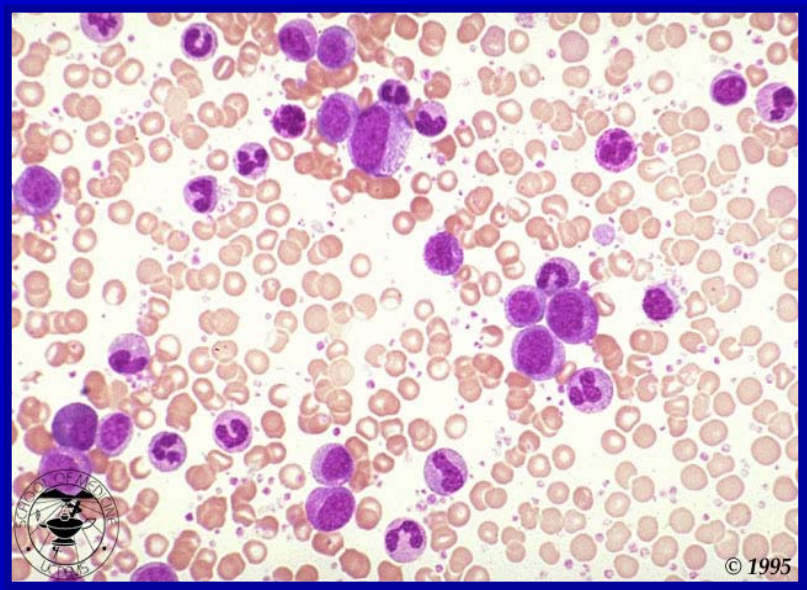
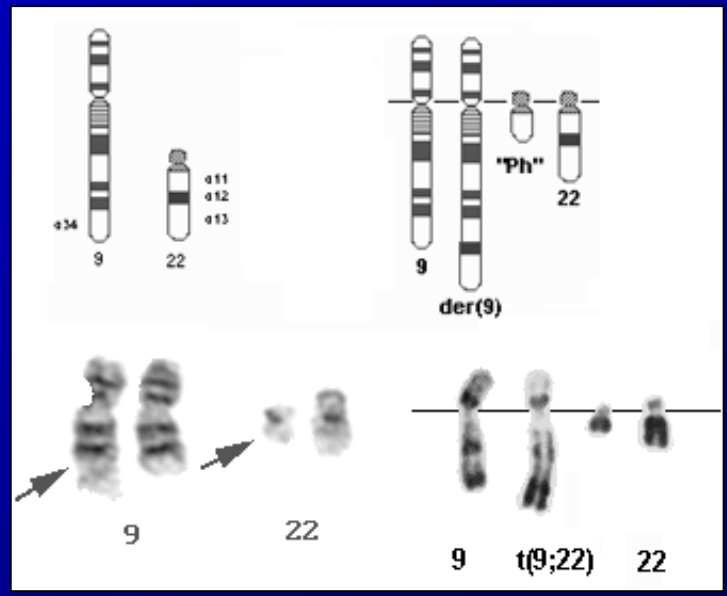
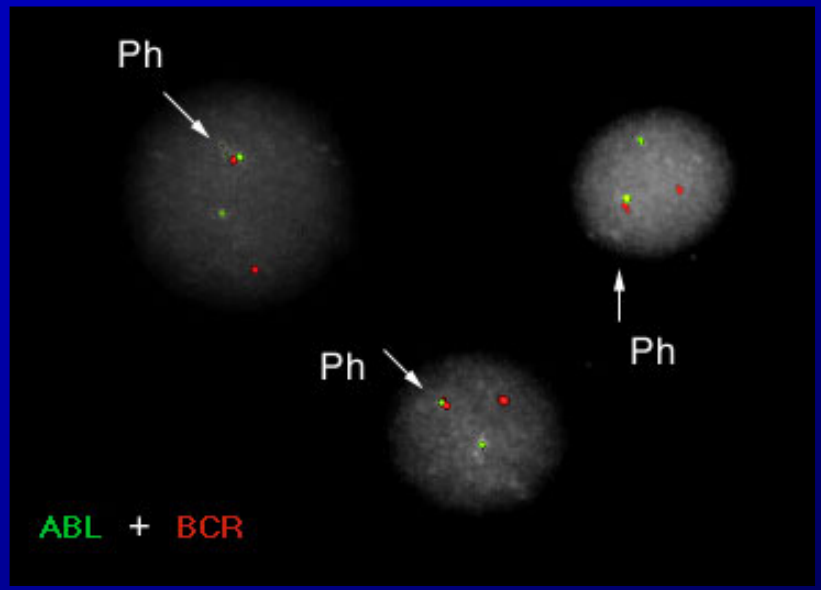
Dasatinib: Una Alternativa Terapéutica en el Tratamiento de la LMCr.

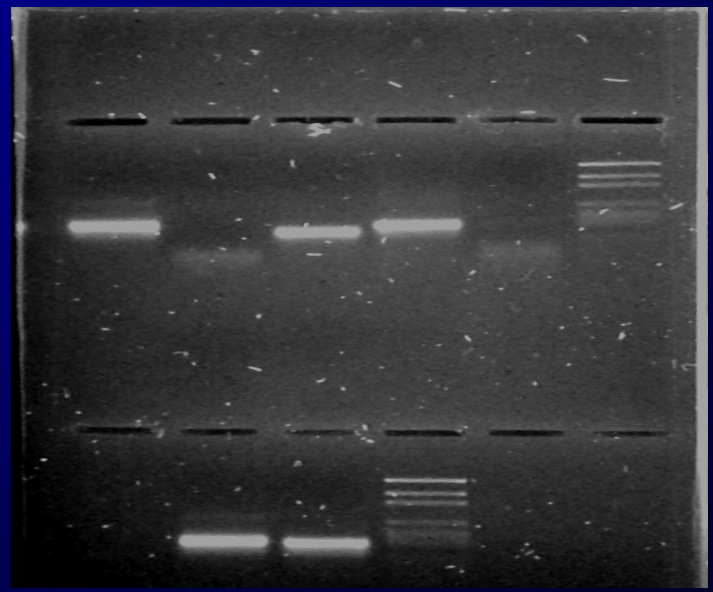
Dr. Jorge Alfaro Lucero
Laboratorio de Terapia Celular
Universidad de Chile
2008

Técnicas para el diagnóstico de LMCr t(9;22)(q34; q11)







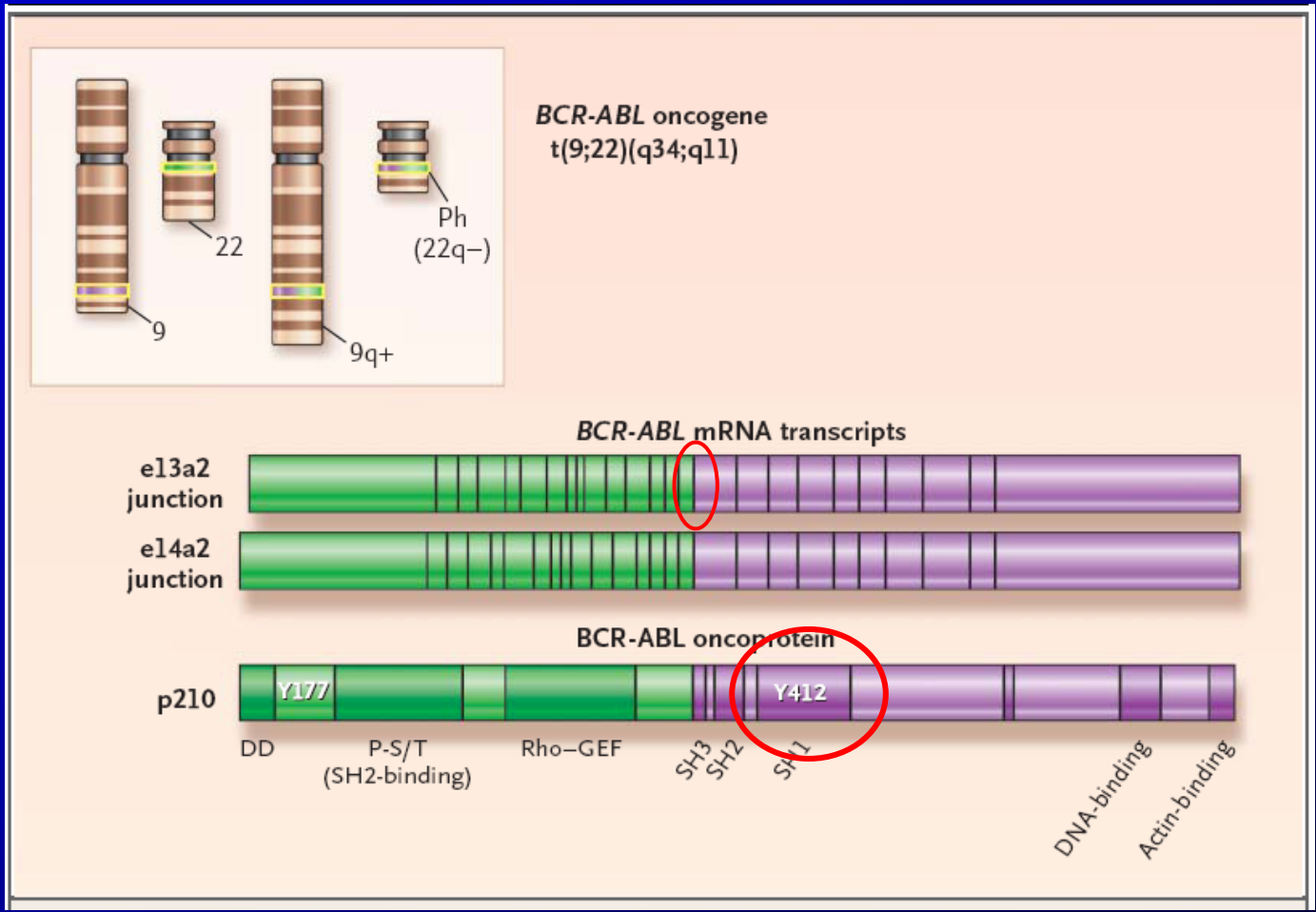


Comparación entre técnicas diagnósticas y de seguimiento

Table 1. Comparison of conventional cytogenetics, FISH, and molecular studies

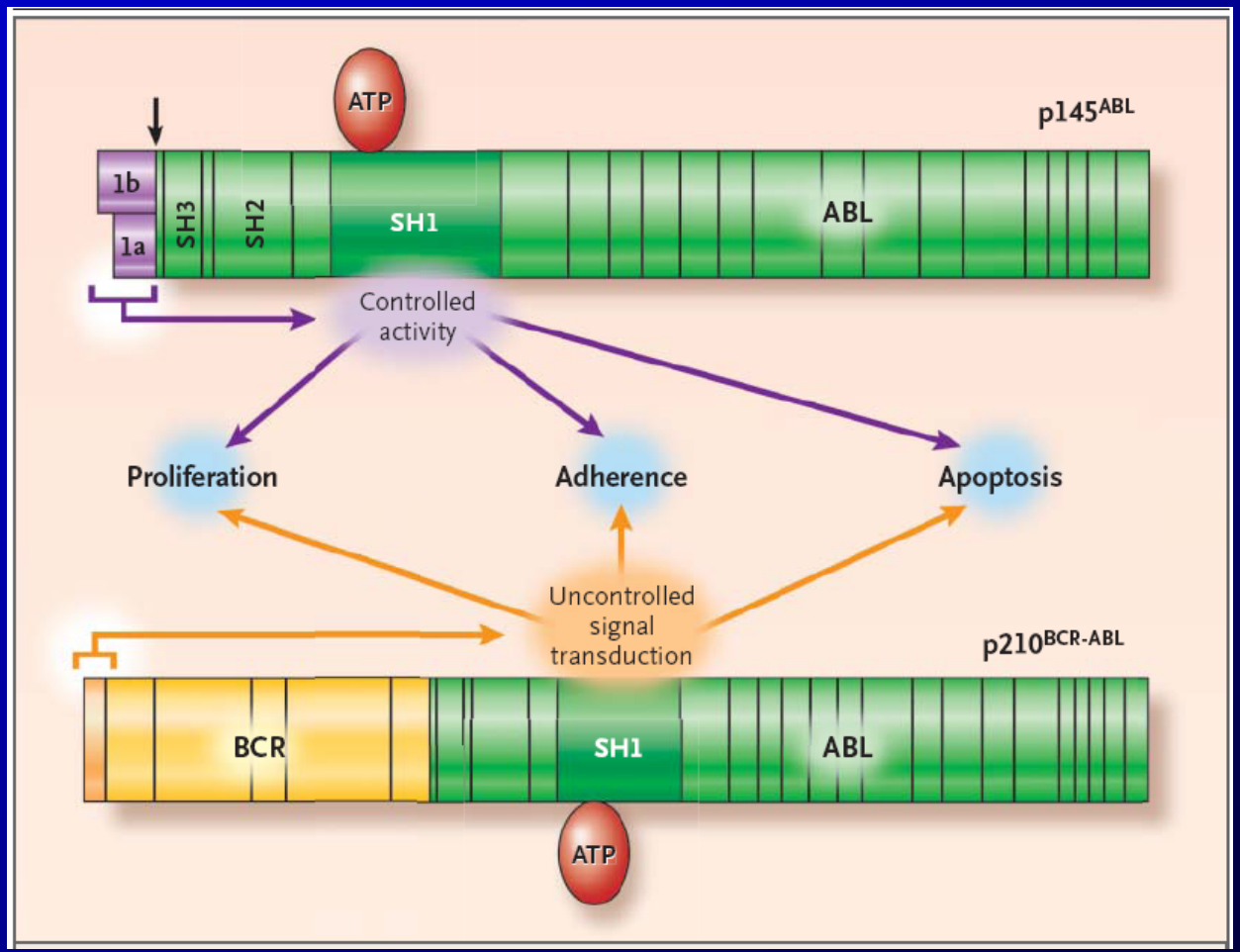
Parameter	Conventional cytogenetics	FISH	QPCR
Sensitivity, % tumor	5%-10%	1%-10%	0.001-0.01
Accuracy of measurement	± 15%	± 2%-5%	± 2- to 5-fold
Metaphases required	Yes	No	No
Marrow sample required	Yes	No	No
Equivalence of blood and marrow results	NA	Yes	Yes
False negativity	Yes	Yes	Yes
False positivity	Rare	Yes, at ≤ 10% level	Yes, at ≤ 0.1% level
Detection of other chromosomal abnormalities	Yes	No	No
Detection of deletions of derivative chromosome 9	No	Yes	No

Transcritos mRNA BCR-ABL

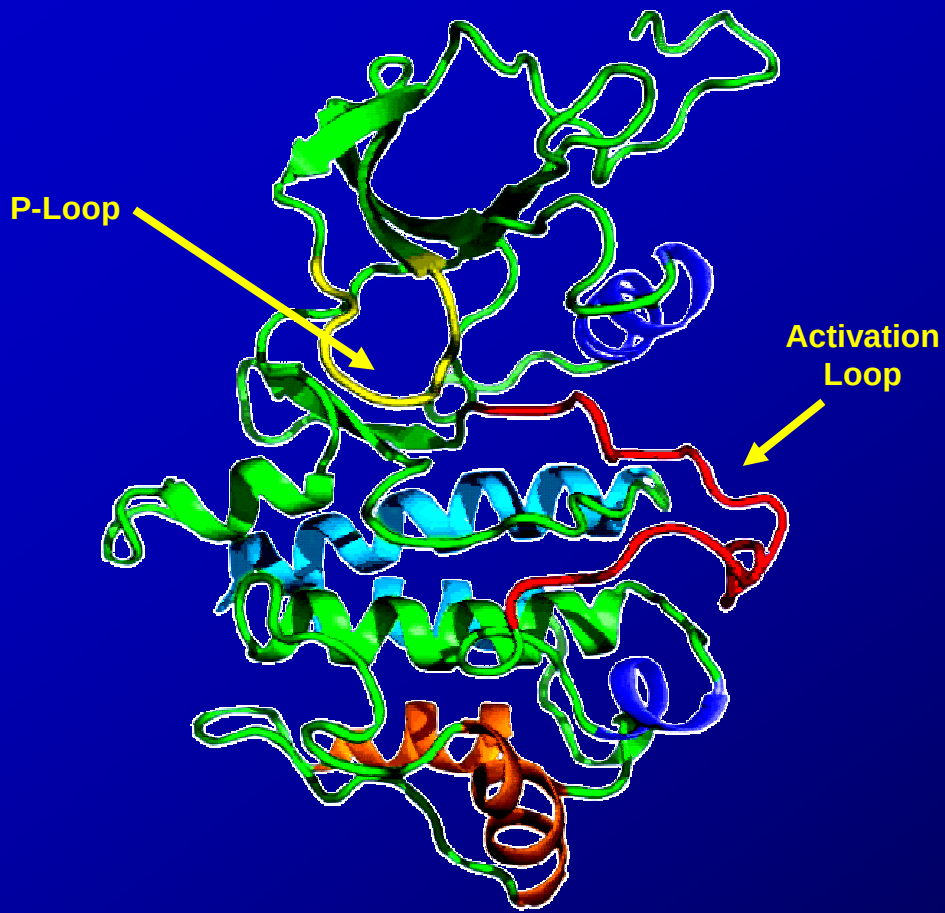


NEJM 2003;349:1451-64

Estructura sitio activo

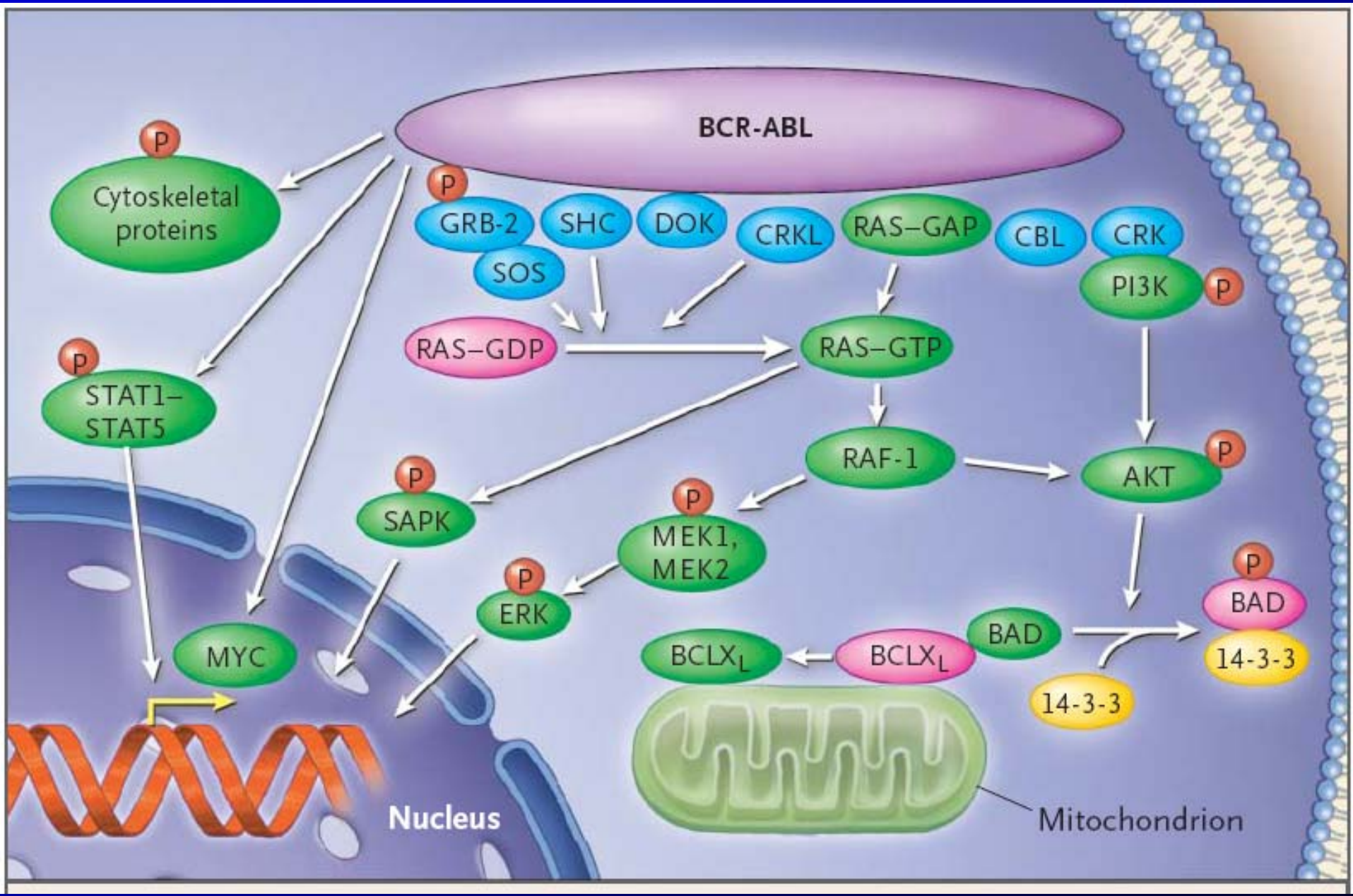


Vías Moleculares en LMCr

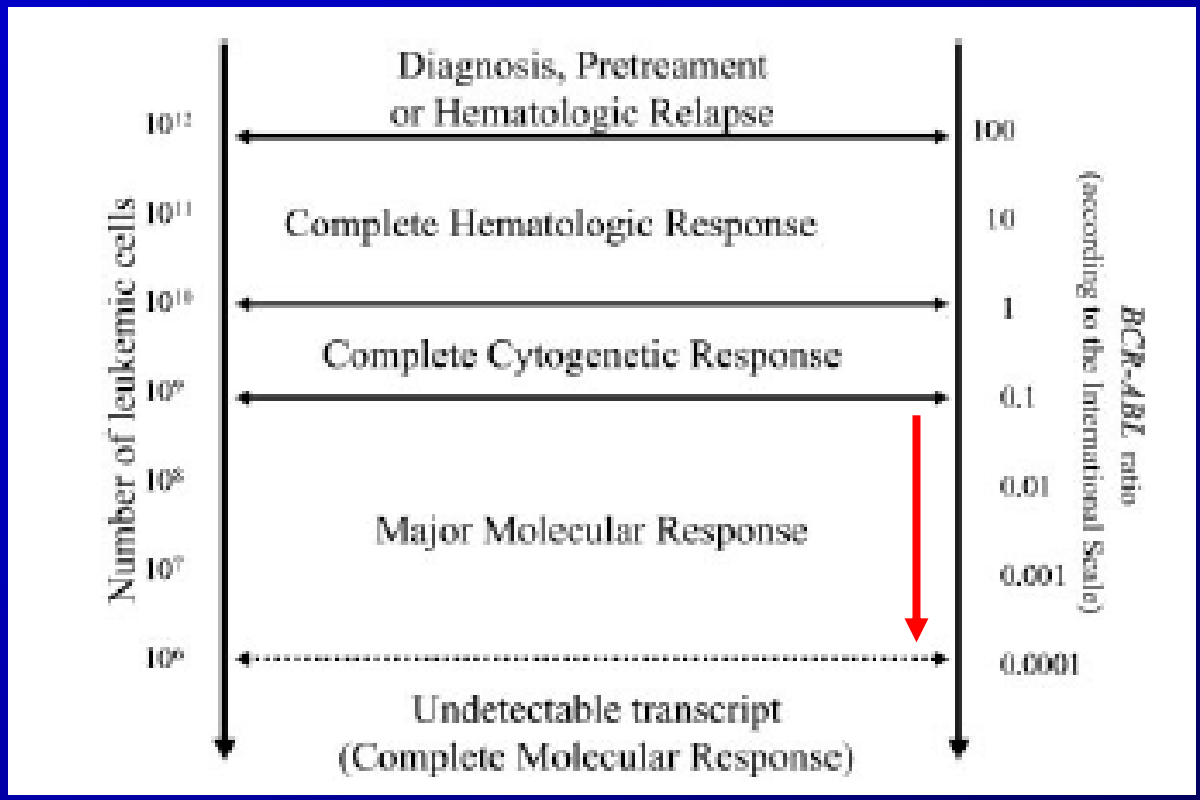


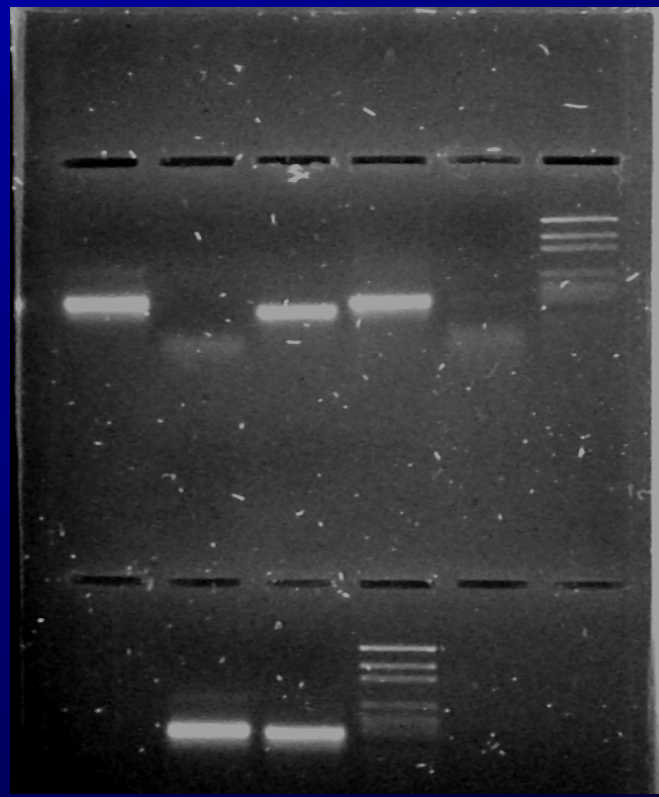
- **La kinasa BCR-ABL es creada por el gen B*CR-ABL* del Cromosoma Philadelphia.[1]**
- **Mientras BCR-ABL es la fuerza manejadora en LMCr, numerosas vías oncogénicas adicionales han sido identificadas.[2]**

1. Sawyers CL. *N Engl J Med.* 1999;340:1330-1340.
2. Melo JV, Deininger MWN. *Hematol Oncol Clin North Am.* 2004;18:545-568.

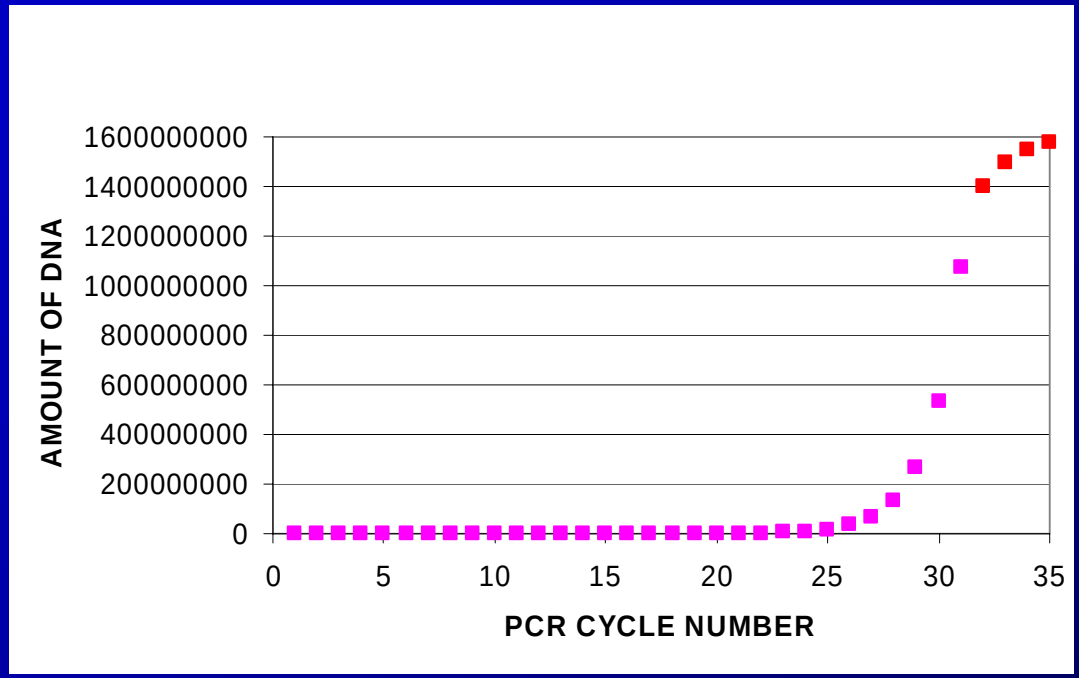

BCR-ABL
P
Cytoskeletal proteins
P
GRB-2
SHC
DOK
CRKL
RAS–GAP
CBL
CRK
SOS
PI3K
P
RAS–GDP
RAS–GTP
P
STAT1–STAT5
RAF-1
AKT
P
P
SAPK
P
MEK1, MEK2
P
ERK
P
BAD
MYC
BCLX$_L$
BCLX$_L$
BAD
14-3-3
14-3-3
Nucleus
Mitochondrion

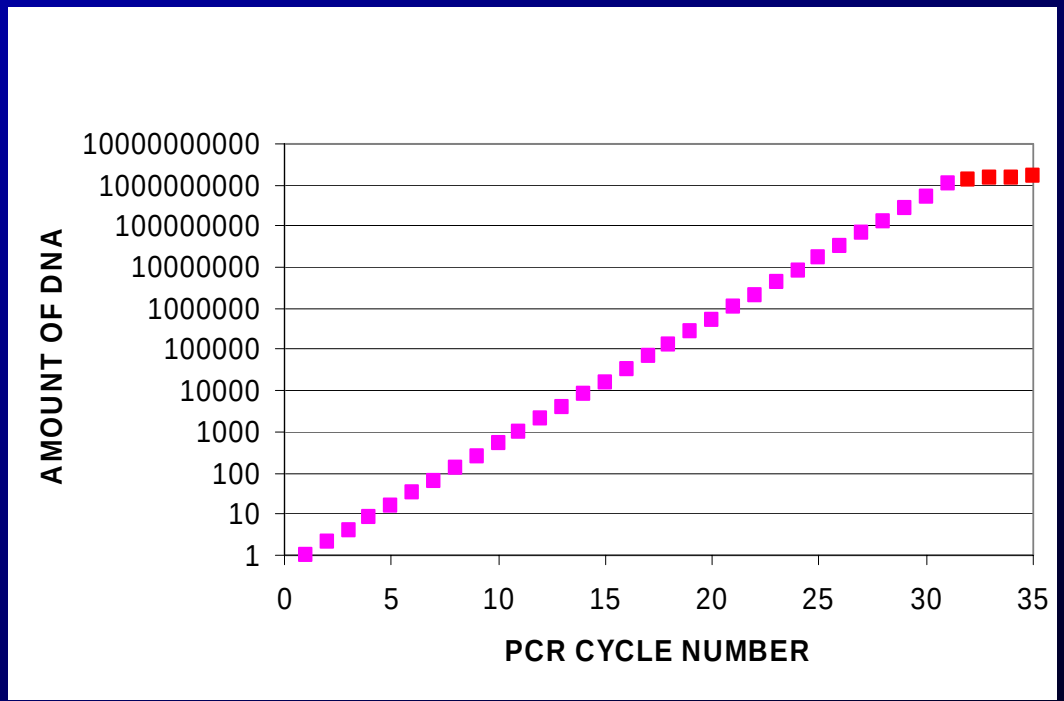
Respuesta Molecular

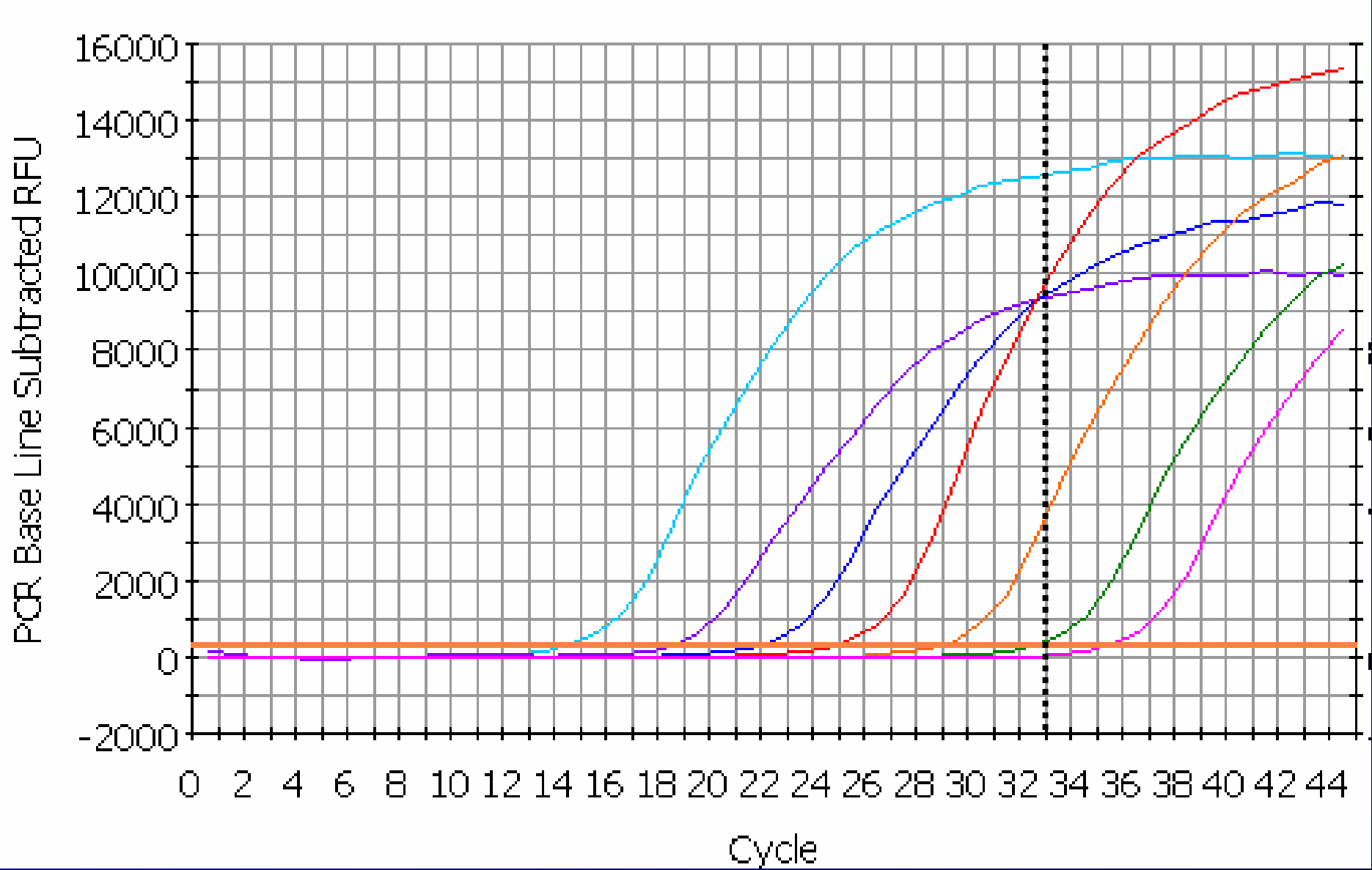




CYCLE NUMBER	AMOUNT OF DNA
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1.024
11	2.048
12	4.096
13	8.192
14	16.384
15	32.768
16	65.536
17	131.072
18	262.144
19	524.288
20	1.048.576
21	2.097.152
22	4.194.304
23	8.388.608
24	16.777.216
25	33.554.432
26	67.108.864
27	134.217.728
28	268.435.456
29	536.870.912
30	1.073.741.824
31	1.400.000.000
32	1.500.000.000
33	1.550.000.000
34	1.580.000.000







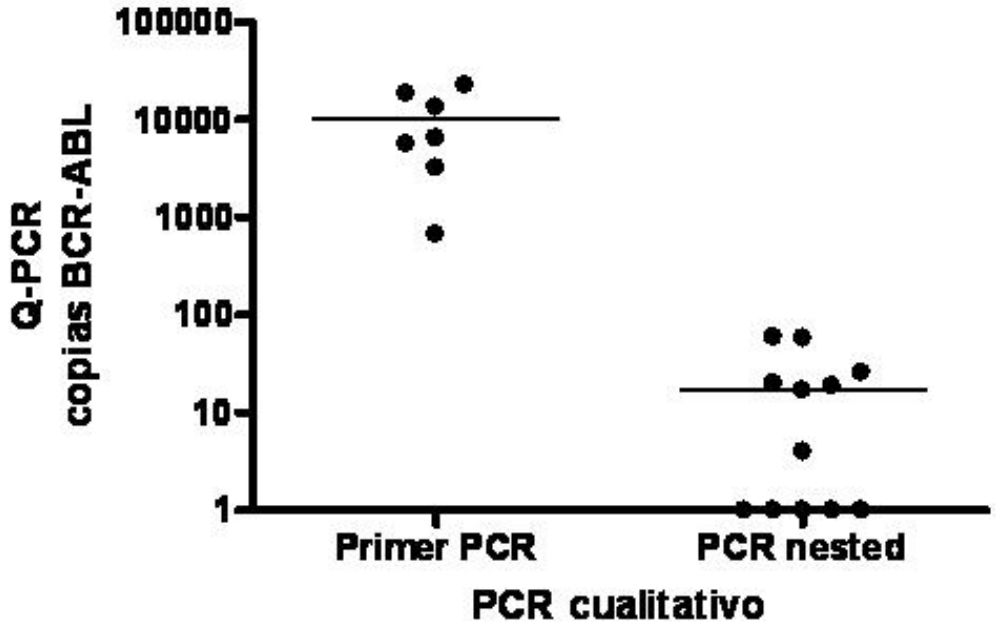
Serie de 10 diluciones

Monitoreo de pacientes con LMCr mediante estudio Q-PCR

- N = 23 pacientes (12 hombres-11 mujeres)
- Relación Hombres/Mujeres = 1,1
- Edad Promedio: 50,4 años (19-74)
- Tiempo de evolución promedio: 56,7 meses
- (0 – 180)
- Pacientes al diagnóstico: 2
- 18 con Imatinib
- 2 con Dasatinib
- 3 Sin tratamiento

N	Codigo	PCR 1	PCR nested	Q-PCR
1	BM069	+	+	32229
2	R23	+	+	22840
3	R22	+	+	18540
4	R10	+	+	13590
5	R07	+	+	6539
6	R03	+	+	5667
7	R02	+	+	699
8	R12	-	+	60
9	R16	-	+	58
10	R13	-	+	26
11	R21	-	+	20
12	R24	-	+	19
13	R19	-	+	17
14	R20	-	+	4
15	R04	-	+	0
16	R05	-	+	0
17	R08	-	+	0
18	R09	-	+	0
19	R15	-	+	0
20	R18	-	-	155
21	R17	-	-	84
22	R06	-	-	0
23	R11	-	-	0

Q-PCR en pacientes con LMCr



Monitoreo de pacientes con LMCr mediante estudio Q-PCR

Colaboradores

• Dr. Carlos Regonesi	Cl. Las Condes
• Dra. Patricia Fardella	FALP
• Dr. Claudio Flores	FALP
• Dr. Jorge Alfaro	Clínica Dávila
• Dr. Denis Suarez	Hosp. Sótero del Rìo.

Laboratorio de Terapia Celular,
Banco de Sangre. Hospital Clínico U. de Chile.

- Dr. Jorge Alfaro
- Dr. Milton Larrondo
- BQ. Claudio Pérez
- TM. Ramón Rabanales

Monitoreo para pacientes en tratamiento con inhibidores de tirosin kinasa. (NCCN v.3.2008)

Indications for cytogenetic and PCR for BCR-ABL mRNA

While a patient appears to be responding to treatment

- BCR-ABL transcript level should be measurable every 3 months.
- Bone Marrow cytogenetics at 6 months and 12 months from initiation of therapy
- Bone Marrow cytogenetics at 18 months if patient not in a complete cytogenetic remission (CCR) at 12 months.

When a patient reaches complete cytogenetic remission (CCR)

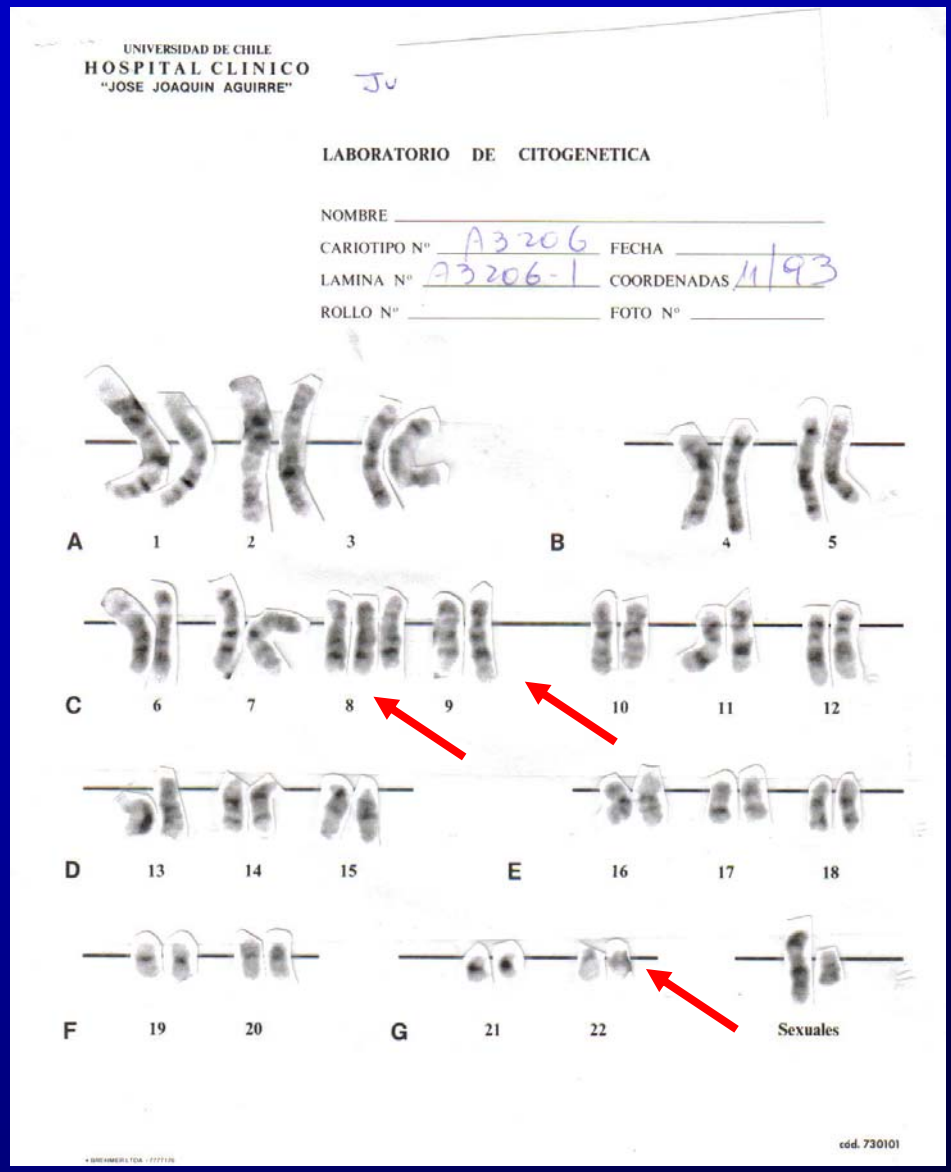
- BCR-ABL transcript level should be measurable every 3 months.
- Bone Marrow cytogenetics should be considered every 12-18 months to check for clonal abnormalities

When appears to have rising level (1 log increase) of BCR-ABL transcripts

- Rising level (1 log increase) should be repeated in one month.
- If a rising of BCR-ABL transcripts is confirmed, the frequency of measurement should be increased to once a month.
- Mutation testing may be considered.

Version 3.2008, 12/20/07 © National Comprehensive Cancer Network

Citogenético en LMCr



UNIVERSIDAD DE CHILE
HOSPITAL CLINICO
"JOSE JOAQUIN AGUIRRE"

JU

LABORATORIO DE CITOGENETICA

NOMBRE _____

CARIOTIPO N° A3206 FECHA _____

LAMINA N° A3206-1 COORDENADAS 11/93

ROLLO N° _____ FOTO N° _____

A 1 2 3 B 4 5

C 6 7 8 9 10 11 12

D 13 14 15 E 16 17 18

F 19 20 G 21 22 Sexuales

cód. 730101

Definición de respuesta

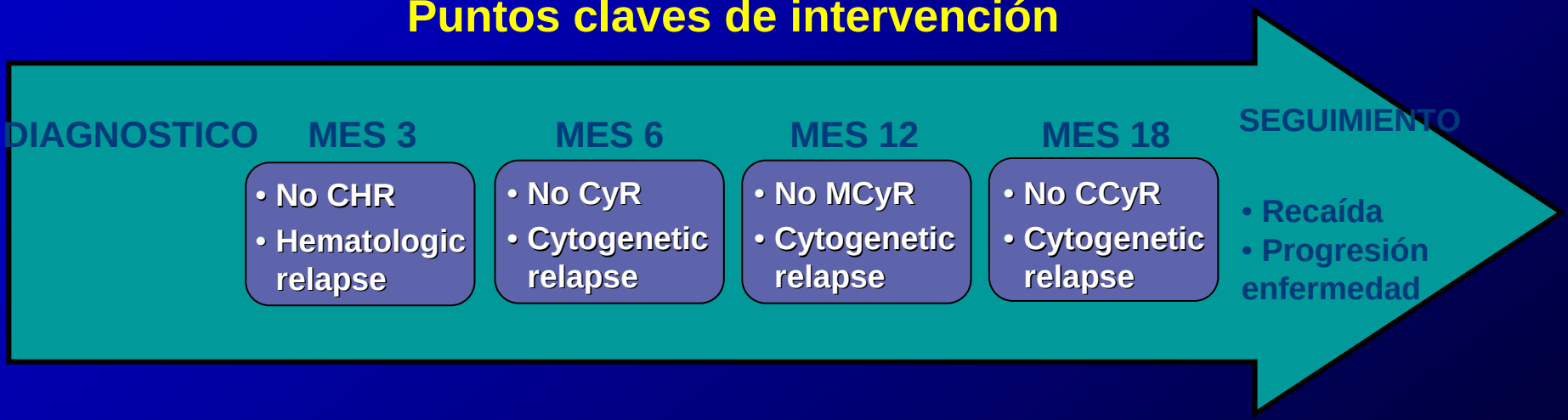
Table 8. Response definition and monitoring

	Hematologic response	Cytogenetic response	Molecular response (BCR-ABL to control gene ratio according to the international scale)
Definitions	Complete: Platelet count < 450 × 10^9/L; WBC count < 10 × 10^9/L; differential without immature granulocytes and with less than 5% basophils; nonpalpable spleen	Complete: Ph^+ 0% Partial: Ph^+ 1%-35% Minor: Ph^+ 36%-65% Minimal: Ph^+ 66%-95% None: Ph^+ > 95%	"Complete" indicates transcript nonquantifiable and nondetectable Major: ≤ 0.10
Monitoring	Check every 2 wk until complete response achieved and confirmed, then every 3 mo unless otherwise required	Check at least every 6 mo until complete response achieved and confirmed, hence at least every 12 mo	Check every 3 mo; mutational analysis in case of failure, suboptimal response, or transcript level increase

Cuando considerar dasatinib

National Comprehensive Cancer Network (NCCN®) Guidelines [1]

Puntos claves de intervención



- MCyR definido como 0% to 35% of cel. Con metafases Ph+

1. The Chronic Myelogenous Leukemia Clinical Practice Guidelines in Oncology (Version 1.2007).

Dasatinib: Indicaciones y uso

- **Dasatinib es un inhibidor de kinasa oral, cuyo blanco es BCR-ABL y la familia de kinasas SRC.**
- **Su indicación es en pacientes adultos con LMCr en fase crónica, acelerada o blástica ya sea linfoide o mieloblástica, con intolerancia o resistencia al Imatinib.**
- **La efectividad del dasatinib está dada en la tasa de respuesta hematológica y citogenética.**
- **Dasatinib está también indicada en LLA Phi+ con resistencia o intolerancia a una terapia previa. [1]**

1. SPRYCEL® (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; November, 2007.

Estudios primarios de eficacia y seguridad de dasatinib

Estudio	Población	Número pacientes
CA180002 (Fase 1)	LMCr fase crónica, acel, blástica, LLA Phi+	92
CA180013 (Fase 2)	LMCr fase crónica	424
CA180017 (Fase 2)	LMCr fase crónica Dasatinib v/s Imatinib	166
CA180005 (Fase 2)	LMCr fase acelerada	197
CA18006 (Fase 2)	LMCr fase blástica	124
CA180015 (Fase 2)	LLA Ph+ o LMCr fase blástica linfoide	101
Total		1104

Imatinib: Resistencia e intolerancia en LMCr

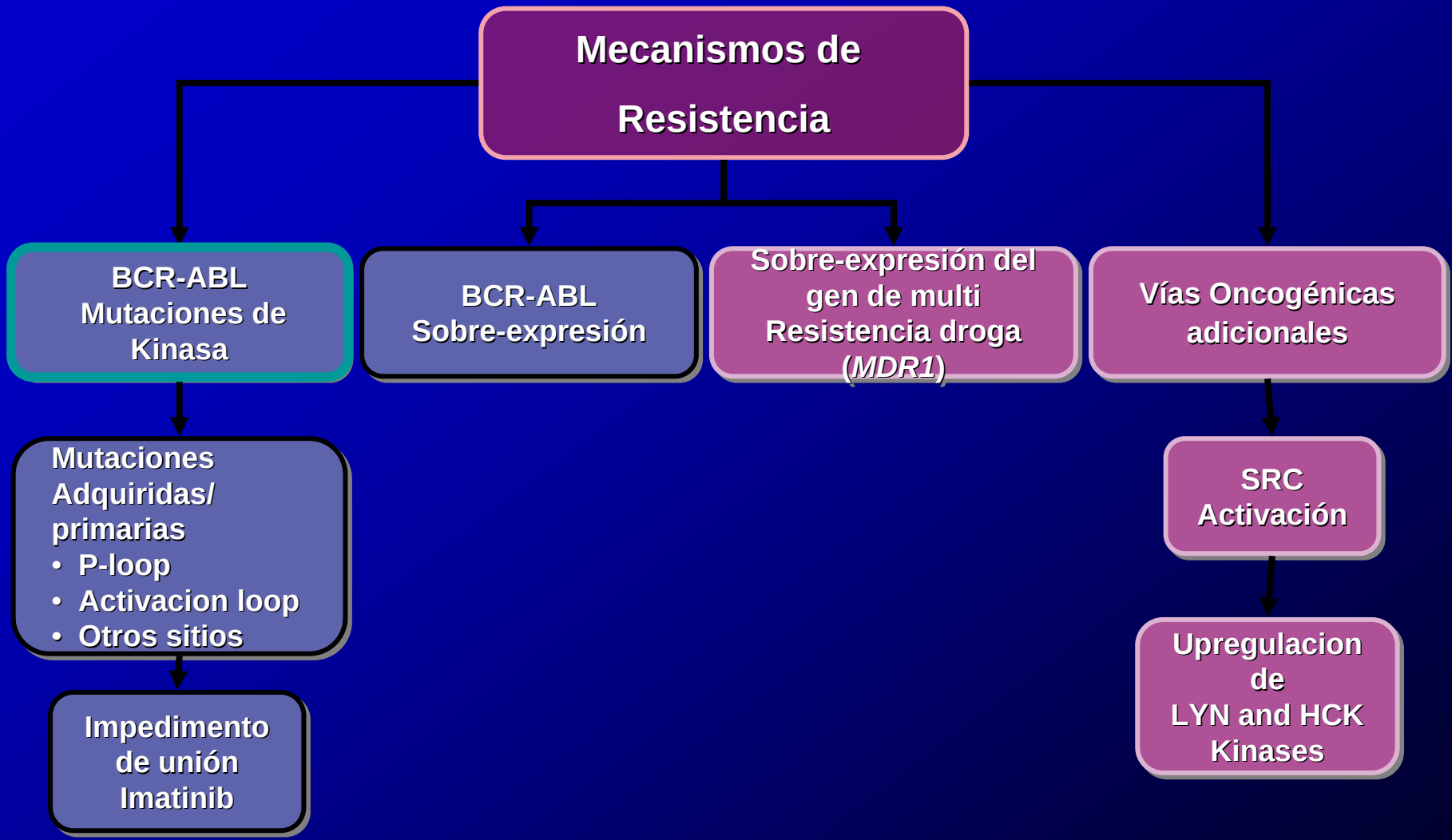
- Falla en alcanzar respuesta hematológica completa (RHC) en 3 a 6 meses de tratamiento.
- Falla en alcanza respuesta citogenética mayor (RCM) a los 12 meses.
- Progresión de enfermedad después de una respuesta hematológica y citogenética.

Intolerancia al Imatinib: definición[1]:

- **Incapacidad de tolerar 400 mg o más de imatinib al día o discontinuación de imatinib a causa de toxicidad.**

1. SPRYCEL® (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; November, 2007.

Mecanismos de Resistencia al Imatinib[1-5]



1. Branford S, Rudzki Z, Walsh S, et al. *Blood.* 2003;102:276-283. 2. Donato NJ, Wu JY, Stapley J, et al. *Blood*. 2003;101:690-698.
3. Weisberg E, Griffin JD. *Blood.* 2000;95:3498-3505. 4. Mahon F-X, Belloc F, Lagarde V, et al. *Blood.* 2003;101:2368-2373.
5. SPRYCEL® (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; November, 2007.

Resistencia al Imatinib dependiente de BCR-ABL

Mutaciones de la kinasa BCR-ABL[1,2]:

- Causa más común de resistencia.
- Mutaciones mantienen BCR-ABL en la conformación activa.
- Imatinib se une solamente a la conformación inactiva de BCR-ABL.
- Mutaciones en el punto de contacto crítico, evita la unión del imatinib.

Sobre-expresión / Amplificación de BCR-ABL en células resistentes al imatinib. [3]:

- La sobre expresión transgénica de BCR-ABL > 5 veces, aumenta 20 veces el mRNA y la proteína BCR-ABL.

1. Branford S, Rudzki Z, Walsh S, et al. *Blood.* 2003;102:276-283.
2. Shah NP, Tran C, Lee FY, et al. *Science*. 2004;305:399-401.
3. Weisberg E, Griffin JD. *Blood.* 2000;95:3498-3505.

Resistencia BCR-ABL Independiente de Imatinib

Sobre-expresión de kinasa SRC Asociada a progresión de enfermedad y resistencia a imatinib.

- Kinasa SRC puede contribuir a la progresión, hacia un estado avanzado de enfermedad.
  - Kinasa SRC está altamente expresada y activada durante la crisis blástica, correlacionándose con la progresión en algunos pacientes con LMCr.

- BCR-ABL sobre-regula significativamente las kinasas SRC (LYN and HCK)[1]

  - LYN su sobre expresión y activación promueve el crecimiento celular y otorga protección anti apoptótica. [1]

  - HCK fosforila c-ABL *in vitro*, con lo cual inhibe la unión y la actividad kinasa inhibitoria. [2]

1. Donato NJ, Wu JY, Stapley J, et al. *Blood*. 2003;101:690-698.
2. Schindler T, Bornmann W, Pellicena P, et al. *Science*. 2000;289:1938-1942.

ABL kinase domain (KD) mutation analysis may be considered (NCCN v.3.2008)

Chronic phase CML

- ABL KD mutation screening may provide additional information if there is inadequate initial response:

- Failure to achieve complete hematologic response at 3 months;
- Minimal cytogenetic response at 6 months or major cytogenetic response at 12 months;
- Sign of loss of response (defined as hematologic relapse, relapse to Ph-positivity or an increase in BCR-ABL transcript ratio)

Accelerated and blast phase CML

- Testing for KD mutation may provide additional information.

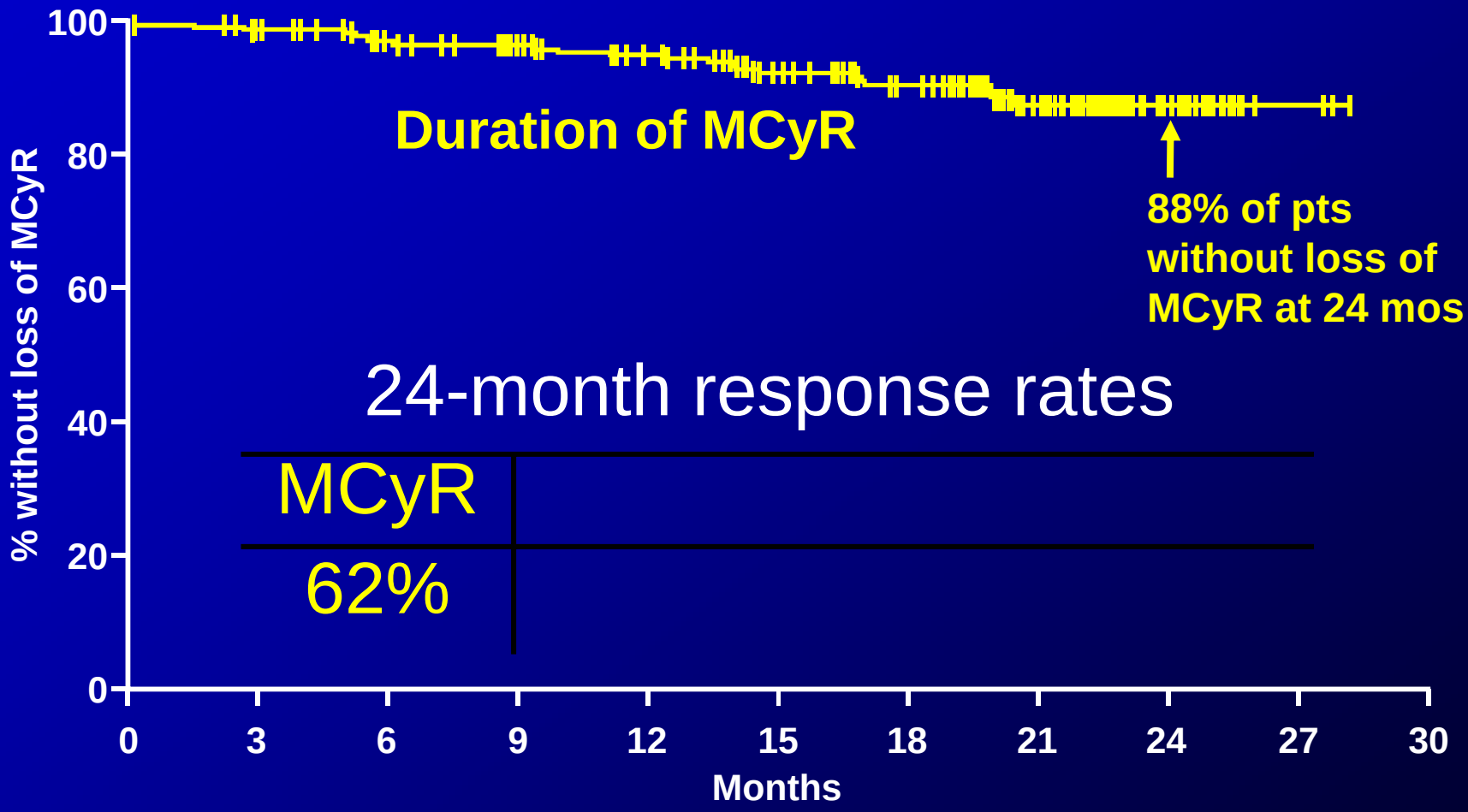
Version 3.2008, 12/20/07 © National Comprehensive Cancer Network

Dasatinib 2-year efficacy in patients with chronic-phase chronic myelogenous leukemia (CML-CP) with resistance or intolerance to imatinib (START-C)

Michael Mauro,[1,2] Michele Baccarani,[2]
Francisco Cervantes,[2] Jeffrey Lipton,[2] Yousif Matloub,[3]
Ritwik Sinha,[3] Richard Stone[2]

[1]Oregon Health and Science University, Portland, OR, USA;
[2]START-C Trial Study Group;
[3]Bristol-Myers Squibb Co., Wallingford, CT, USA

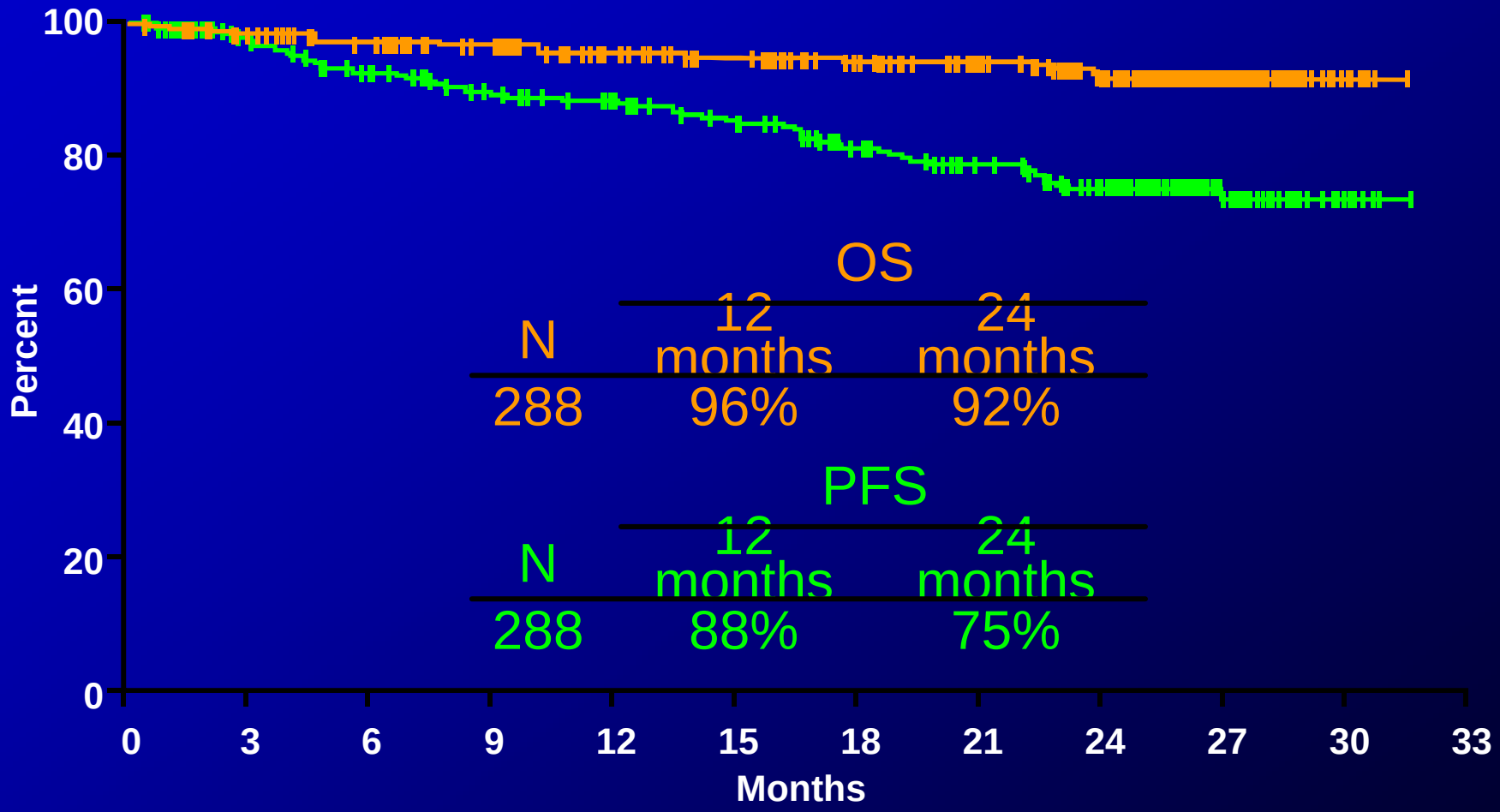
Rate and durability of MCyR with dasatinib



24-month response rates

MCyR
62%

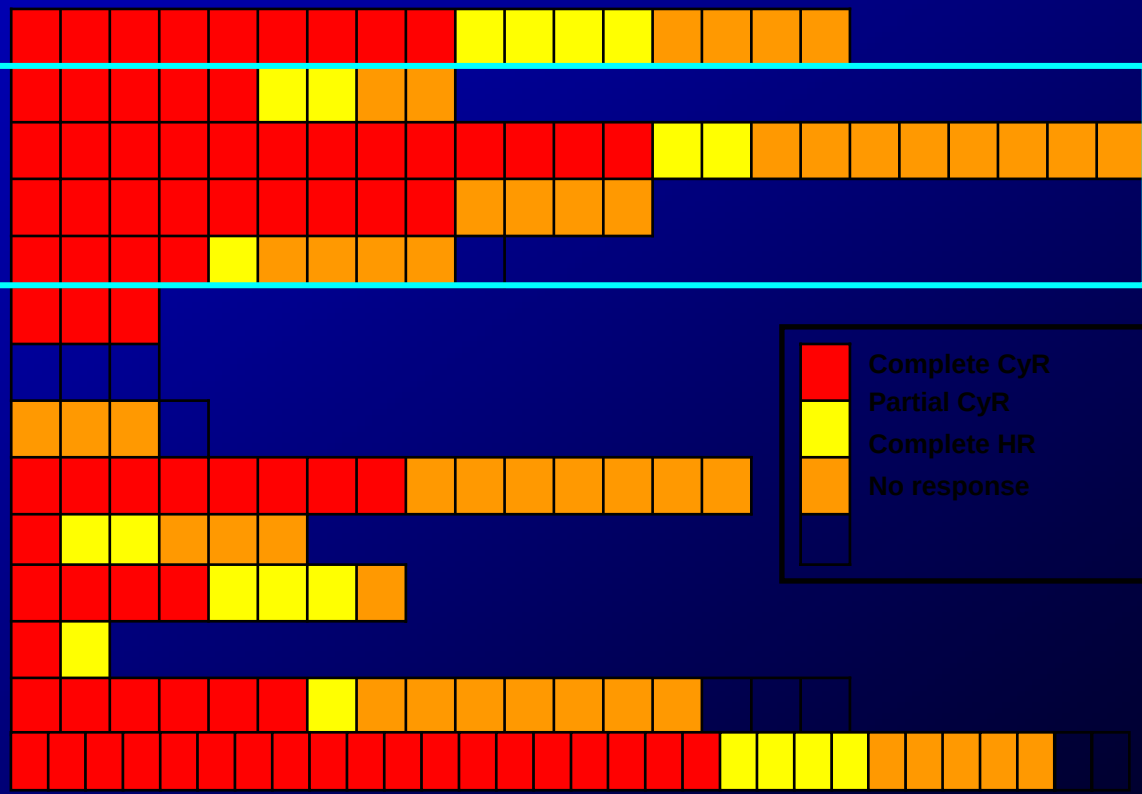
Imatinib resistant patients: PFS and overall survival with dasatinib



Progression was defined as increasing WBC count, loss of CHR / MCyR, confirmed AP / BP, or death

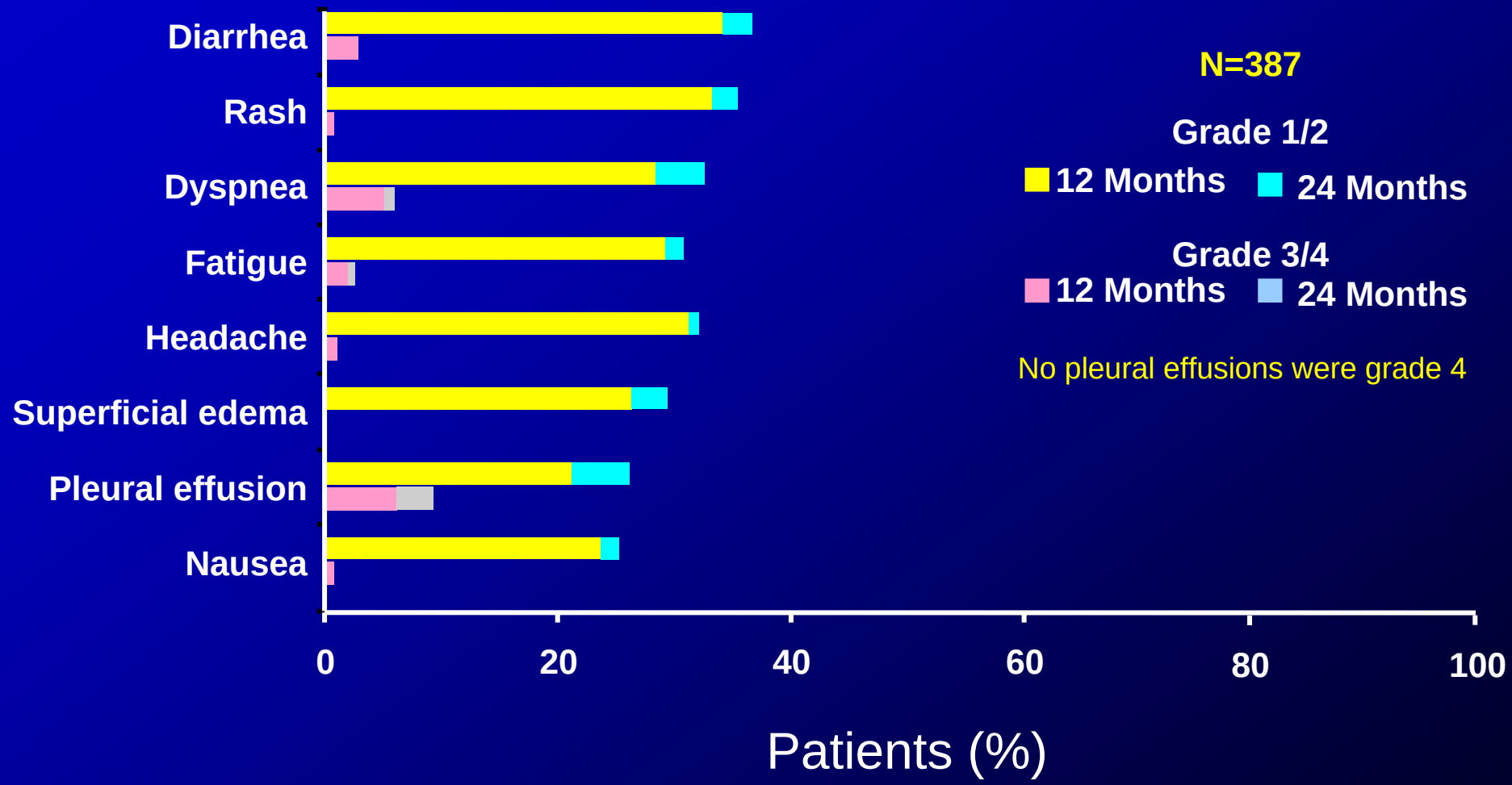
Response by individual baseline BCR-ABL mutation

	Cellular IC_{50} (nM)		
	Dasatinib	Imatinib	n
M244V	1.3	2000	17
L248V		1500	9
G250E/V	1.8	1350–3900	23
Y253F/H/K	1.3–10	>10000	14
E255K/V	5.6–13	4400–8400	10
D276G		1500	3
T315I	>1000	>10000	3
F317L	7.4	1050	4
M351T	1.1	930	15
E355G		400	6
F359C/I/V	2.2	1200	8
L387M	2.0	1000	2
H396P/R	0.6–1.13	850–4200	17
Other			30



P-loop residues 248-256 are highlighted

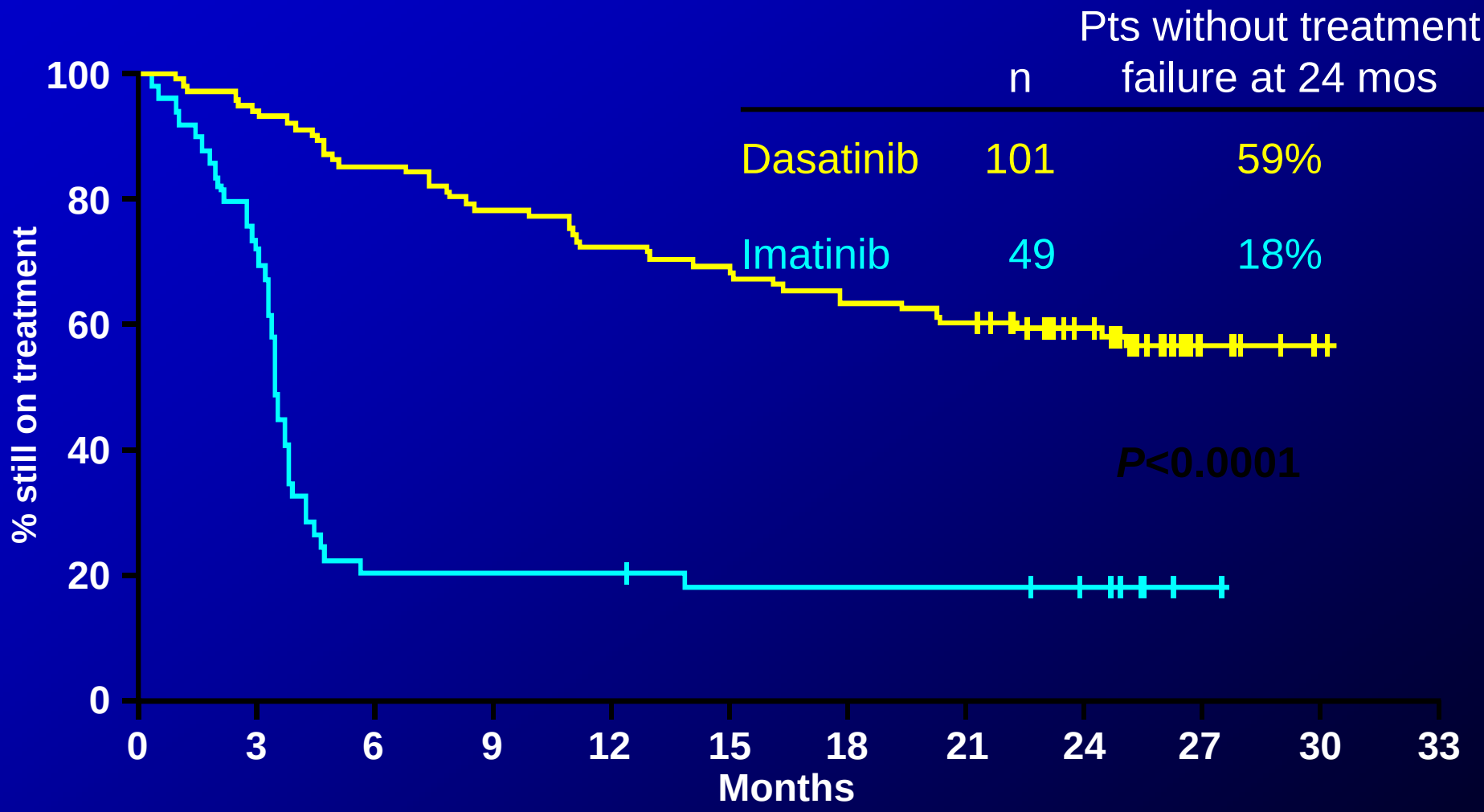
Non-hematologic side-effects



Conclusiones: LMCr fase crónica

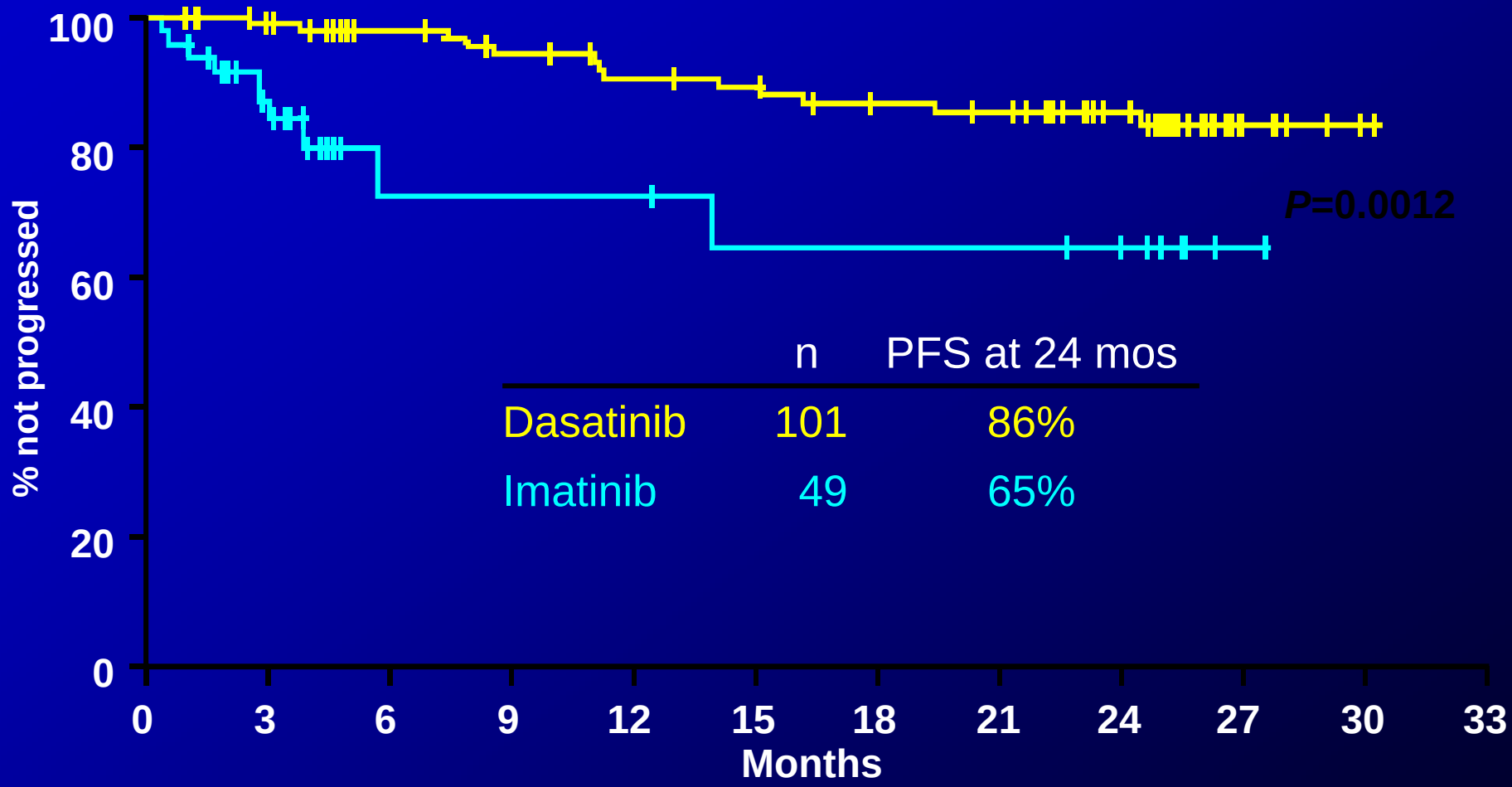
- 2 años de seguimiento (N=387) demostró eficacia contínua de dasatinib
  - Total: MCyR 62%, CCyR 53%, MMR among CCyR 79%
  - 88% continua en MCyR, 90% in CCyR at 2 years
  - PFS 80% at 2 years
  - OS 94% at 2 years
- Dasatinib demostró eficacia en todos los subgrupos incluídos:
  - Todos menos uno de 43 mutaciones diferentes
  - Pacientes con mutaciones P-loop o no previa CyR to imatinib
- Dasatinib 70 mg BID fue bien tolerado.
  - Ausencia de dasatinib cross-intolerance con imatinib

Time to treatment failure: Dasatinib v/s Imatinib



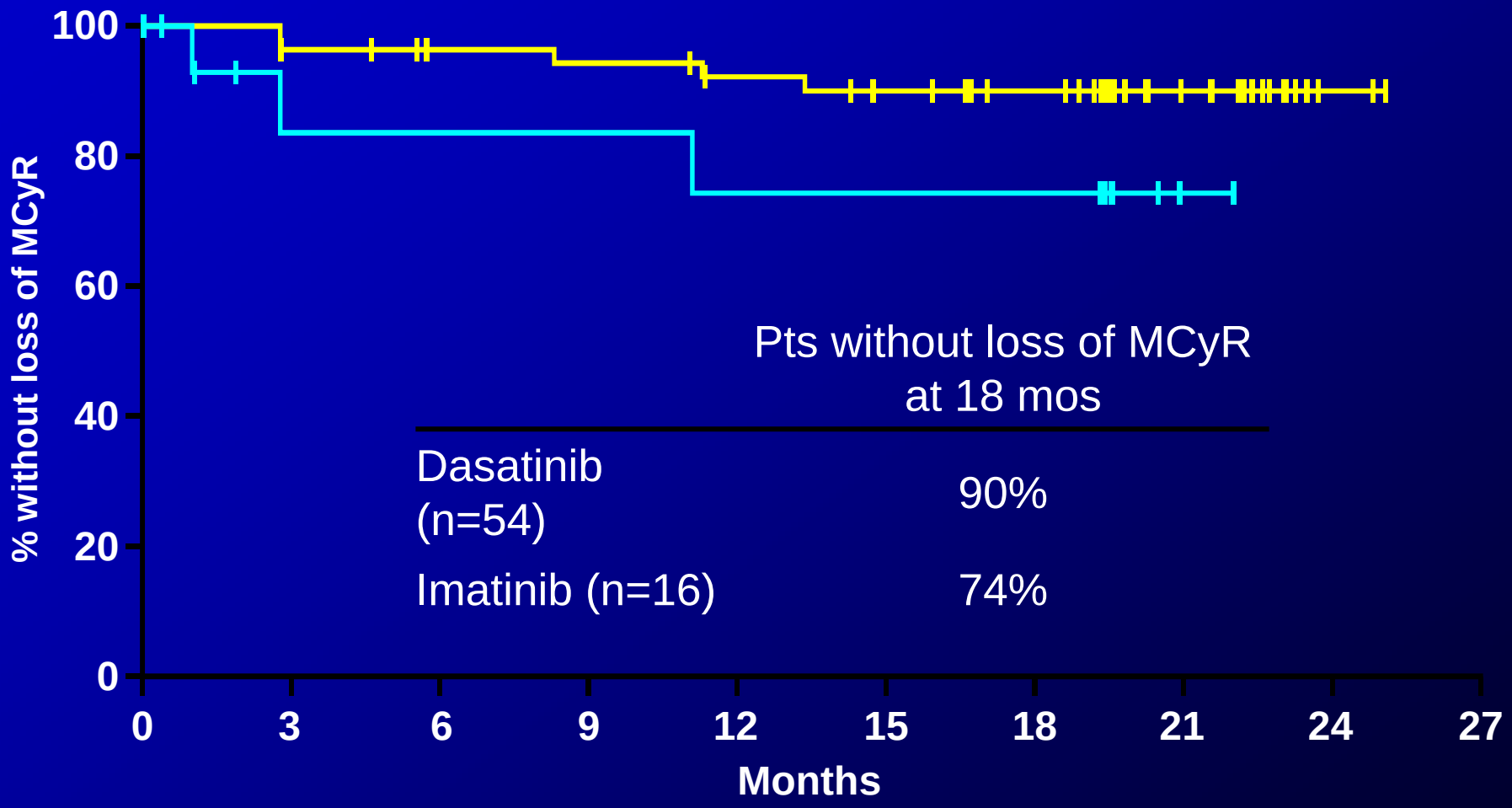
Failure: progression, lack of response, cross-over, or off treatment
Progression: increasing WBC count, loss of CHR / MCyR, confirmed AP / BP, or death

Progression-free survival

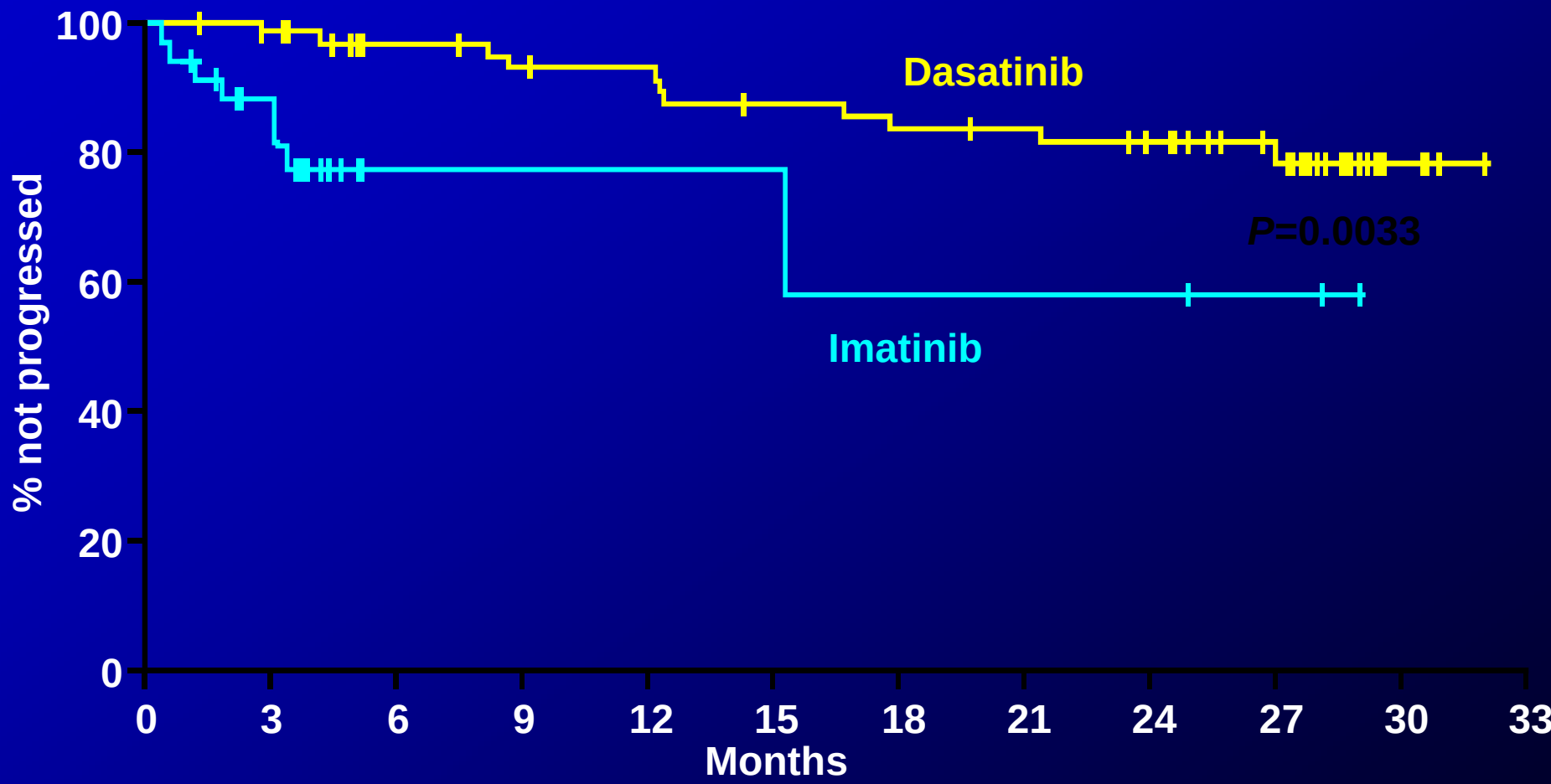


Progression: increasing WBC count, loss of CHR / MCyR, confirmed AP / BP, or death

Duración de MCyR



PFS by prior imatinib dose of 600 mg/day



Seguridad

	Dasatinib	Imatinib
Median Rx duration (mos)	23	3
Discontinuation due to toxicity (%)	22	20
Grade 3/4 cytopenia (%)		
Neutropenia	63	39
Thrombocytopenia	57	14
Grade 3/4 non-hematologic AEs (%)		
Fatigue	3	4
Diarrhea	3	2
Pleural effusion	5	0
Superficial edema	4	0
Headache	2	2
Musculoskeletal pain	1	2
Bleeding	1	0

Conclusiones

- Dasatinib is superior to imatinib 800 mg post-imatinib failure (400–600 mg/day)
  - CCyR: dasatinib 44% vs imatinib 18%, *P*=0.0025
  - MMR: dasatinib 29% vs imatinib 12%, *P*=0.028
  - 24-month PFS: dasatinib 86% vs imatinib 65%, *P*=0.0012
  - demonstrated efficacy regardless of prior imatinib dose

- Dasatinib 70 mg dos veces al día fué bien tolerado.
  - La dosis de 100 mg vo al dia fue aprobada mostrando eficacia similar a 70 mg vo cada 12 hr con menos efectos adversos. [4]

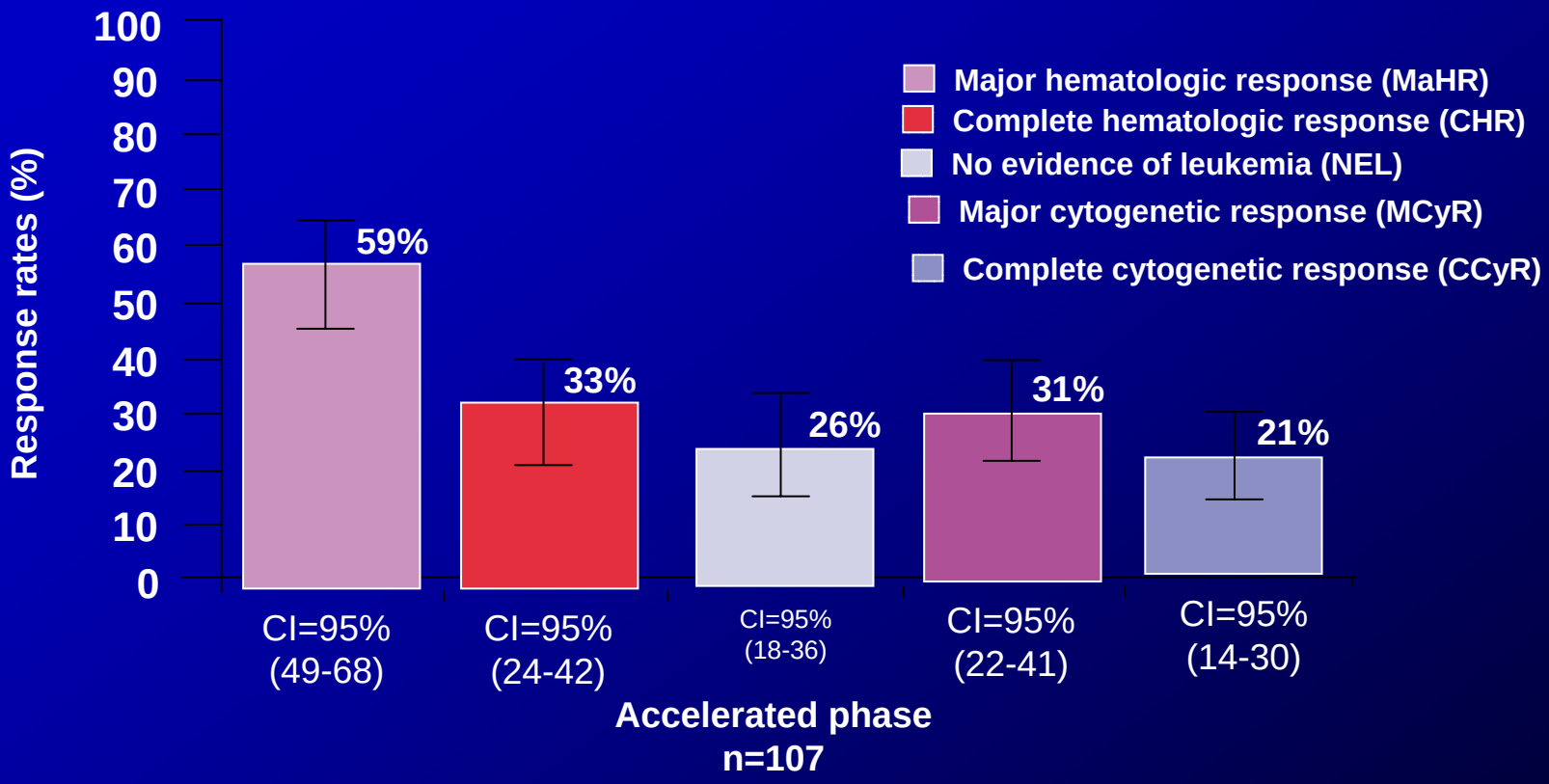
The **START** Trials en LMCr avanzada[1]

	Accelerated (n=107)	Myeloid Blast (n=74)	Lymphoid Blast (n=42)	Ph+ ALL (n=36)
Median time since diagnosis, mo (range)	91 (4-355)	49 (3-216)	28 (2-186)	20 (3-97)
Imatinib resistant	93%	92%	88%	94%
Imatinib intolerant	7%	8%	12%	6%
Imatinib >3 years	68%	47%	24%	3%
Imatinib >1 year	92%	85%	52%	56%
Cytotoxic chemotherapy	67%	66%	79%	92%
Interferon	75%	55%	48%	8%
Stem cell transplant	18%	12%	33%	42%

START : SRC/ABL Tirosin kinase inhibition Activity: Research Trial of Dasatinib.

Eficacia del Dasatinib

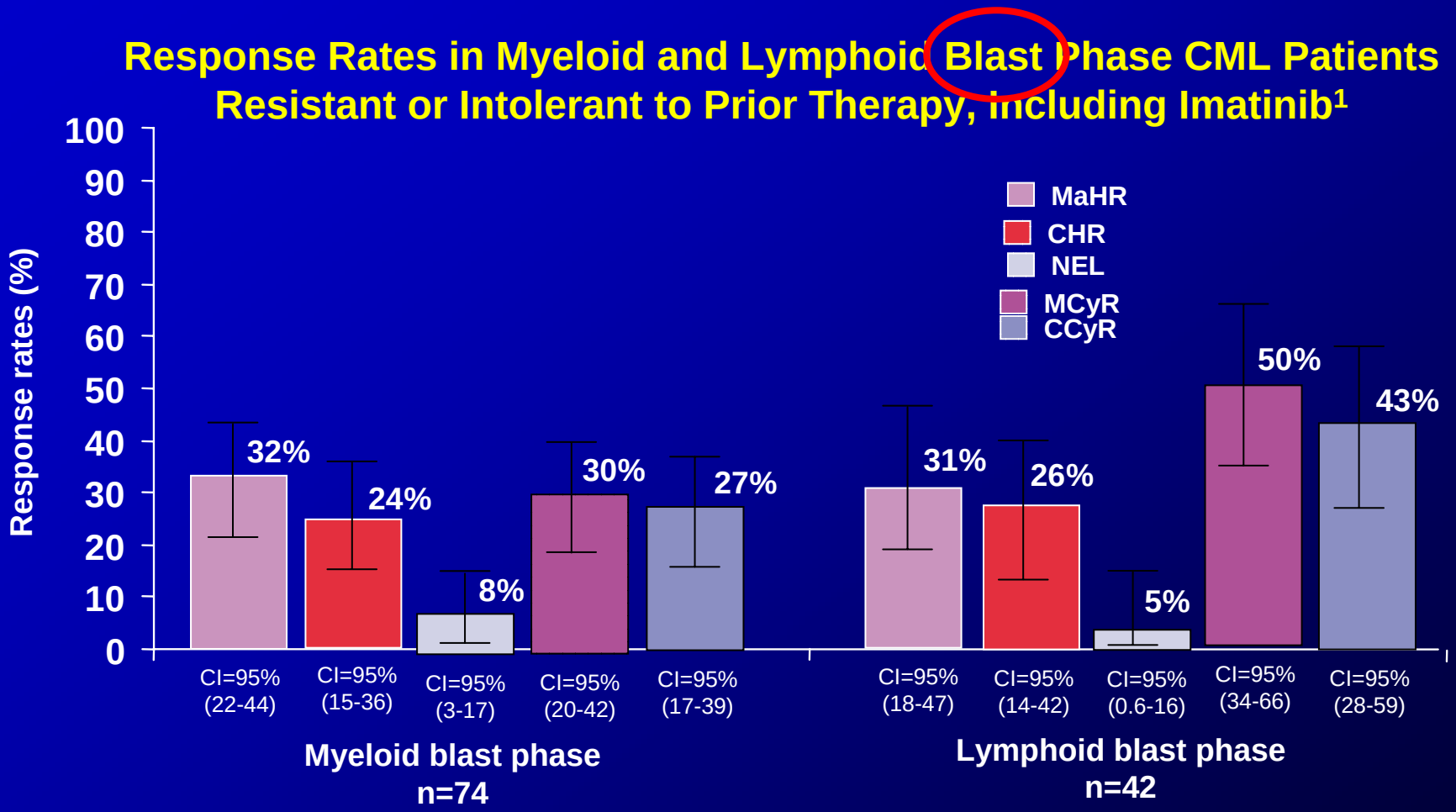
Response Rates in Accelerated Phase CML Patients Resistant or Intolerant to Prior Therapy, Including Imatinib[1]



- The primary endpoint was MaHR[1]
- Median duration of treatment was 5.5 months[1]

1. SPRYCEL® (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; July 2006.

Eficacia del Dasatinib



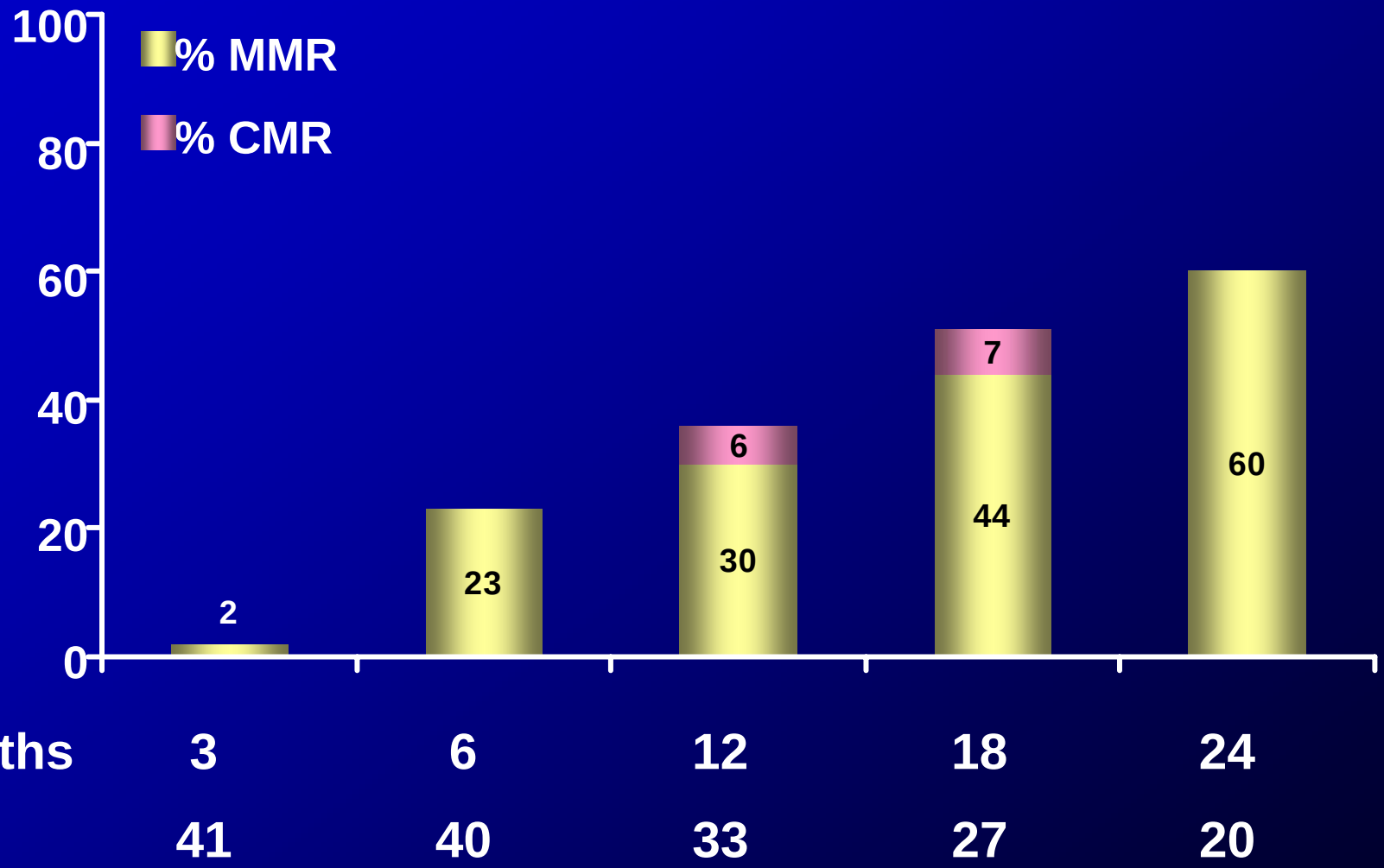
- The primary endpoint was MaHR[1]
- Median duration of treatment was 3.5 months and 2.8 months in myeloid blast phase and lymphoid blast phase, respectively[1]

1. SPRYCEL® (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; July 2006.

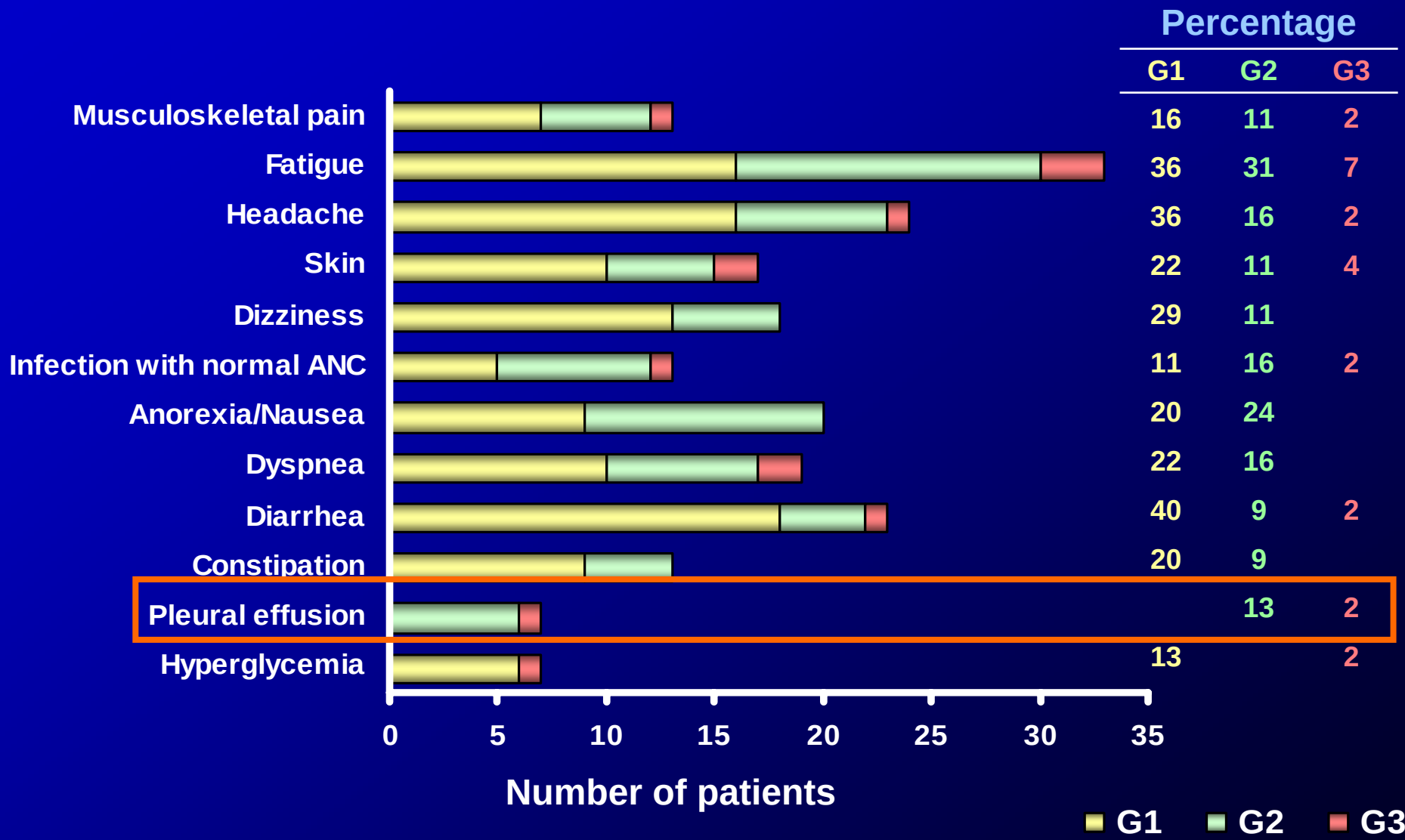
Resumen de eficacia LMCr fases avanzadas

	Accelerated (n=107)	Myeloid Blast (n=74)	Lymphoid Blast (n=42)
MaHR (95% CI)	59 (49-68)	32 (22-44)	31 (18-47)
CHR (95% CI)	33 (24-42)	24 (15-36)	26 (14-42)
NEL (95% CI)	26 (18-36)	8 (3-17)	5 (0.6-16)
MCyR (95% CI)	31 (22-41)	30 (20-42)	50 (34-66)
CCyR (95% CI)	21 (14-30)	27 (17-39)	43 (28-59)

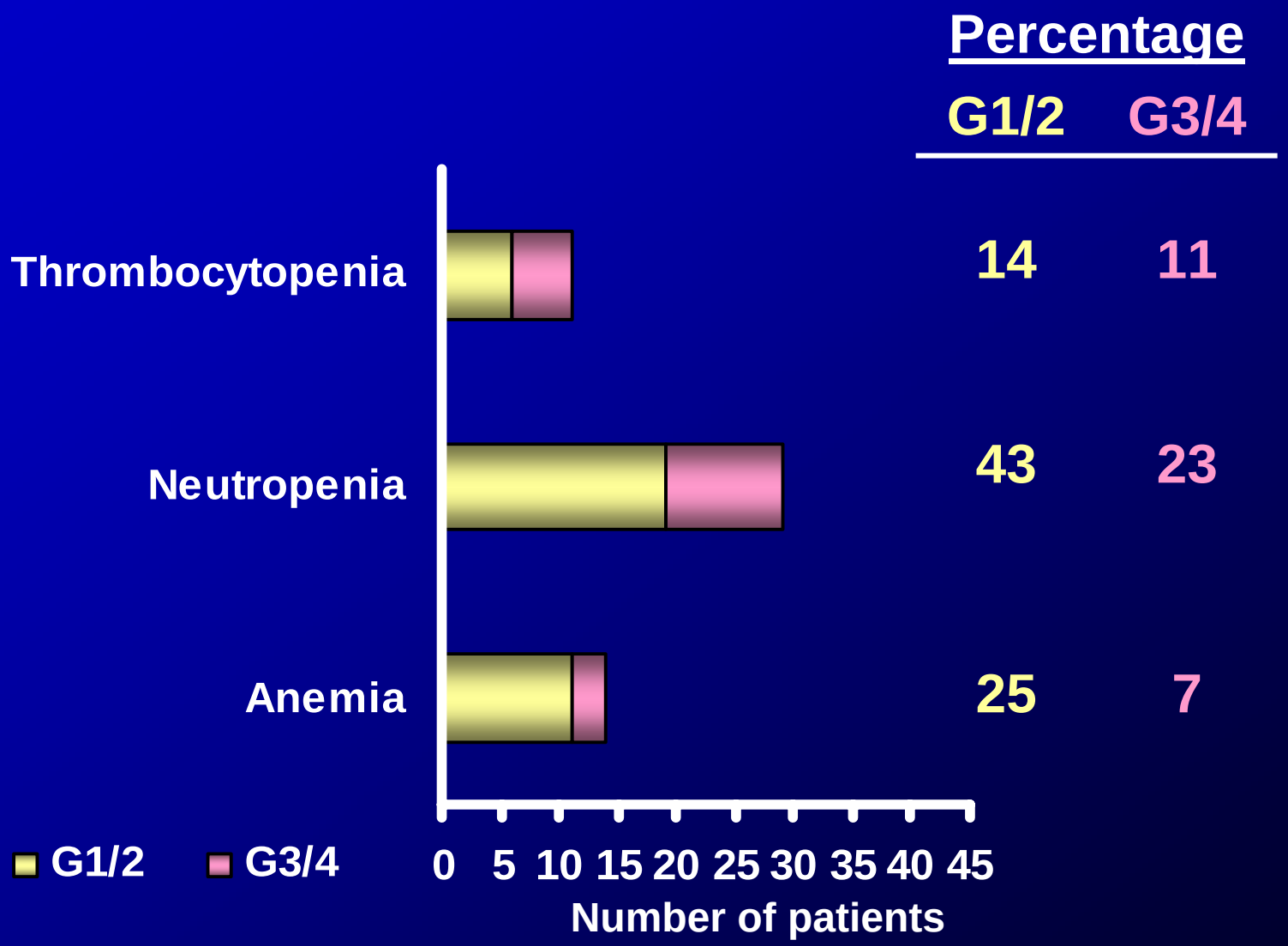
Dasatinib in Early CP CML
Molecular Responses



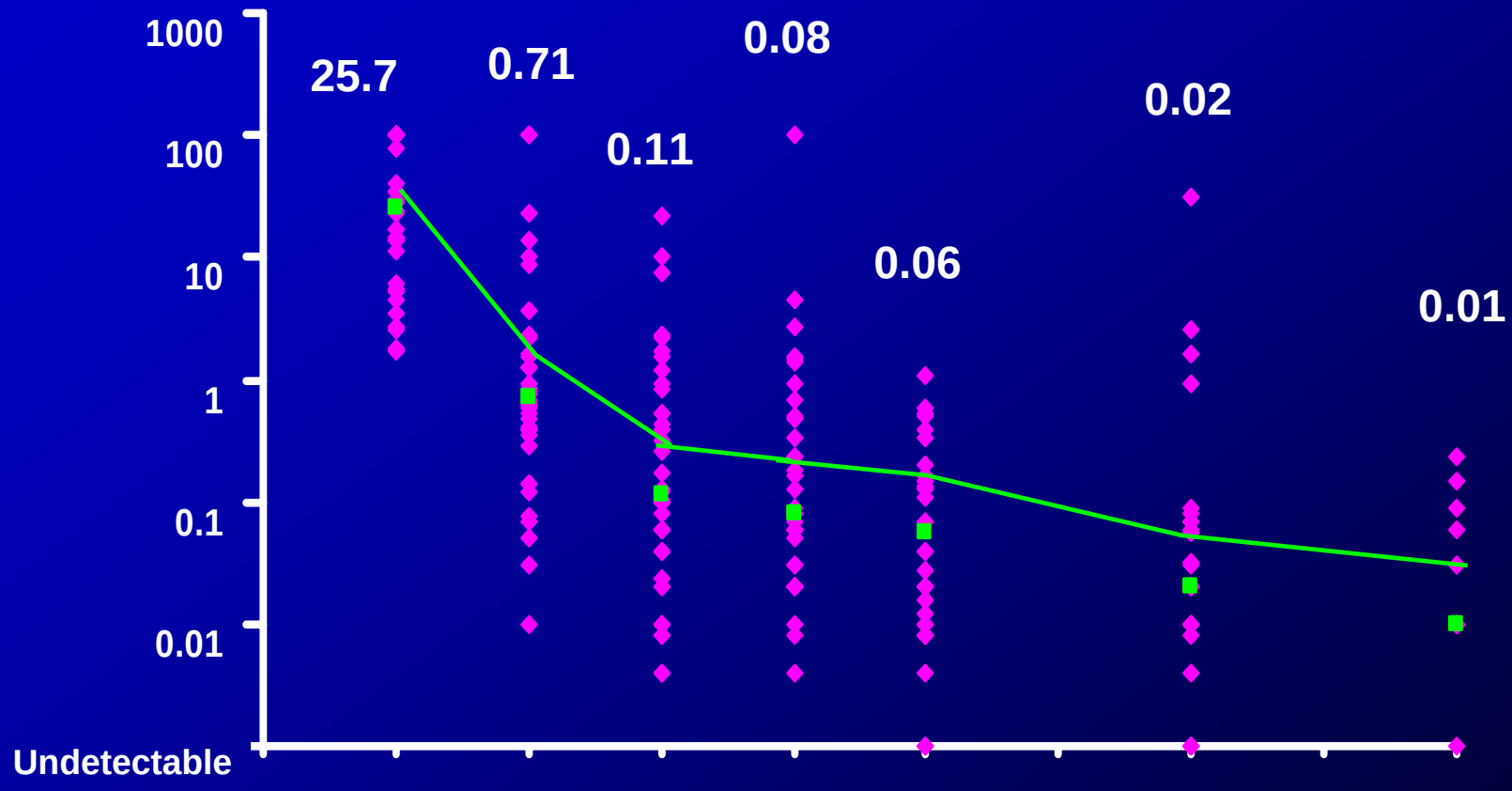
Dasatinib in Early CP CML
Non-Hematologic Adverse Events



Dasatinib in Early CP CML
Hematologic Adverse Events



Dasatinib Respuestas Moleculares



Month	0	3	6	9	12	18	24
N	44	41	40	35	33	27	20

Terminamos