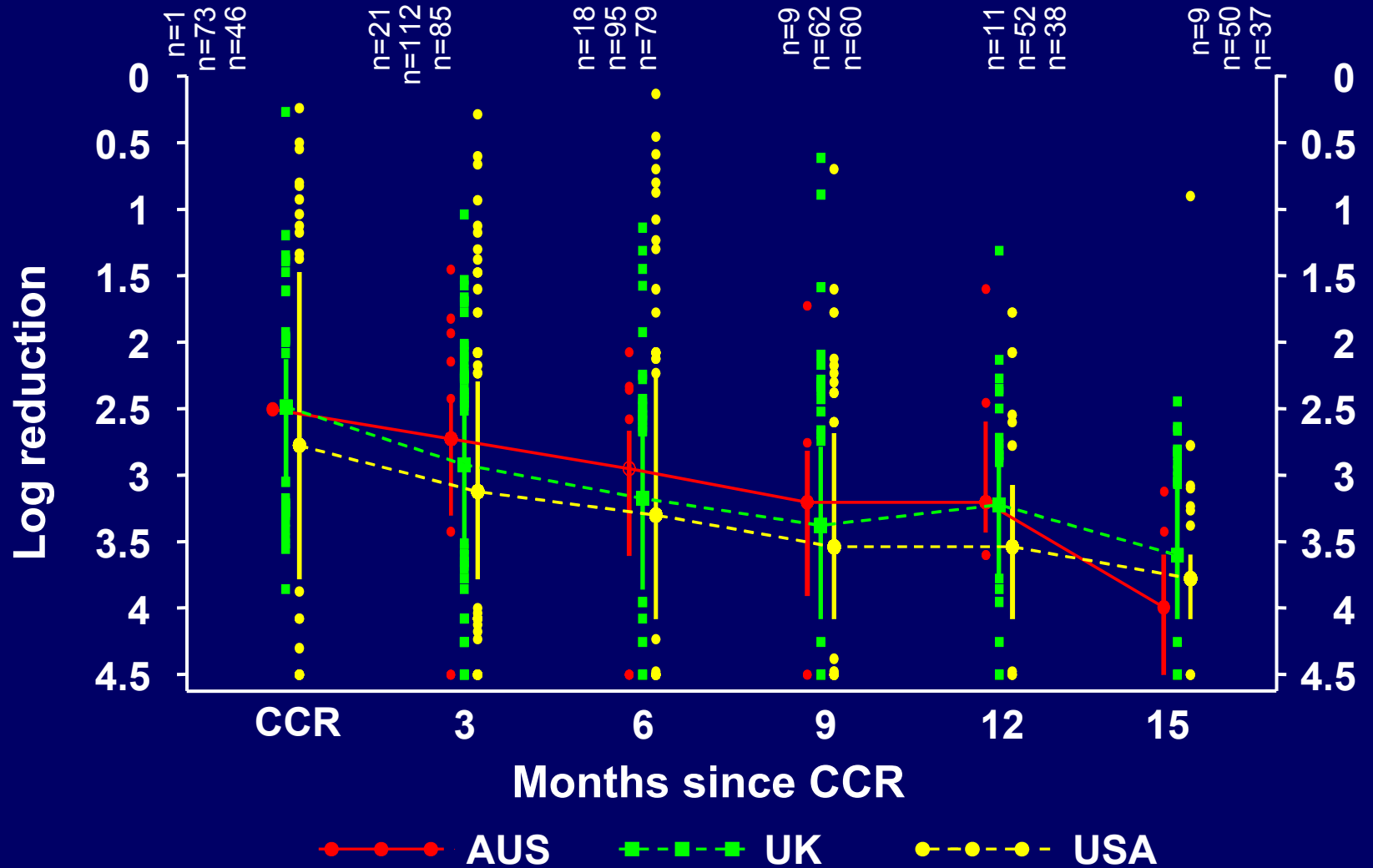
Individual calculation of relative molecular response:

MRD level after therapy/pretherapeutic level,
eg, individual $\Delta \log$ ratio *BCR-ABL/G6PD* (%)

Variability of the Individual “Pretherapeutic *BCR-ABL* Level”



Standardized Log Reduction - by Laboratory

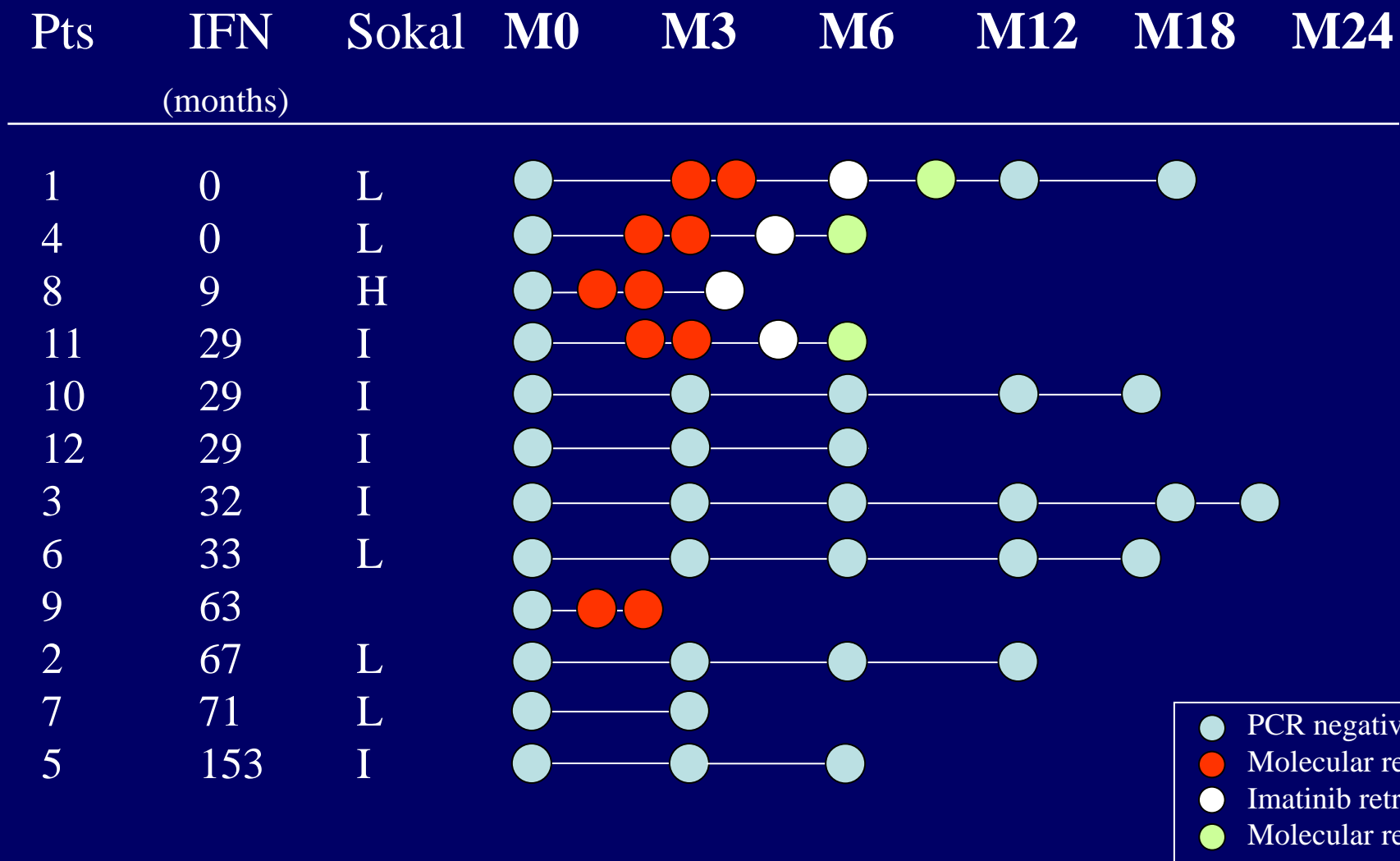


Recommendations for standardization

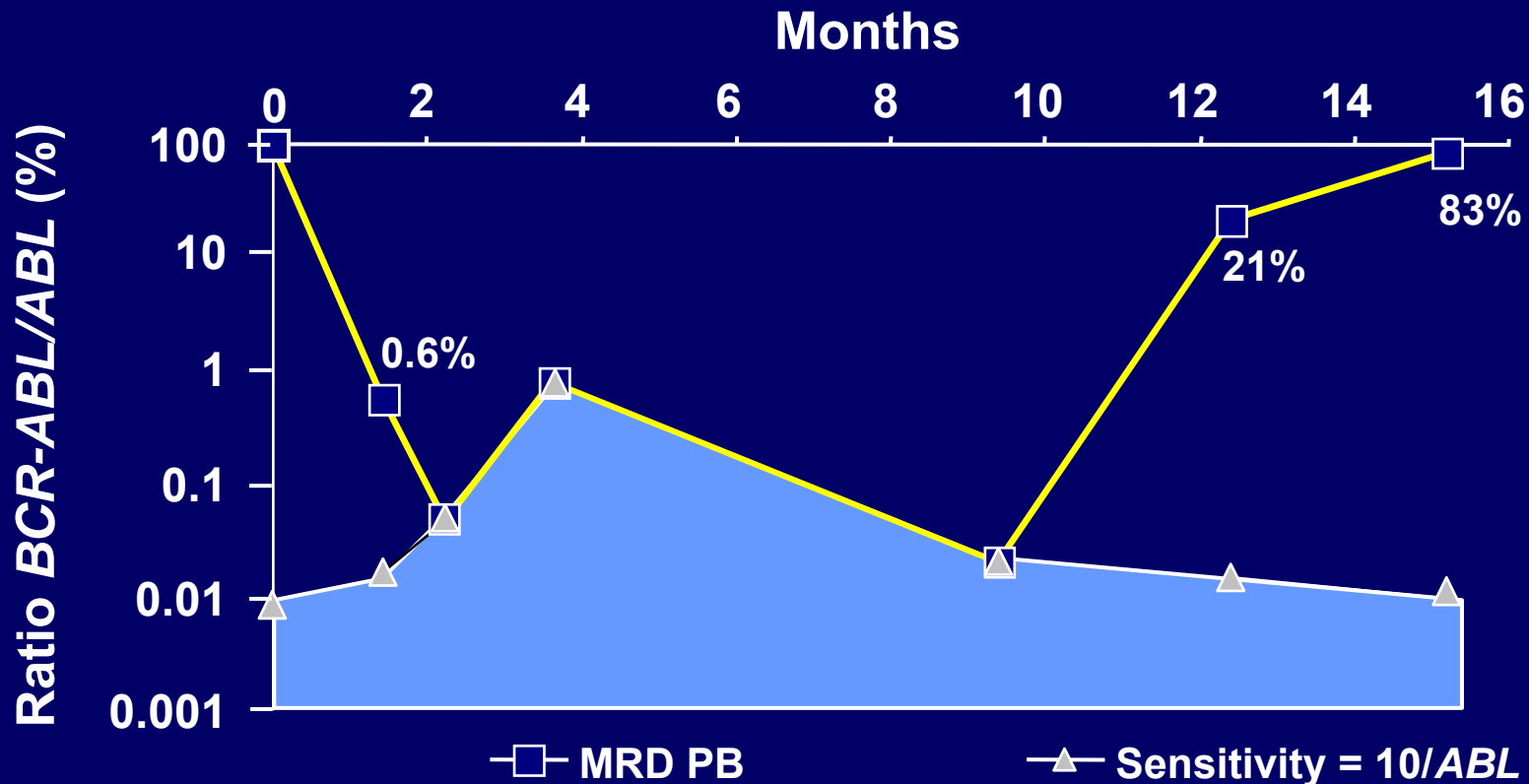
(Torino, March 2003; San Diego December 2003 and 2004)

- Use of 10 ml peripheral blood ($\sim 5 \times 10^7$ 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 genes for routine use: total ABL, BCR or *beta*-GUS

CMR = cure?



Sensitivity Depends on the Quality of the Individual Sample



Disease detectable:
 $BCR-ABL/ABL$ (%)

Disease undetectable:
 Negative / [no. of *ABL* transcripts]

Definition of failure and suboptimal response

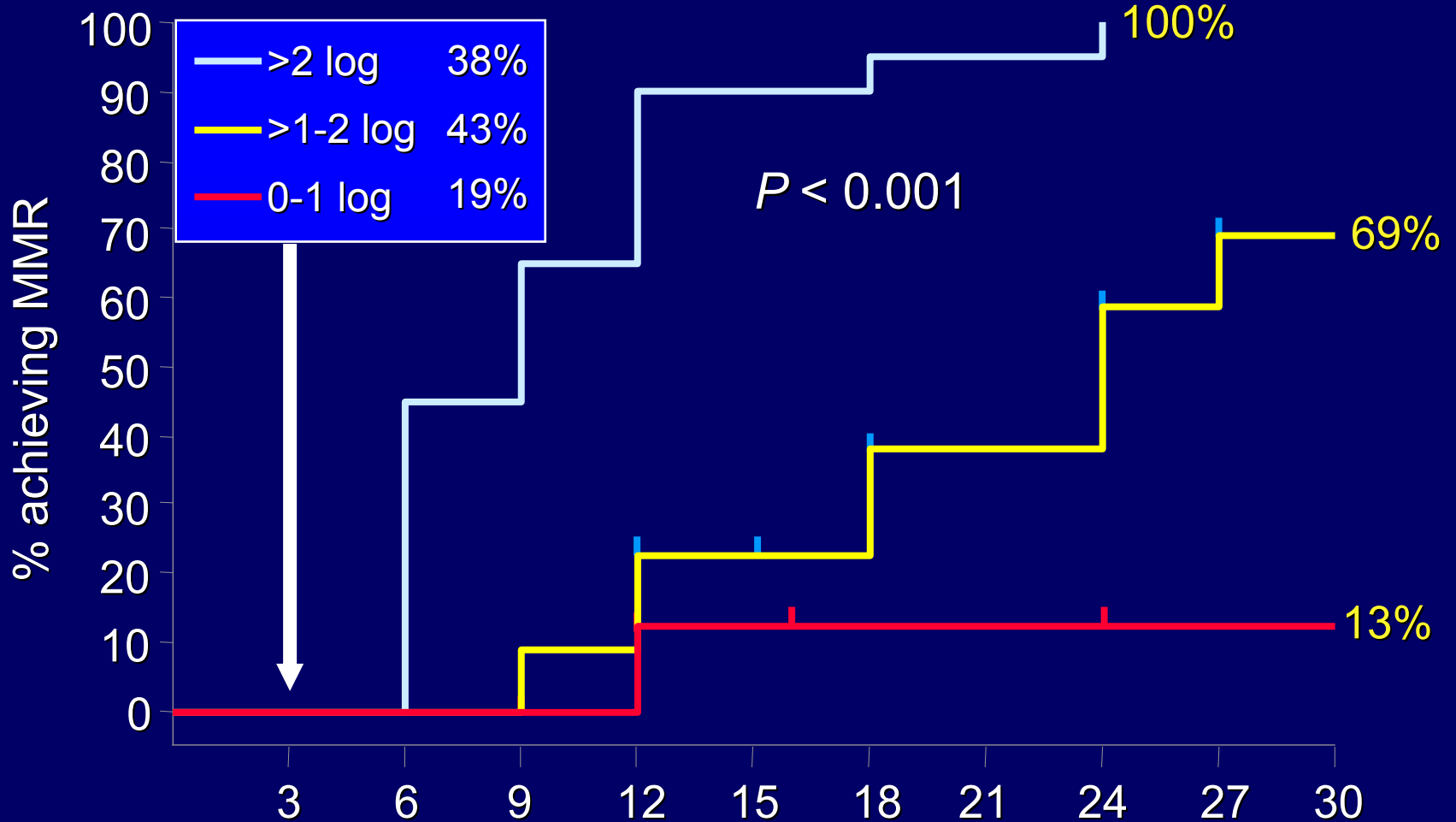
Time	Failure	Subopt Resp	Warnings
Diagnosis	-	-	High risk Del(9q) ACA in Ph+ cells
3 mos	No CHR	No CgR	
6 mos	< CHR No CgR	< PCgR	
12 mos	< PCgR	< CCgR	< MMoIR
18 mos	< CCgR	< MMoIR	
Anytime	ACA in Ph+ cells Loss of CHR Loss of CCgR Mutation (IM-insensit.)	Loss of MMoIR Mutation (IM-sensit.)	Any ↑ BCR-ABL transcript level OCA in Ph- cells

Rationale for response monitoring

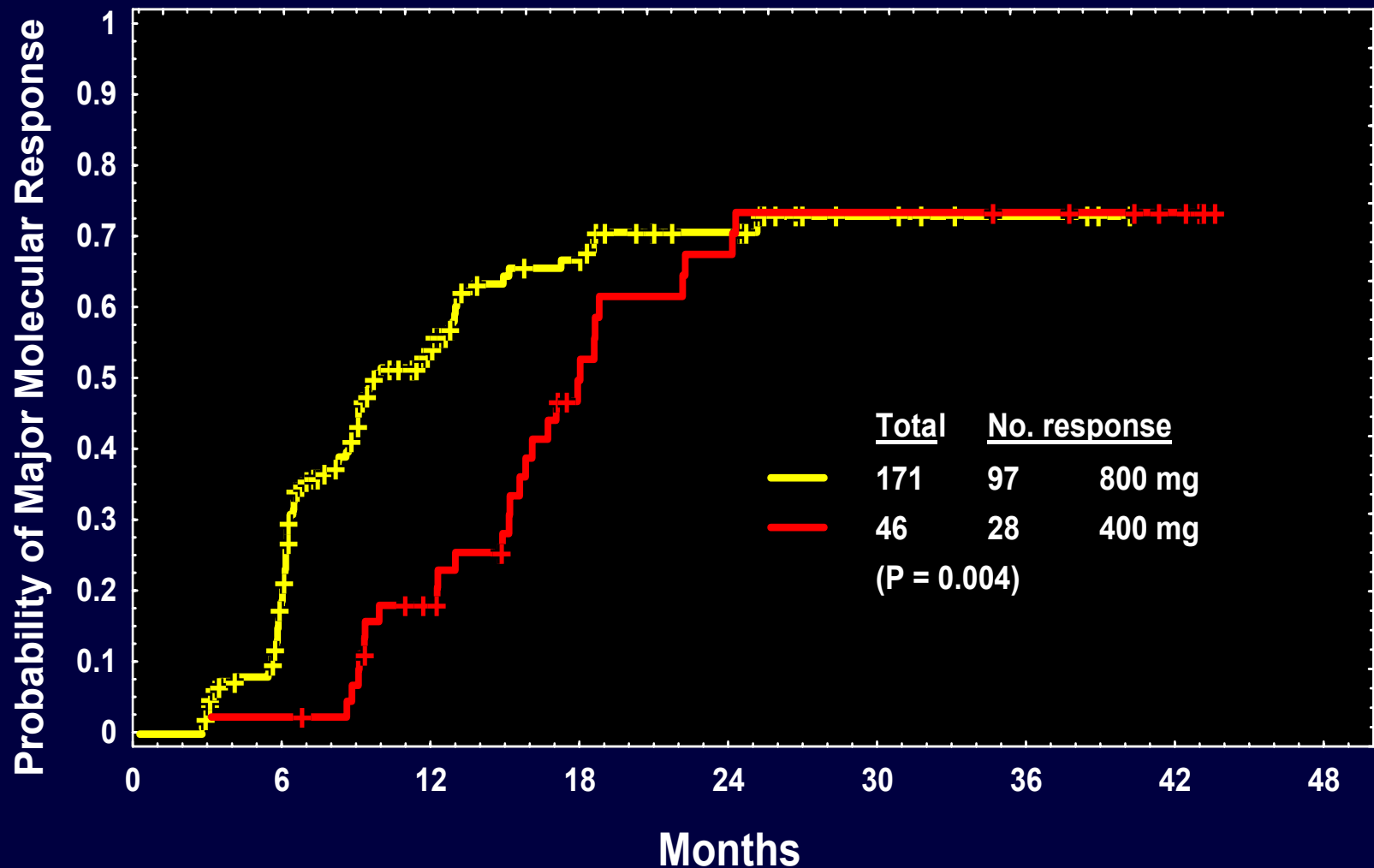
- Most patients in CP have an excellent response to imatinib
- Some patients, however, still progress
- Identify patients who will ultimately experience relapse
- Early identification of relapse or progression provides an opportunity for alternative therapy

Australian IRIS trial: Probability of achieving MMR

3 month *BCR-ABL*



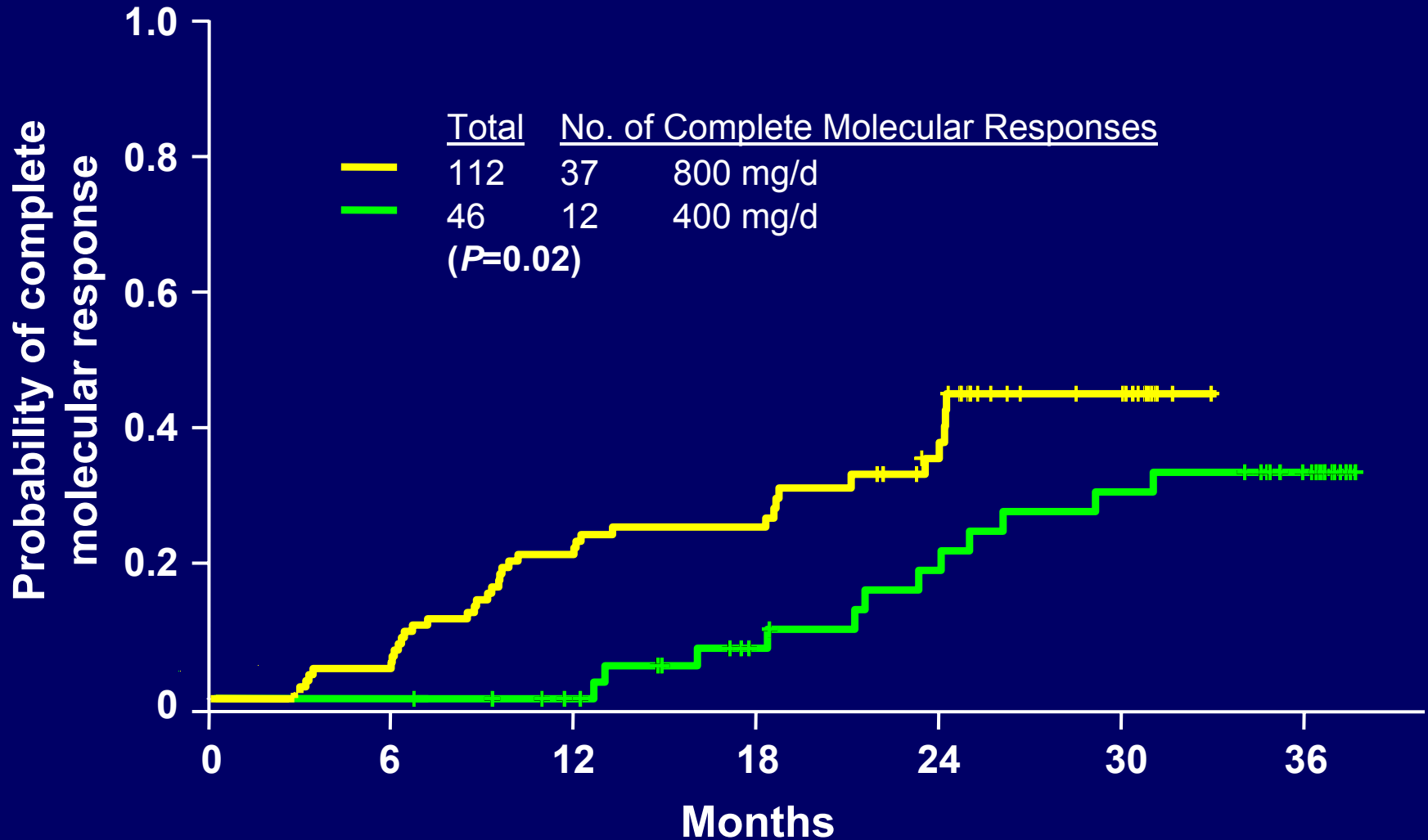
Cumulative Incidence of Patients with Major Molecular Response



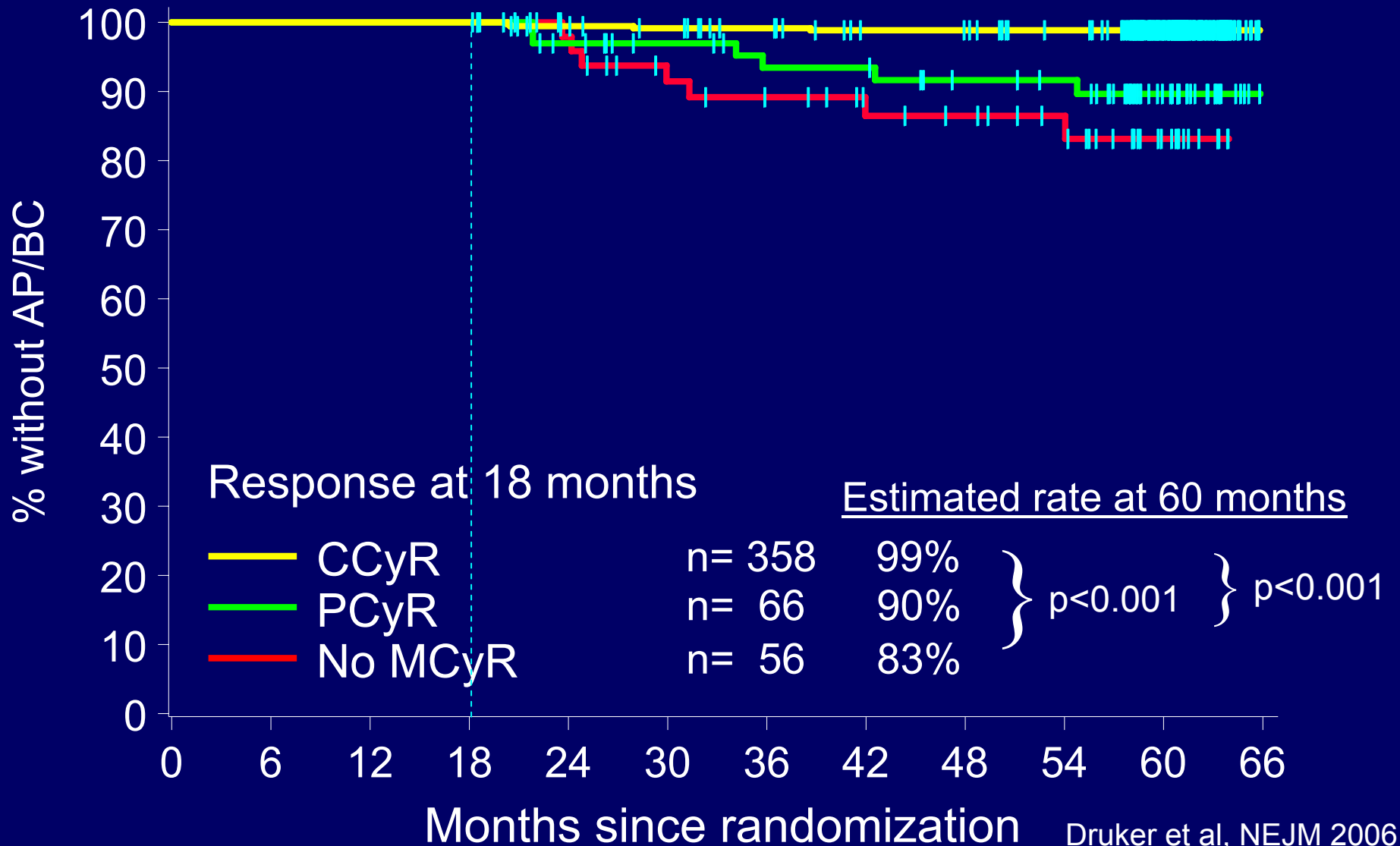
Cortes et al. ASH 2004; Kantarjian et al. Blood 2003; 101: 97-100

Kantarjian et al. Blood 2004; 103: 2873-2878

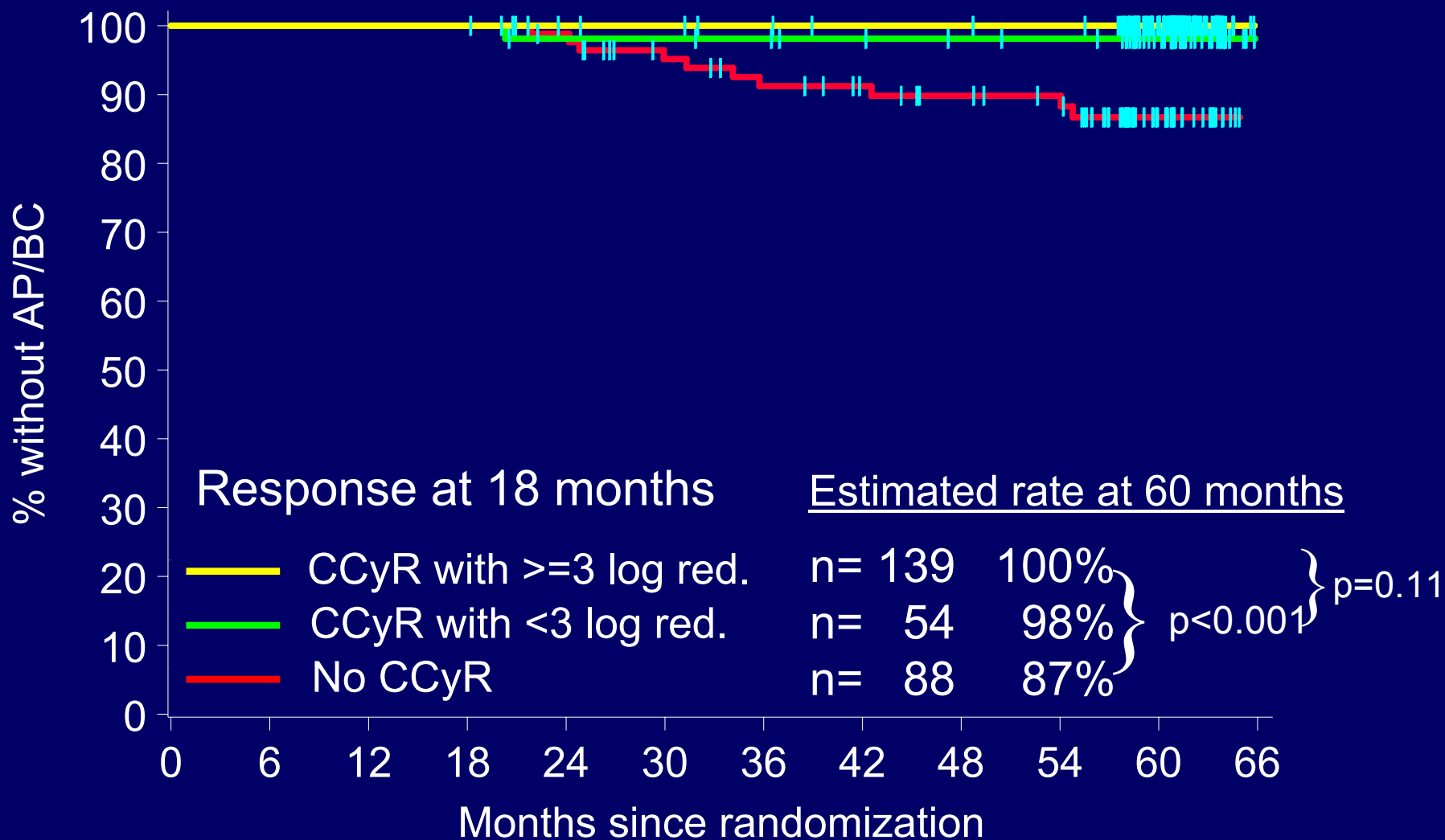
Cumulative Incidence of Undetectable *BCR-ABL*



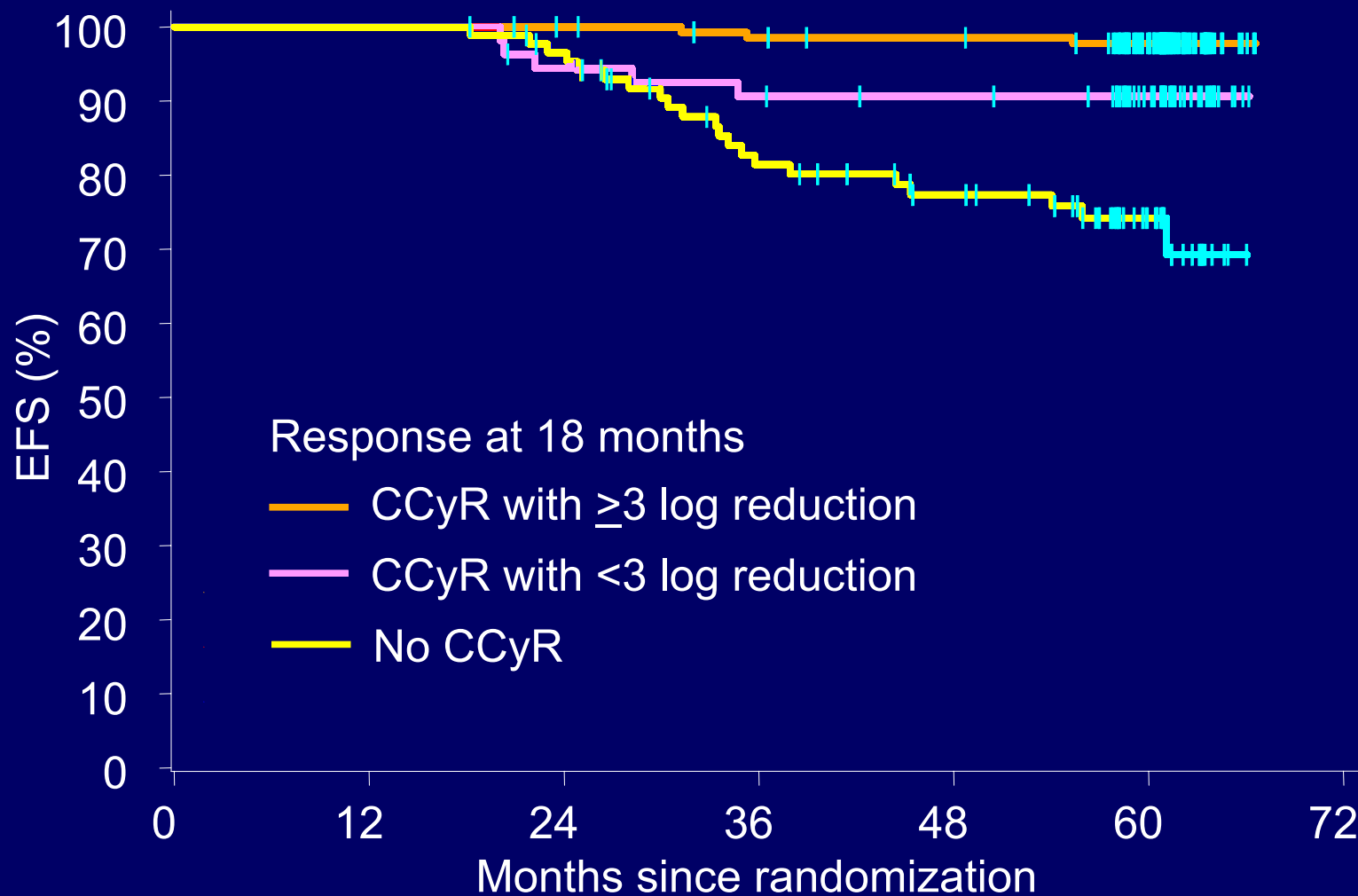
Survival without AP/BC by cytogenetic response at 18 months on 1st-line imatinib



Survival without AP/BC by molecular response at 18 months on first-line imatinib



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



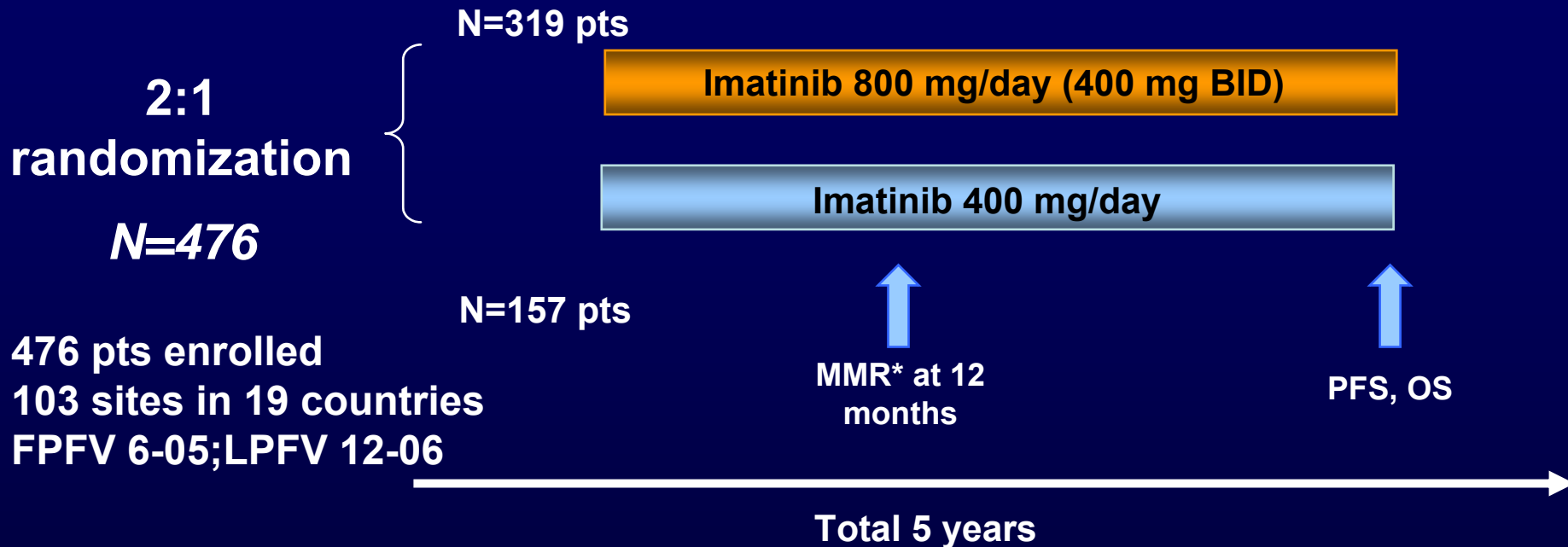
Primary endpoints in 1st line studies

- Imatinib 400 mg vs.
- IFN/AraC (IRIS) CCR @ 1 year
- Imatinib 800 mg (TOPS) MMR @ 1 year
- Nilotinib (2 dosages) MMR @ 1 year
- Dasatinib CCR @ 1 year
- Bosutinib CCR @ 1 year
- Nilotinib 2x300 mg (Phase II) CCR @ 6 months

Primary endpoints in 2nd line studies

- Imatinib 800 mg vs.
- Dasatinib MMR @ 1 year

Imatinib 400 mg vs 800 mg in CML-CP: Study Design



Detect a difference of 20% for the MMR rate at 12 months (i.e., from 40% to 60% with a 90% power)

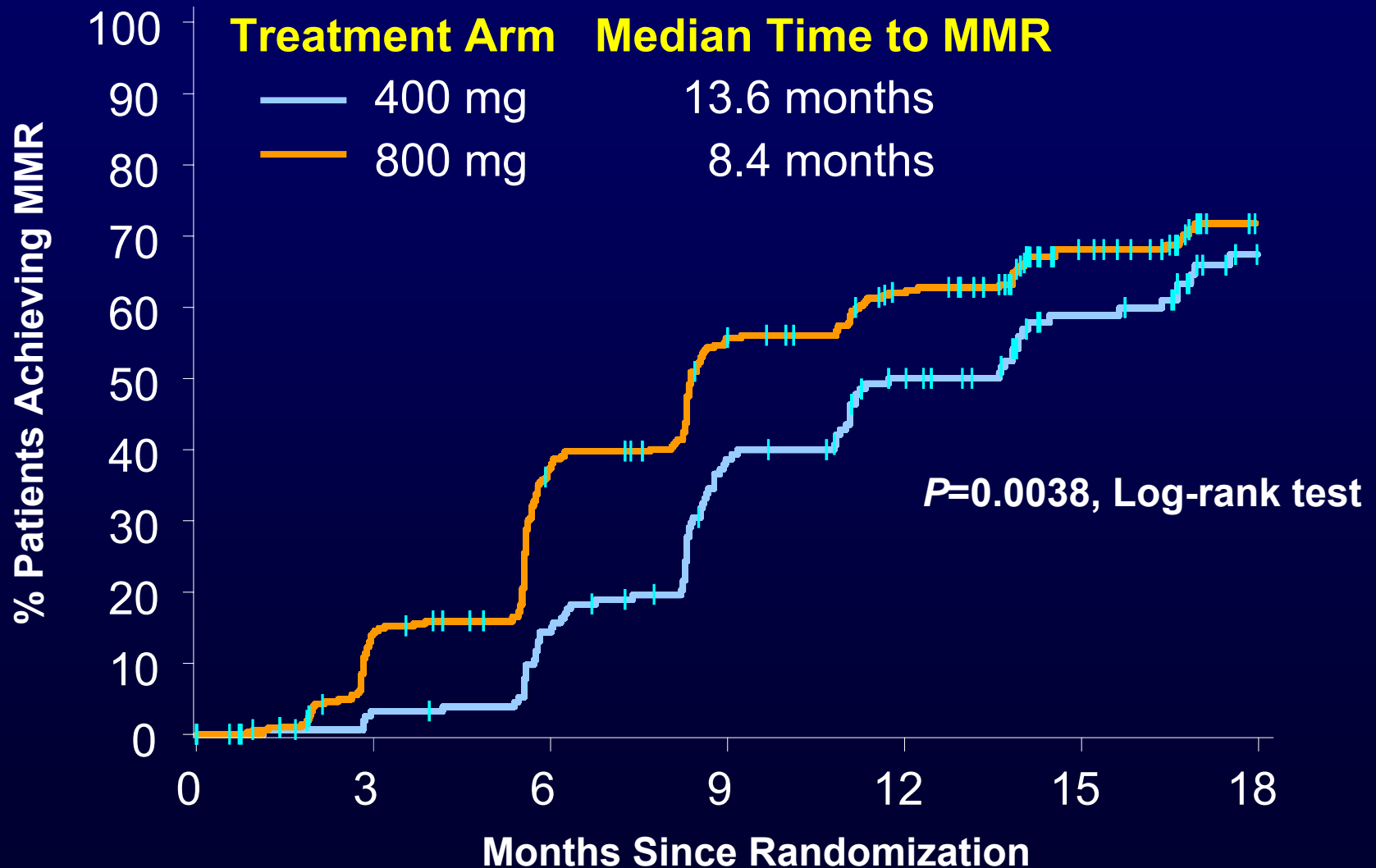
- Cytogenetic analysis every 6 months until CCyR, then every 12 months
- Molecular analysis by PCR every month x 3, then every 3 months

FPFV, first patient first visit; LPFV, last patient first visit

Data cut-off of December 31st, 2007

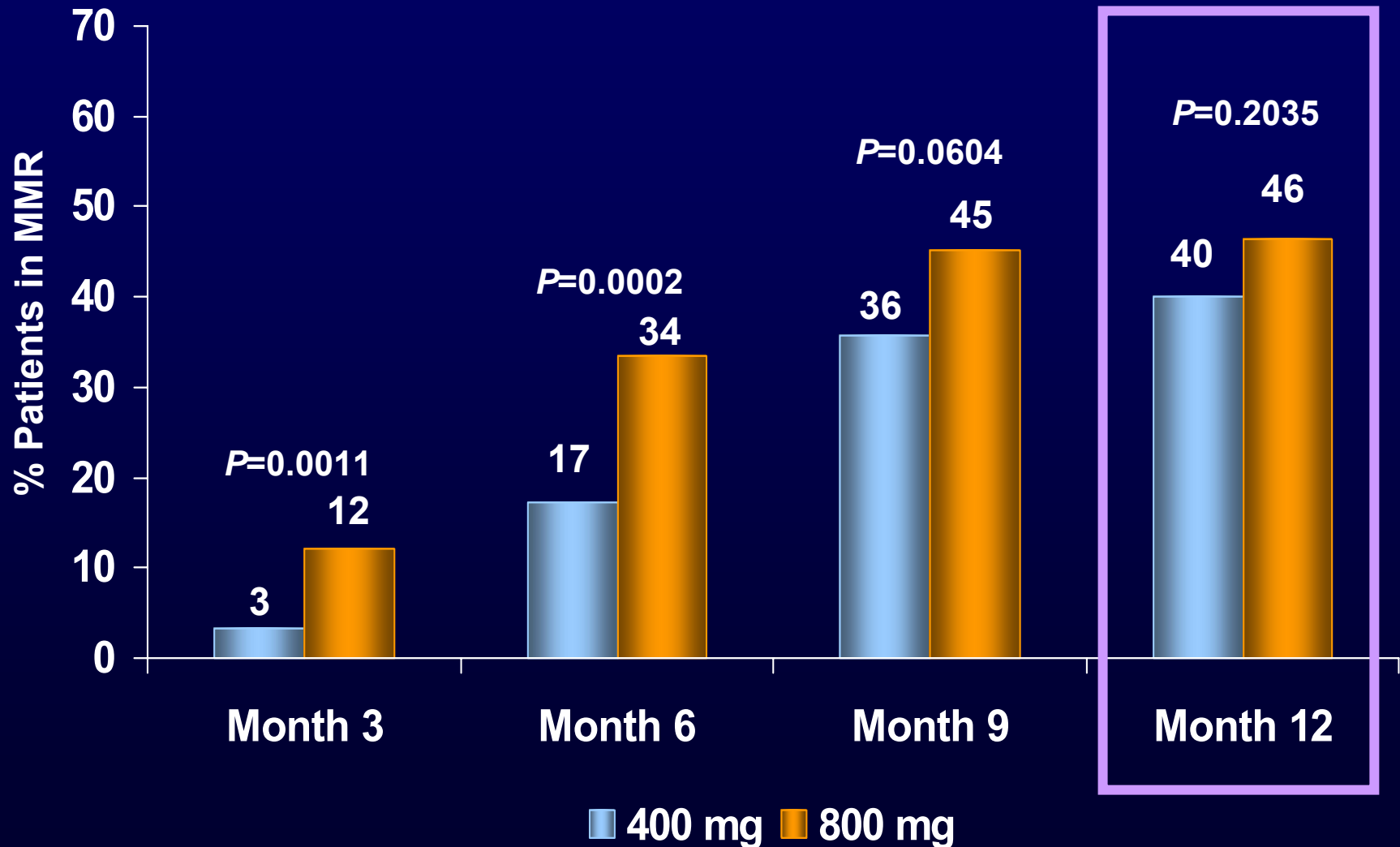
*BCR-ABL/control gene $\leq 0.1\%$ utilizing the International Scale (IS)

Imatinib 400 mg vs 800 mg in CML-CP: Time to First MMR* by Treatment Arm

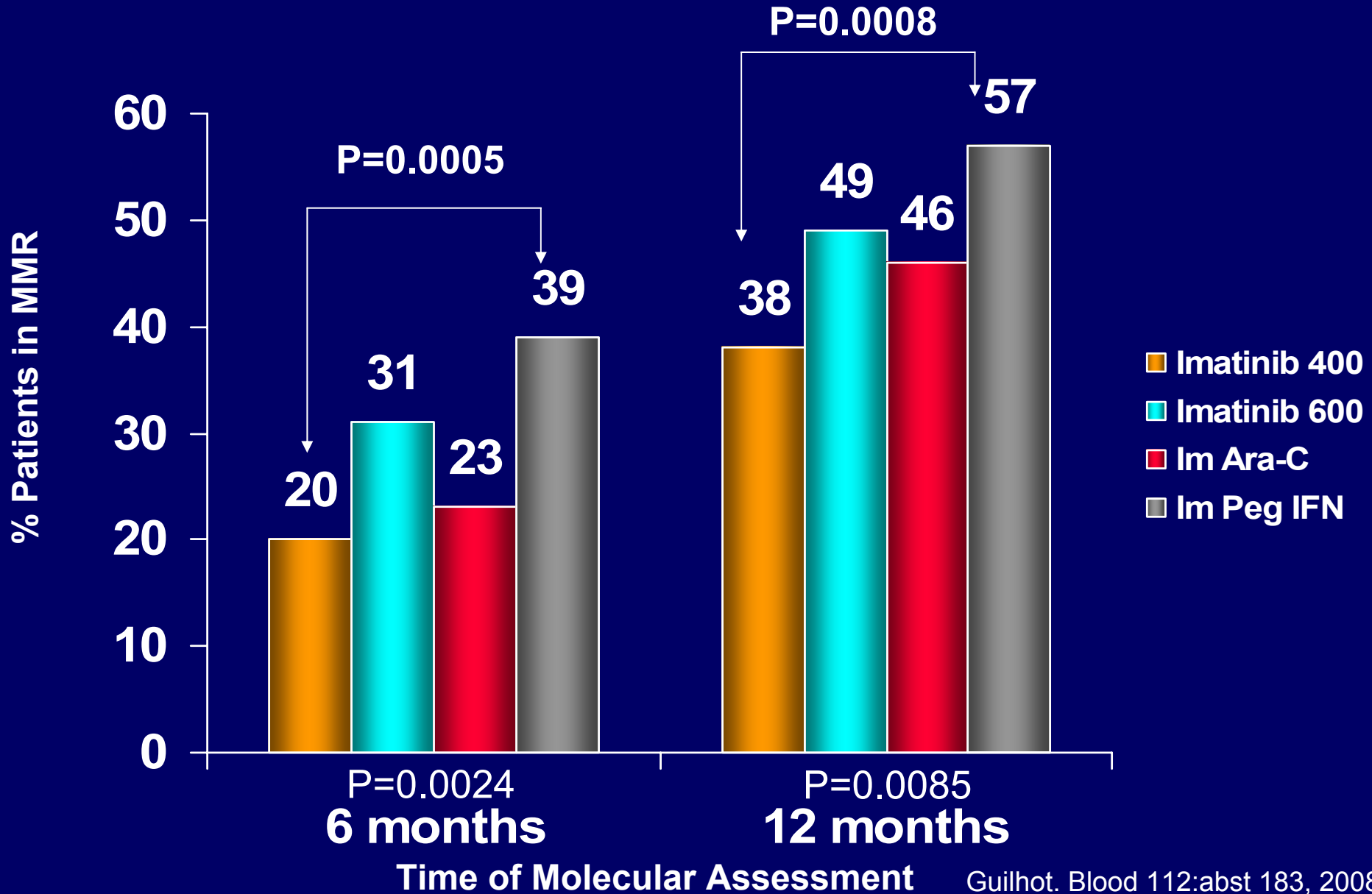


*MMR = BCR-ABL/control gene $\leq 0.1\%$ utilizing the International Scale (IS)

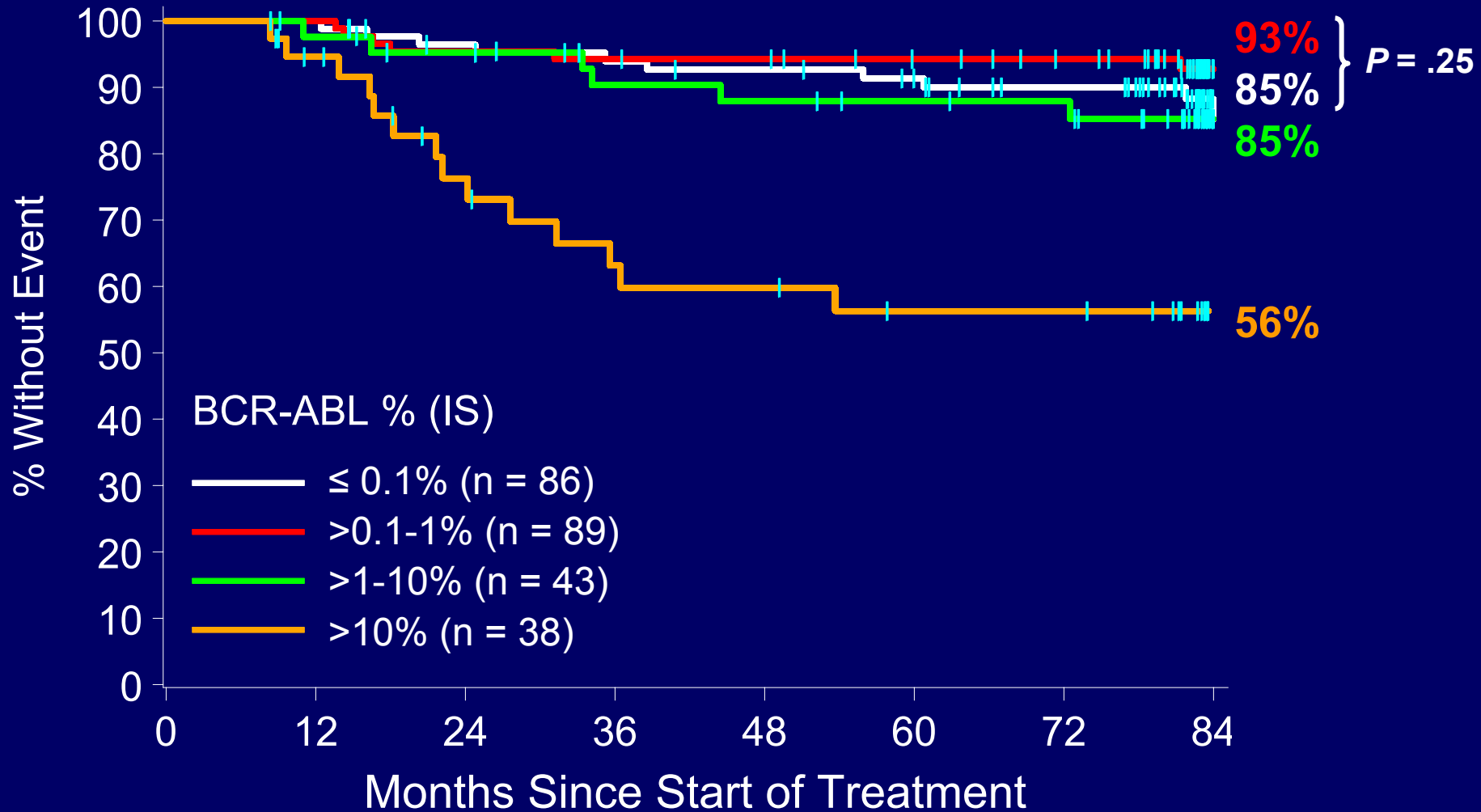
Imatinib 400 mg vs 800 mg in CML-CP: MMR Rates Over Time (ITT)



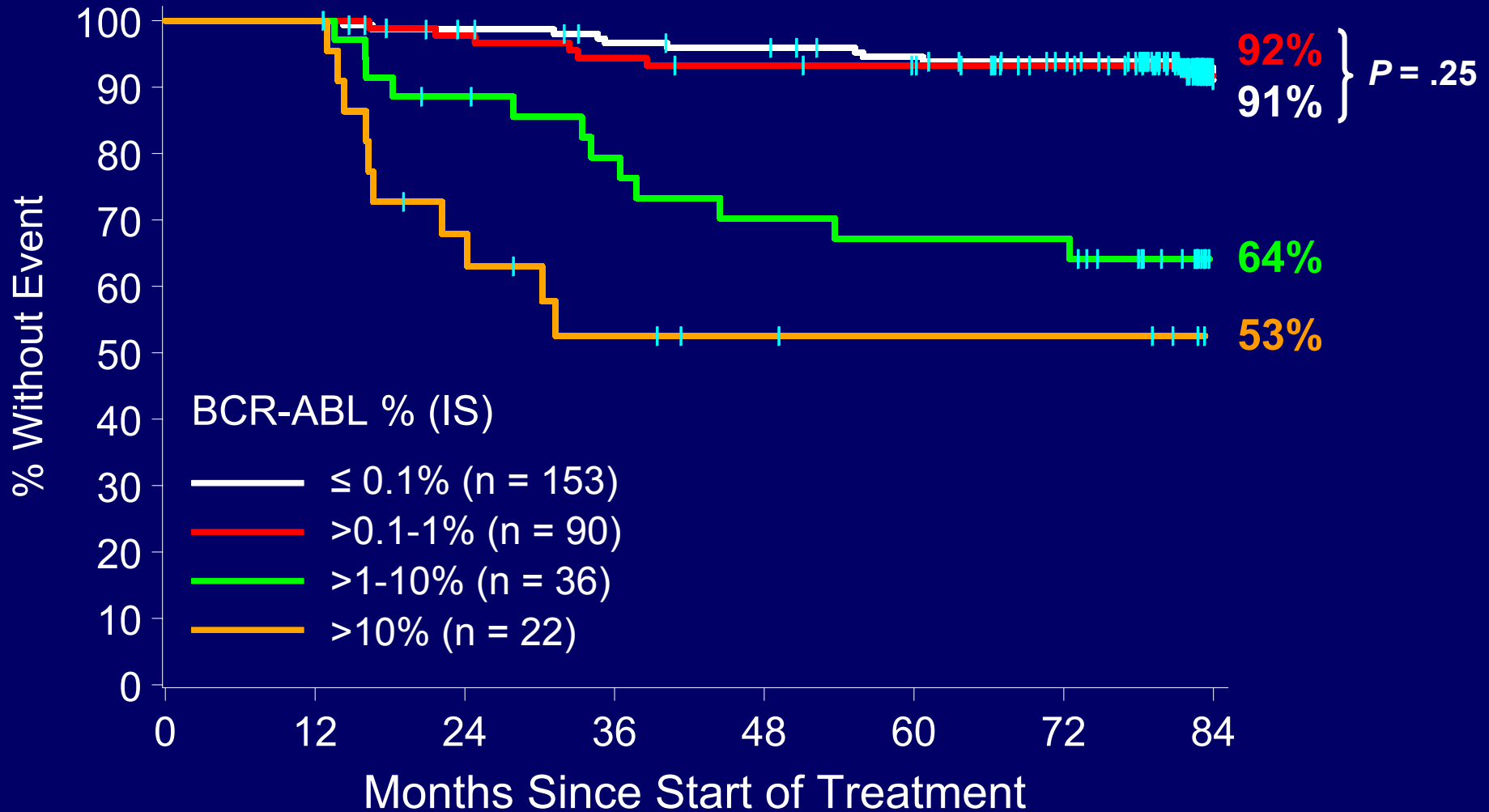
Major molecular response rates at 6 and 12 months



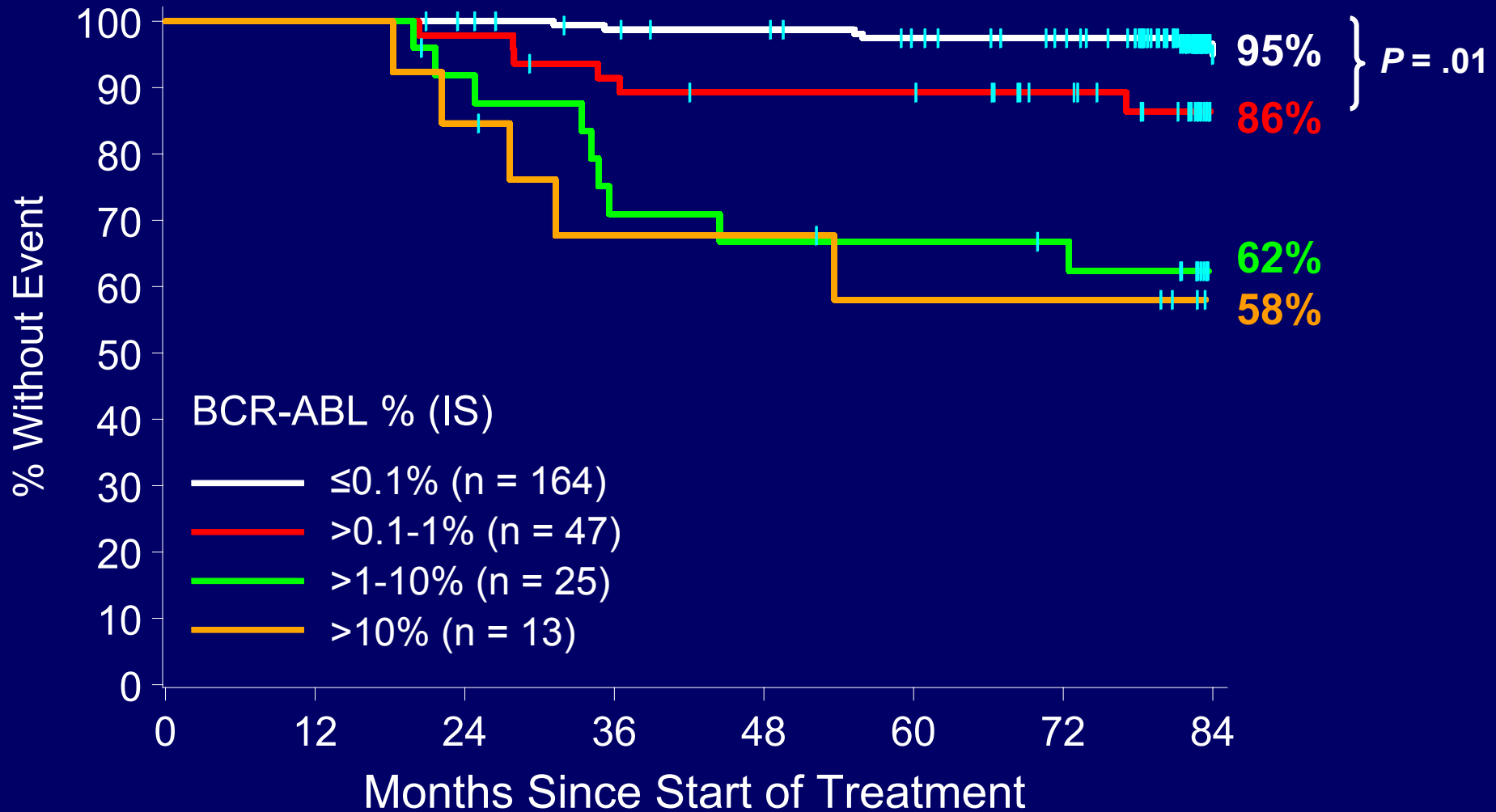
EFS: 6-Month Landmark Analysis



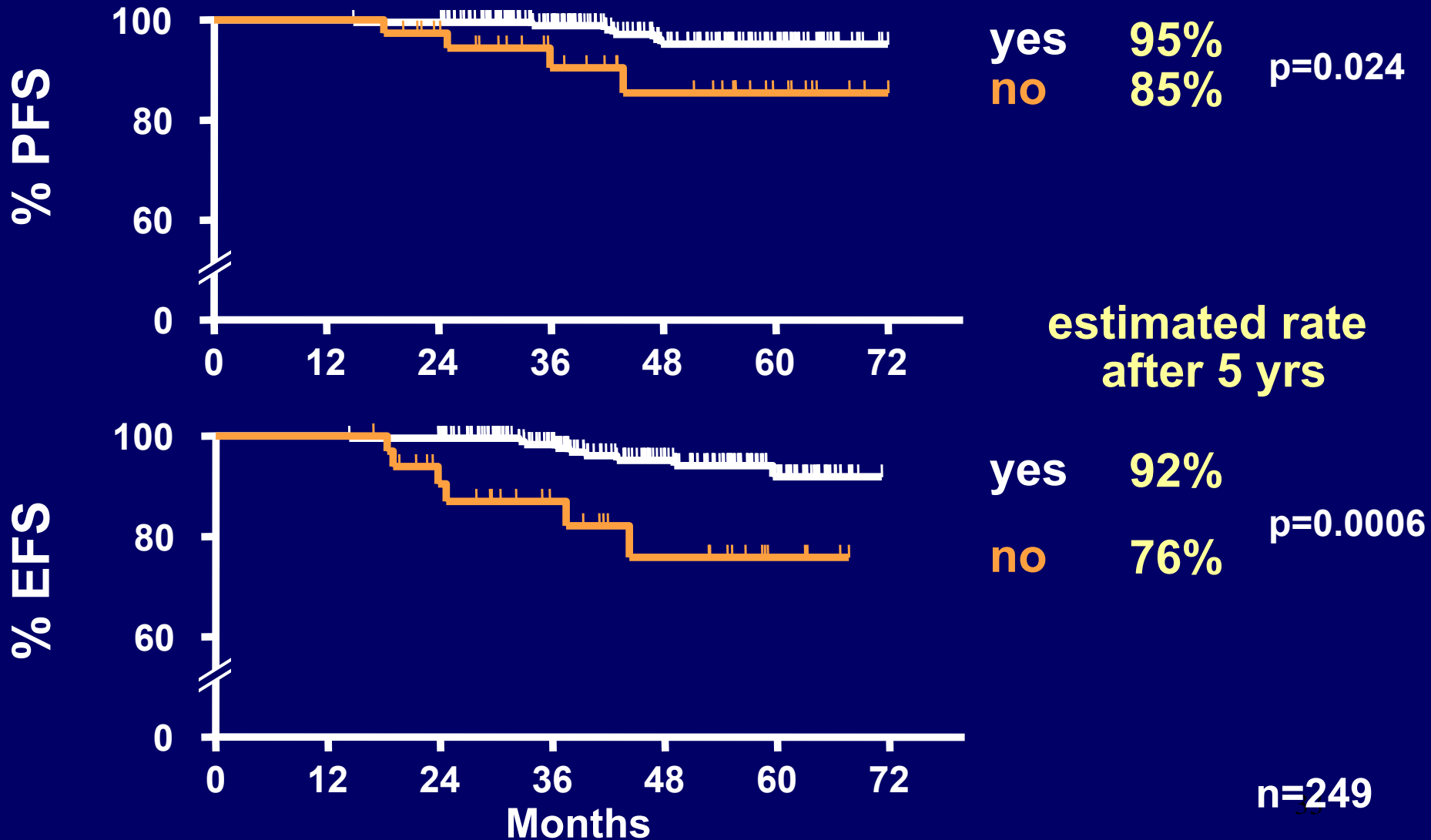
EFS: 12-Month Landmark Analysis



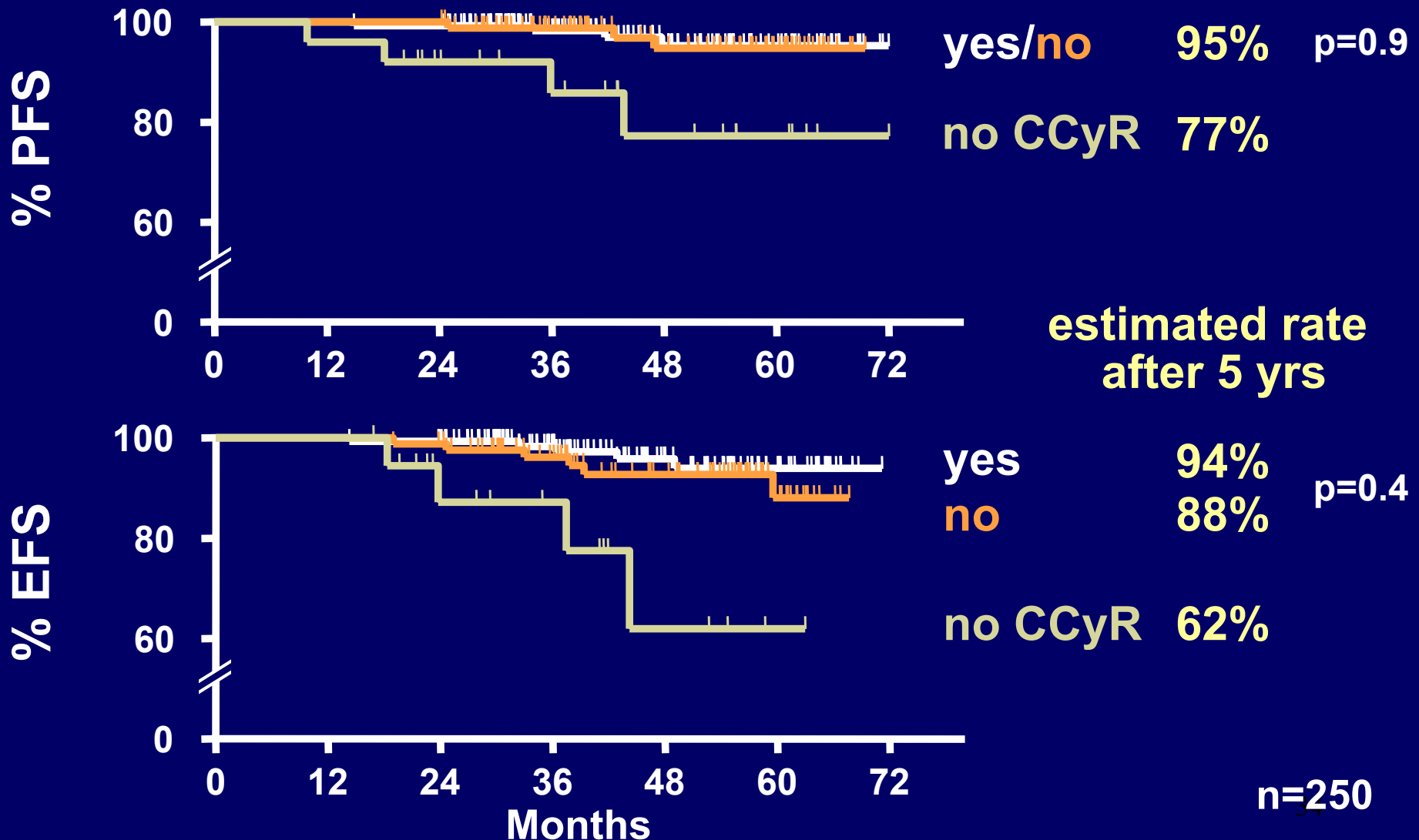
EFS: 18-Month Landmark Analysis



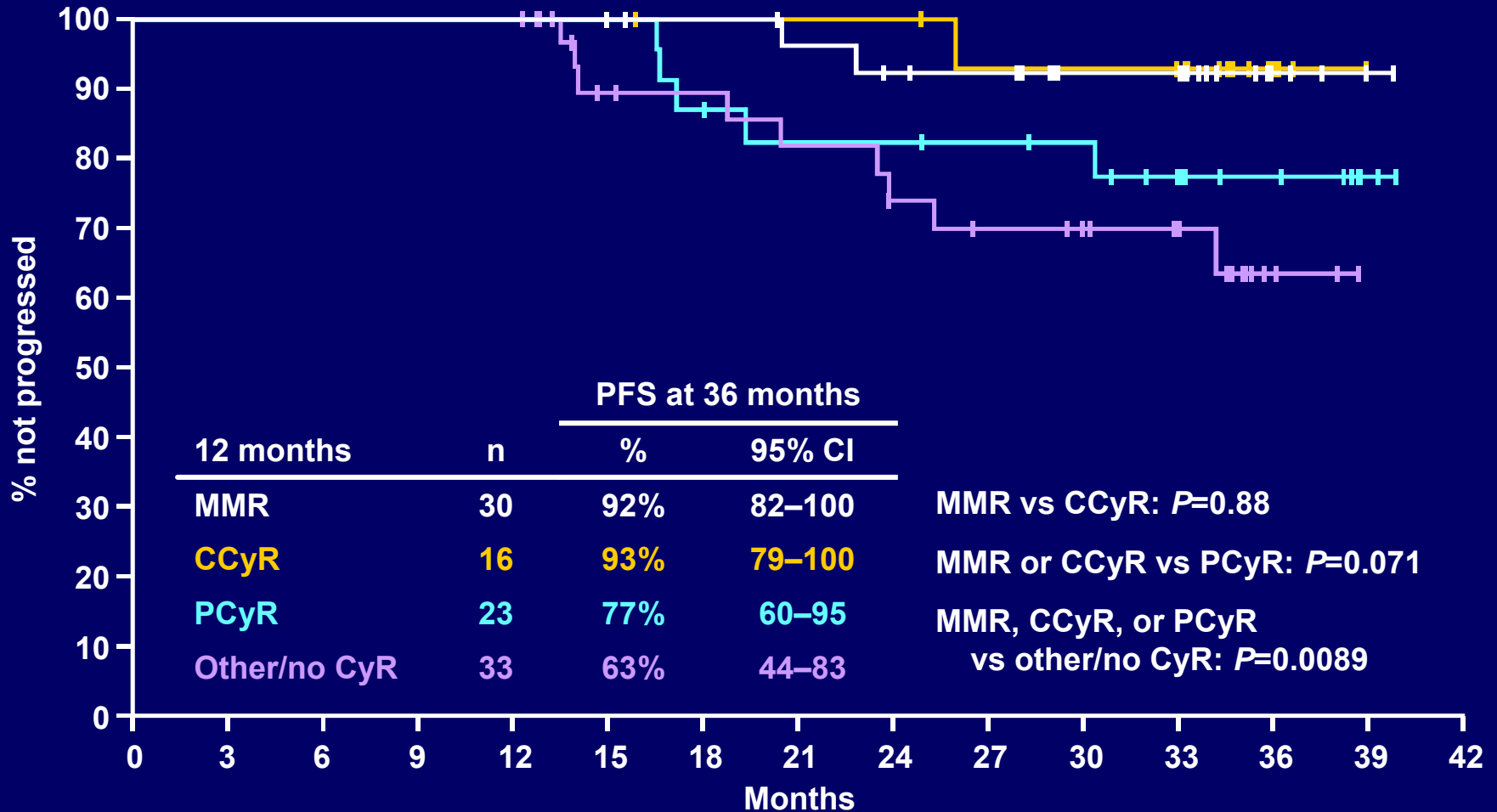
PFS and EFS according to achievement of 1% BCR-ABL^{IS} after 18 months



PFS and EFS according to achievement of 0.1% BCR-ABL^{IS} after 18 months

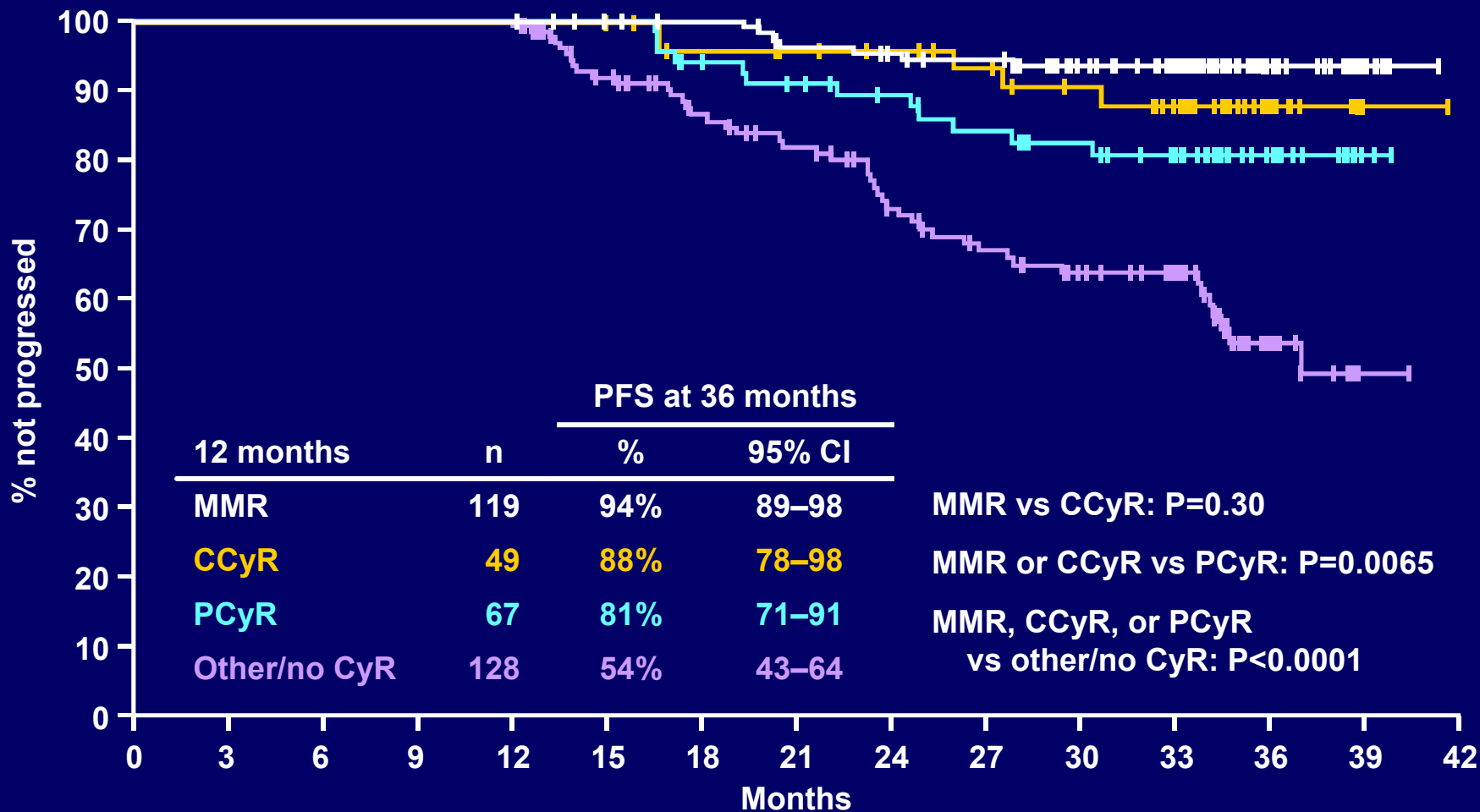


Landmark analysis of PFS according to response at 12 months (100 mg once daily)



Patients not assessed at the landmark (± 1.5 months) or with Ph(-) BCR-ABL(+) disease were excluded
 Progression was defined as increasing WBC count, loss of CHR or MCyR, $\geq 30\%$ increase in Ph(+) metaphases, confirmed AP/BP disease, or death

Landmark analysis of PFS according to response at 12 months (all doses)



Patients not assessed at the landmark (± 1.5 months) or with Ph(-) BCR-ABL(+) disease were excluded
 Progression was defined as increasing WBC count, loss of CHR or MCyR, $\geq 30\%$ increase in Ph(+) metaphases, confirmed AP/BP disease, or death

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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 "

PCR
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- [CML Discussion](#)
- [Childhood CML](#)
- [GIST Discussion](#)
- [Gleevec FAQ](#)
- [Gleevec History](#)
- [Thanks Dr. "D"](#)

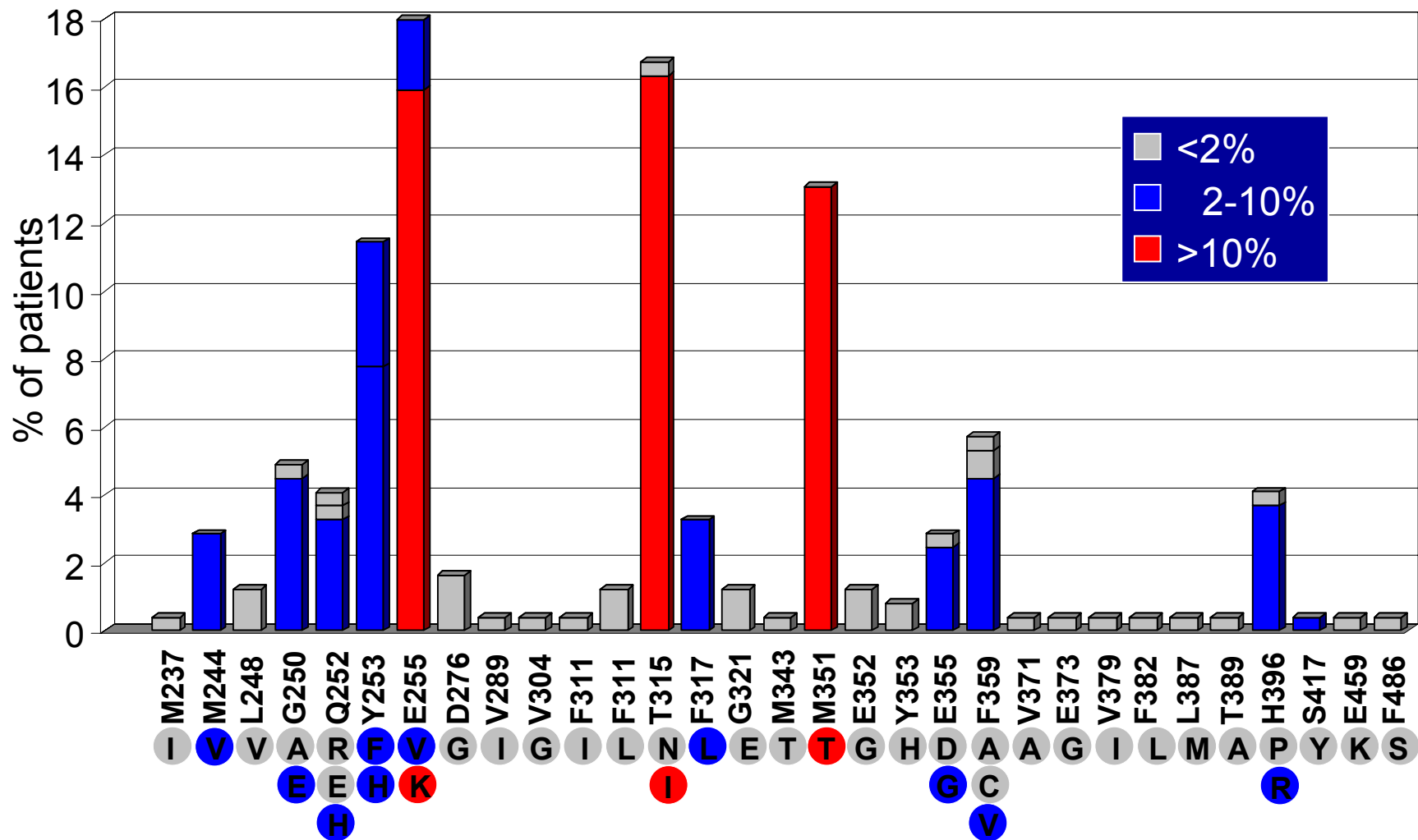
three months i had my first PCR quantative test and the results were 0.001%. Today it was 0.027% My Dr. says it is a very sensitive test and that I should not be worried. I will have another in three months. Is 0.027% a bad number?
 Help?
 Sharon T

Name:

Email:

Sensitive detection of BCR-ABL mutations

Kinase domain mutations associated with imatinib resistance



Amino acid (ABL-B)

Value of Q-PCR as a Screen for Mutations

214 patients with serial Q-PCR tests

	>2-Fold Rise in <i>BCR-ABL</i>	
	YES 56 (26%)	NO 158 (74%)
<i>BCR-ABL</i> 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%)

Outcome of patients by increase in Q-PCR

Table 3. Outcome of Patients in CGCR by Increases in QPCR

QPCR Log Increase	No. of Patients	Imatinib Dose Escalation	CML Progression	Follow-Up From QPCR Increase (months)	
				Median	Range
Persistent MMR					
Any	28	0	0	36	3-62
Loss of MMR					
> 0.5-1	12	0	0	34	14-59
> 1-2	25	0	2	31	6-52
> 2	11	4	4	45	20-57
Not in MMR					
< 1	32	3	1	35	10-70
> 1	8	1	4	25	12-56

Abbreviations: CGCR, cytogenetic complete response; QPCR, quantitative polymerase chain reaction; CML, chronic myelogenous leukemia; MMR, major molecular response.

Table 1. Outcome of Patients in CGCR on Imatinib Starting Dose of 400 mg Daily With Significant Increases in QPCR

QPCR Level by QPCR Log Increase	No. of Patients	Imatinib Dose Escalation	CG Relapse	CML Progression
Loss of MMR				
> 0.5-1	1	0	0	0
> 1-2	7	0	1	0
> 2	4	2	2	1
Not in MMR				
< 1	3	0	0	0
> 1	3	0	3	2

Abbreviations: MMR, major molecular response; CGCR, cytogenetic complete response; CML, chronic myelogenous leukemia; QPCR, quantitative polymerase chain reaction.

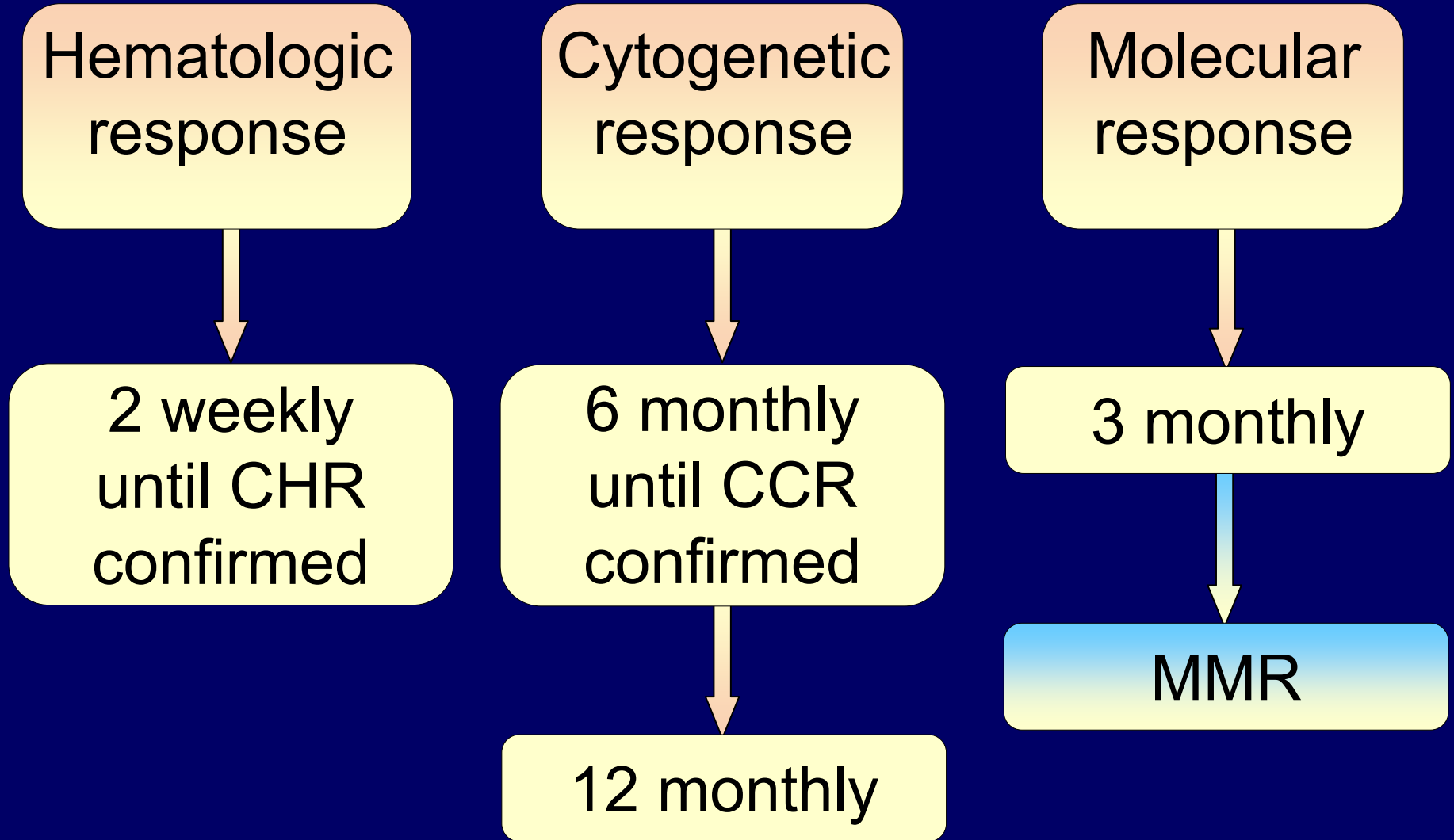
Houston recommendations

In summary, this study suggests that for patients with CML who are in durable CGCR on imatinib therapy, monitoring by QPCR is appropriate every 6 months. More frequent monitoring (eg, every 3 months) may be reasonable in patients who lose a MMR or never achieve a MMR, and who show higher than 1 log increase of CML burden (expected progression rate of 23%), although the benefit of earlier therapeutic intervention (v intervention at cytogenetic relapse) is not proven. Such patients may benefit from mutational analysis studies and may be good candidates for programs evaluating continuation of the same imatinib dose schedule versus escalating the imatinib dose or changing to second generation TKIs. Outside such investigations, therapeutic interventions, particularly a change to a second generation TKI or consideration of an allogeneic stem-cell transplant, are questionable in patients in CGCR.

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%

Recommendations by an Expert Panel



Recommendations by an Expert Panel

