XXV Congreso de la SEAP-SEC-SEPAF, Zaragoza, Mayo 2011

ENFERMEDAD RESIDUAL MINIMA EN HEMATOPATOLOGÍA

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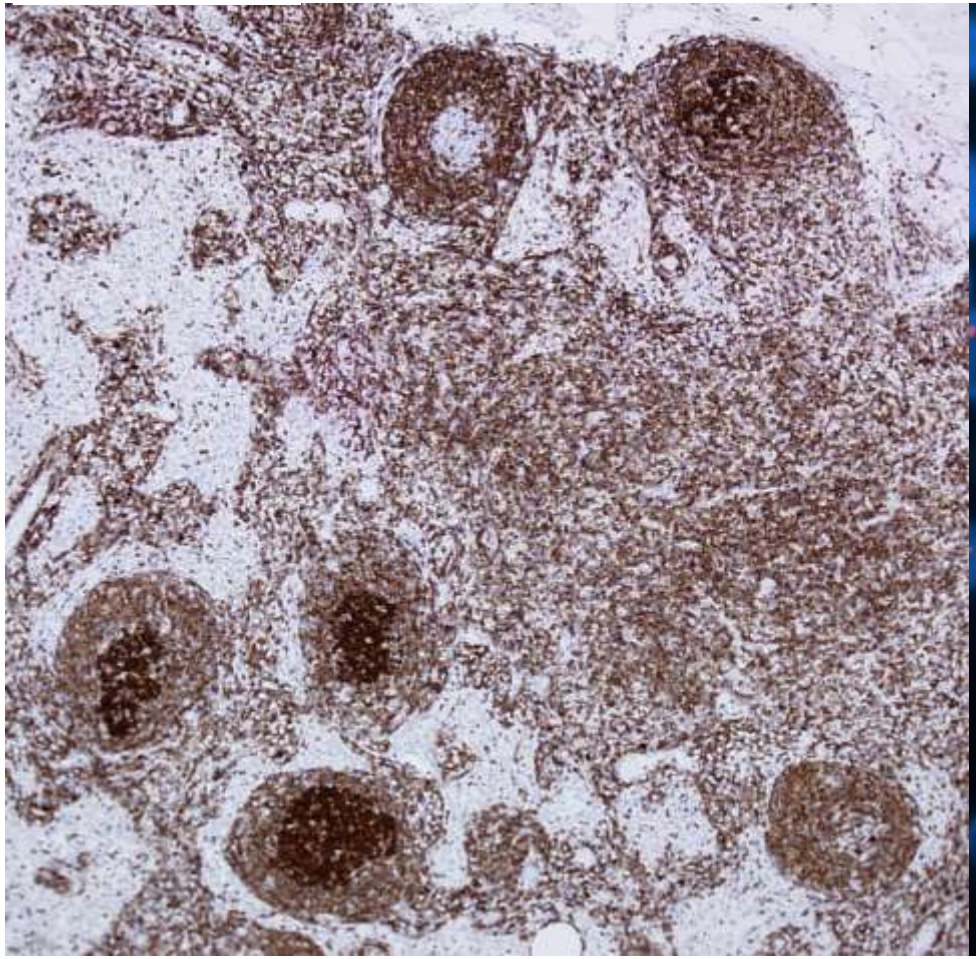
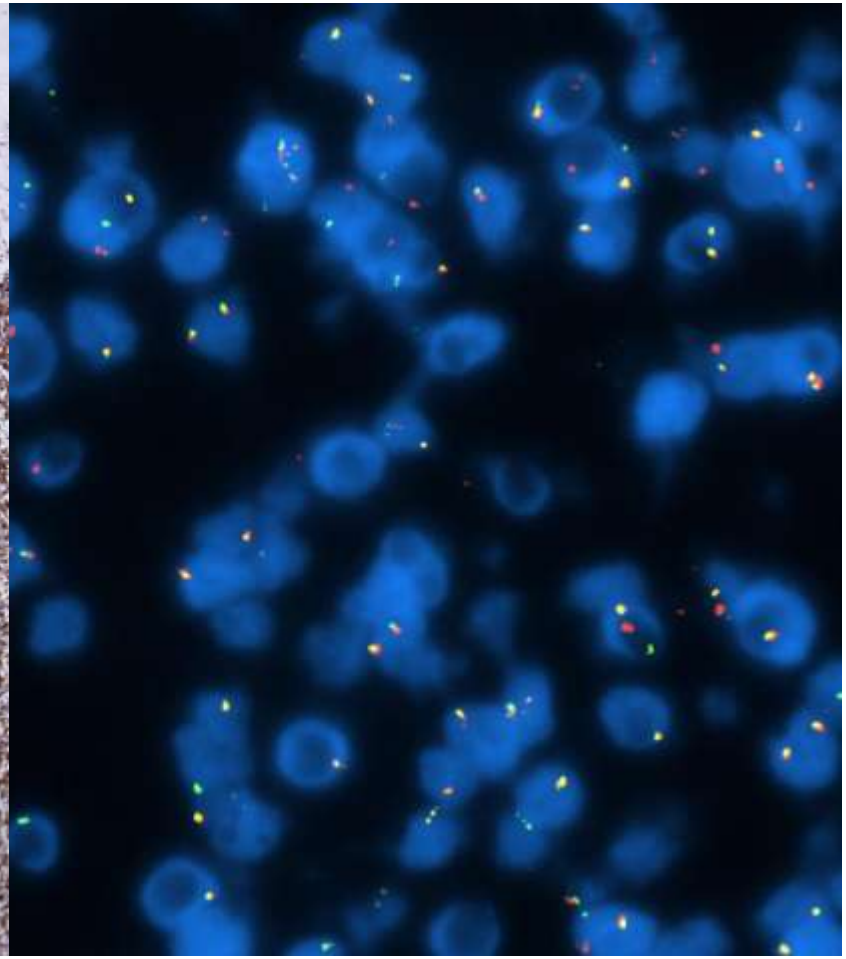
RTICC Red Temática de Investigación Cooperativa en Cáncer

FUNDACIÓN aecc INVESTIGACIÓN CONTRA EL CÁNCER

IHQ BCL2





FISH (sonda BA, BCL2)

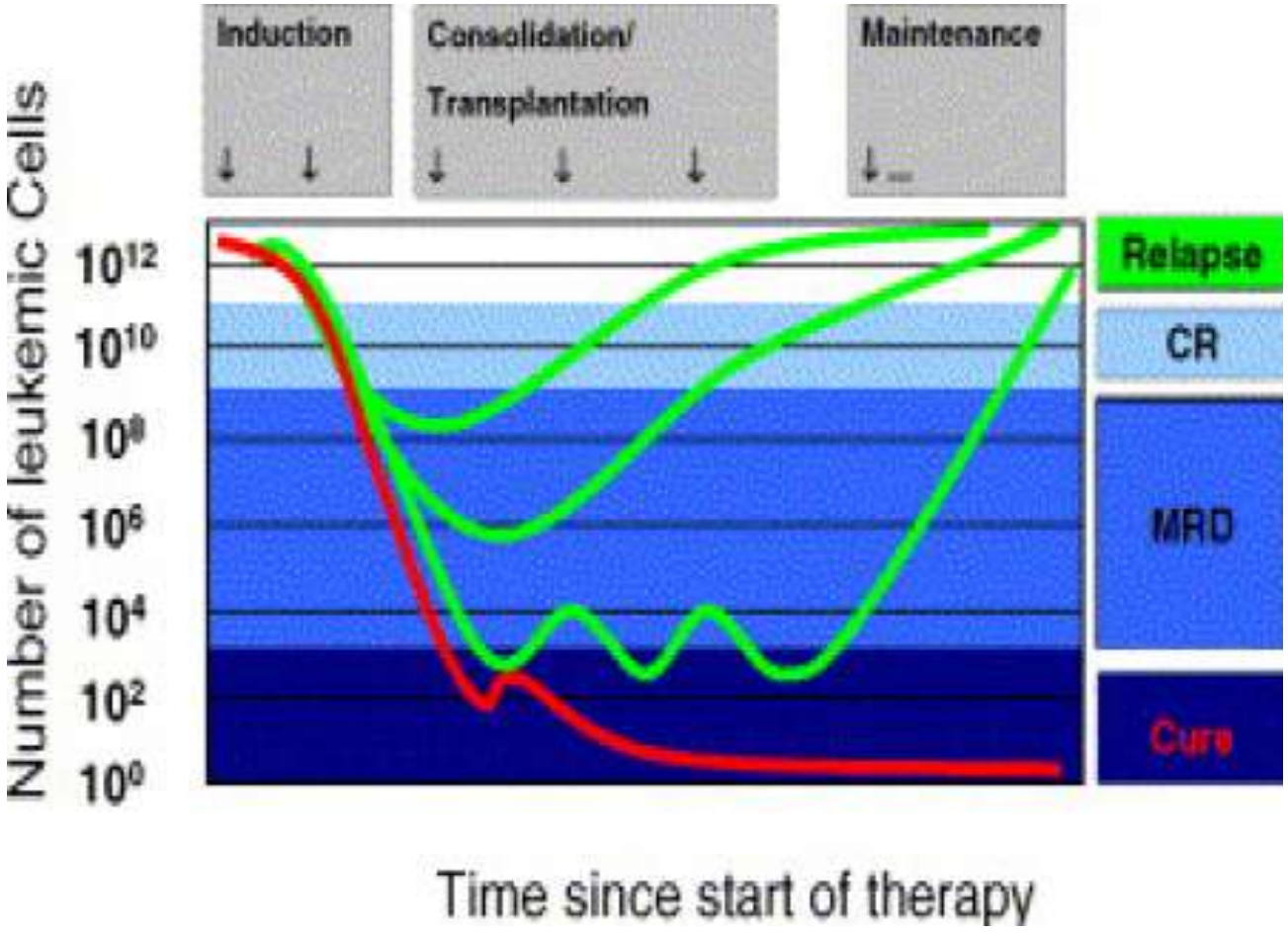
Enfermedad Mínima Residual (EMR) y lesiones precoces (“in situ” (in situ FL, in situ MCL, Linfocitosis B monoclonal) plantean el mismo problema conceptual y de manejo. ¿Cuando empieza a ser relevante clínicamente un hallazgo molecular?

ENFERMEDAD MÍNIMA RESIDUAL

Paciente **en remisión clínica** (sin síntomas de la enfermedad) durante (o tras) su tratamiento.

Persistencia de un pequeño número de células malignas indetectables por criterios morfológicos y/o inmunológicos convencionales.

Puede ser la causa de recaída de muchos pacientes con cáncer.



Su medición requiere identificar a las células neoplásicas usando técnicas de biología molecular, basadas en ADN o ARN, suficientemente sensibles para evaluar la masa residual, que en remisión completa, puede ir desde 1 célula tumoral entre 1 millón de células normales.

ENFERMEDAD MÍNIMA RESIDUAL

En el tratamiento del cáncer, la detección de EMR tiene papeles importantes:

-**Detectar** precozmente las neoplasias.

-**Diagnosticar y pronosticar con precisión** las neoplasias.

-Comparar la eficacia de distintos tratamientos a nivel molecular → **Estratificar los tratamientos**.

-Monitorizar el grado de remisión del paciente → **Estratificar los pacientes de riesgo y anticipar las recaídas**.

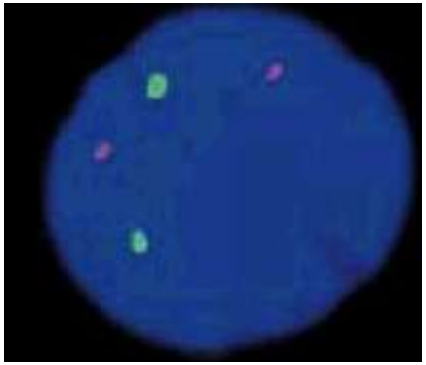
-**Adecuar el tratamiento** de consolidación en cada caso específico evitando sobre o infra tratamientos → **Medicina personalizada**.

-**Introducir nuevas terapias** (e.g. biológicas) cuando la carga tumoral es baja.

DIAGNÓSTICO DE EMR: SENSIBILIDAD

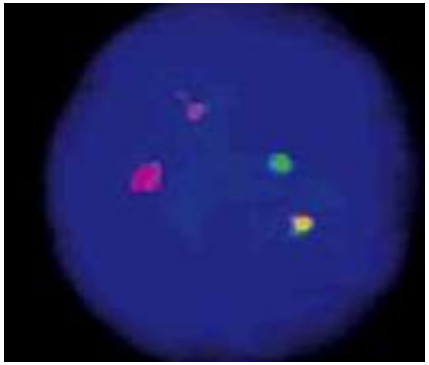
TÉCNICA	SENSIBILIDAD	DETECTA 1 célula entre
MORPHOLOGÍA (Microscopia convencional)	5 - 1%	20-100
SOUTHERN BLOT	$10^{-1} – 10^{-2}$ (10 - 1%)	10-100
CYTOGENÉTICA	$10^{-1} – 10^{-2}$ (10 - 1%)	10-1.000
CULTIVOS CELULARES	$10^{-1} – 10^{-3}$ (10 - 0.1%)	10-1.000
FISH	$10^{-1} – 10^{-4}$ (10 - 0.01%)	10-10.000
PCR cualitativa (p.ej Clonalidad)	$10^{-3} – 10^{-4}$ (0.1 - 0.01%)	1.000-10.000
CITOMETRÍA DE FLUJO (Inmunofenotipado)	$10^{-3} – 10^{-5}$ (0.1 - 0.001%)	1.000-100.000
qRT – PCR	$10^{-4} – 10^{-6}$ (0.01 - 0.0001%)	10.000-1.000.000

BCR/ABL por FISH



NEGATIVO

BCR
ABL
BCR/ABL



POSITIVO

DIAGNÓSTICO DE EMR: APLICABILIDAD

Table 2. Molecular Markers of Minimal Residual Disease in Hematologic Malignancies*

Disease	Marker	Applicability, %
ALL	Rearranged *IgH* and *TCR* genes	>90
	t(1;19) *PBX1-E2A*	5
	t(4;11) *AF4-MLL*	5
	t(9;22) *BCR-ABL*	<5–30†
	t(8;14) *MYC-IgH*	3
	t(11;19) *MLL-ENL*	<1
	Tal interstitial deletion *TAL1*	15–20
AML	Rearranged *IgH* and *TCR* genes	10–15
	t(15;17) *RARα-PML*	>95‡
	t(8;21) *AML1-ETO*	<5§
	Inv(16) *CBFβ-MYH11*	<5–10
	t(6;9) *DEK-CAN*	<5
CML	t(9;22) *BCR-ABL*	>90
CLL	Rearranged *IgH* and *TCR* genes	>95
	t(11;19) *BCL-3*	Unknown
Non-Hodgkin lymphoma	Rearranged *IgH* and *TCR* genes	>95
MCL	t(11;14) *BCL-1*	
FCL	t(14;18) *BCL-2*	
DLCL	t(3;14) *BCL-6*	
BL	t(8;14) *MYC*	
ALCL	t(2;5) *NPM-ALK*	

* From references 16–18. ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FCL, follicular cell lymphoma; DLCL, diffuse large cell lymphoma; BL, Burkitt lymphoma; and ALCL, anaplastic large cell lymphoma.

† Up to 30% in adults.

‡ In cases of acute promyelocytic leukemia (APL, AML-M3).

§ In up to 20% of adult and 40% of pediatric AML-M2.

Faderl et al. Arch Pathol Lab Med. (1999)

MARCADORES DE EMR: PROS Y CONTRAS

Table 4 Advantages and disadvantages of MRD-PCR targets

MRD-PCR target	Advantages	Disadvantages
Ig/TCR gene rearrangements (DNA level)	Patient specific Low-risk of contamination High stability of DNA DNA amount per cell is relatively constant	Labor intensive target identification Loss of target because of clonal evolution (oligoclonality and ongoing rearrangements) Background dependent on type of sample and follow-up time point Target not related to oncogenesis
Fusion genes (DNA level)	Patient specific Low-risk of contamination High stability of DNA DNA amount per cell is relatively constant Related to oncogenesis Stable targets No (or very low: $<10^{-6}$) background in normal cells Target not affected by type of sample and follow-up time point	Labor intensive identification of exact breakpoints, except for some translocations with small breakpoint regions, such as *SIL-TAL1*, 70% of *BCL2-IGH*, and 30% of *BCL1-IGH*
Fusion gene-transcripts (mRNA level)	Can be identified with limited set of primers Related to oncogenesis No (or very low: $<10^{-6}$) background in normal cells	Not patient specific Relatively high chance of contamination Expression level may be affected by therapy Instability of RNA

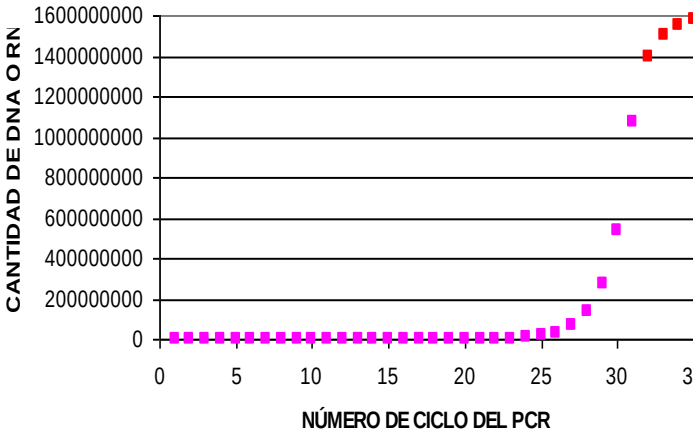
VHJ Van der Velden et al. Leukemia (2003)

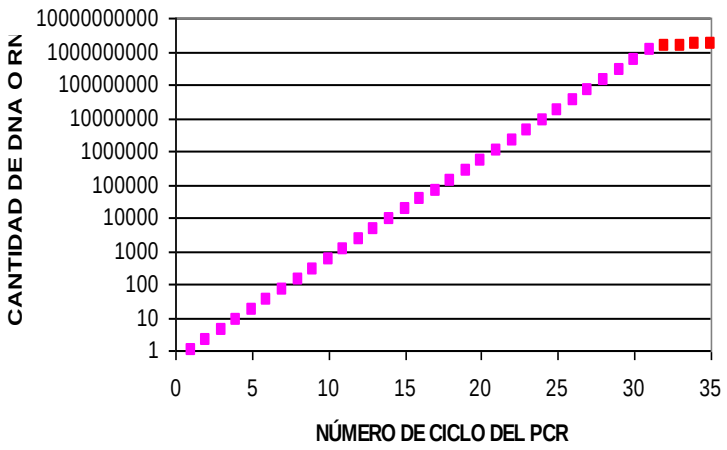
TÉCNICA BÁSICA: PCR A TIEMPO REAL (RT-PCR)

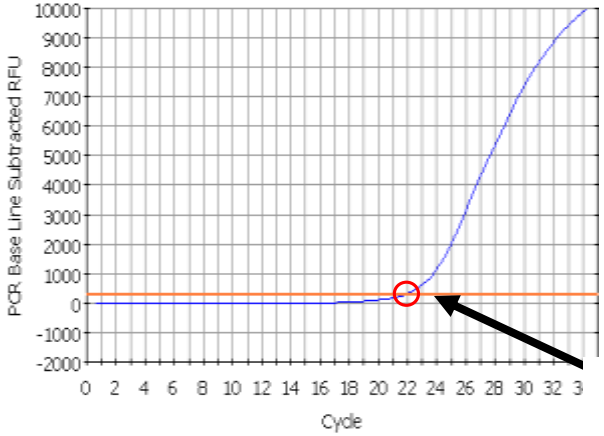
CICLO	CANTIDAD DNA
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1.024
11	2.048
12	4.096
13	8.192
14	16.384
15	32.768
16	65.536
17	131.072
18	262.144
19	524.288
20	1.048.576
21	2.097.152
22	4.194.304
23	8.388.608
24	16.777.216
25	33.554.432
26	67.108.864
27	134.217.728
28	268.435.456
29	536.870.912
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

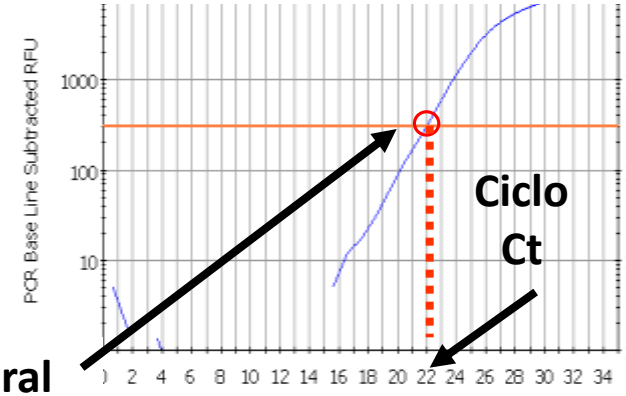
Es la capacidad de monitorizar el progreso de la PCR según ocurre mediante la detección de fluorescencia.

Las reacciones se caracterizan por el punto en el que se detecta por primera vez la amplificación de DNA.







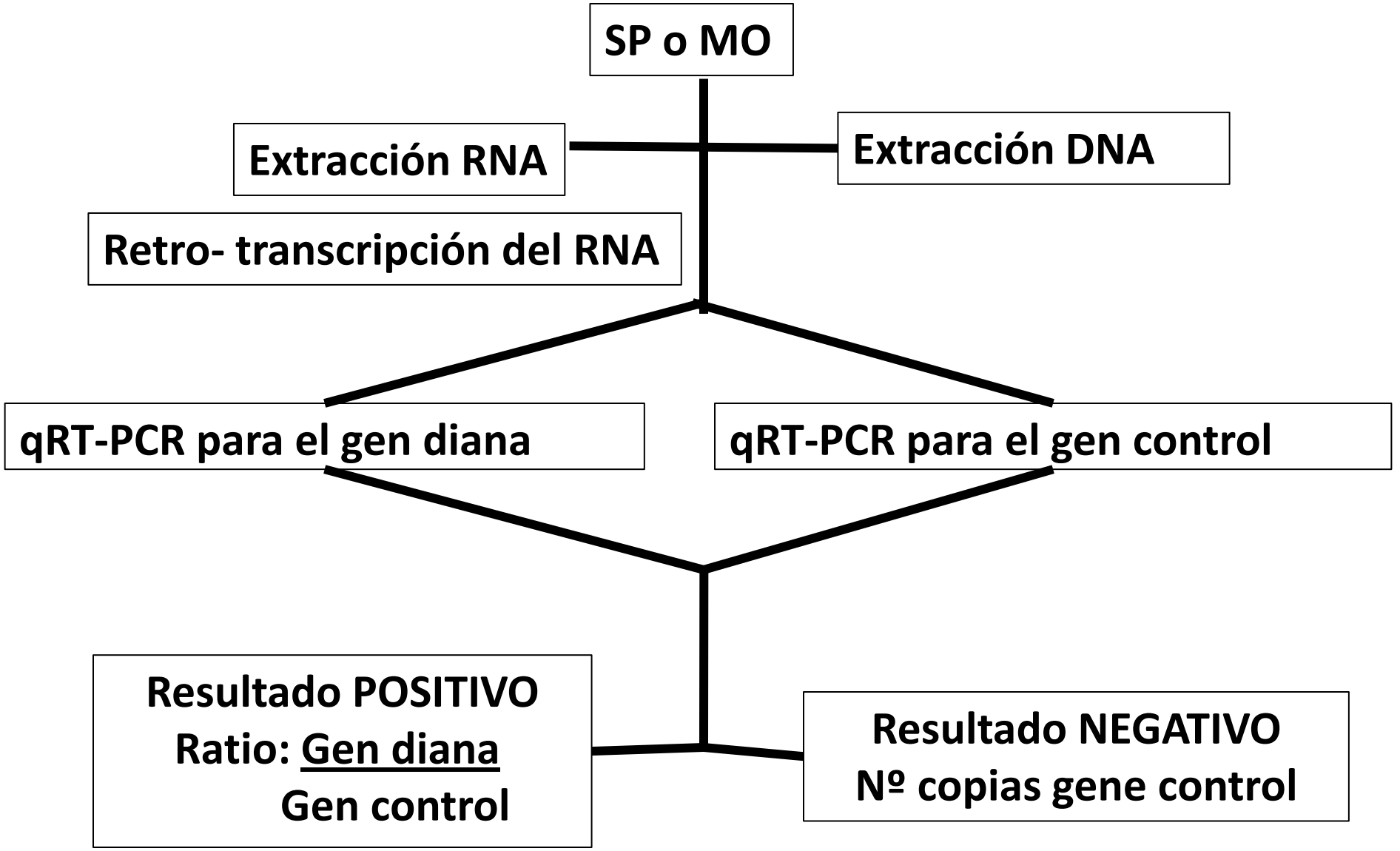


Umbral

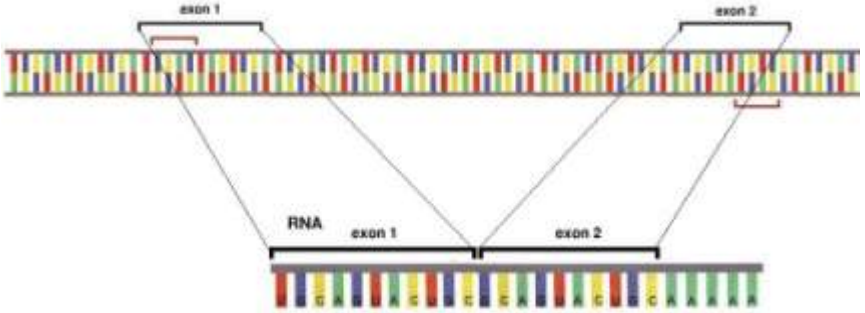
Se correlaciona con la cantidad inicial de DNA presente.

Se determina durante la fase exponencial.

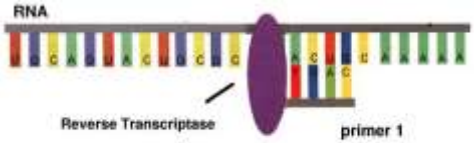
PCR A TIEMPO REAL: el flujo de trabajo



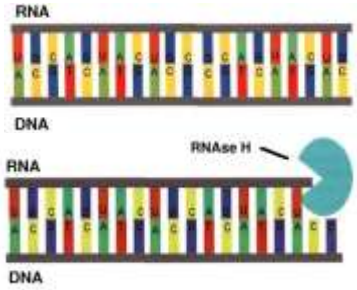
TRANSCRIPCIÓN REVERSA



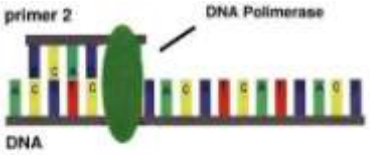
Transcripción del gen en mRNA



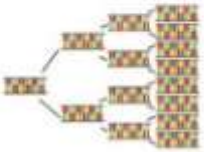
Unión del primer cebador al mRNA
La transcriptasa reversa sintetiza el cDNA



Degradación de la cadena de RNA.



Unión del segundo cebador al cDNA
Elongación mediante DNA Polimerasa.



Amplificación mediante PCR.

PCR A TIEMPO REAL: ventajas y desventajas

- Mayor reproducibilidad, precisión, especificidad (con sondas) y sensibilidad

↓

Mayor fiabilidad

- Señal de fluorescencia directamente proporcional al número de transcritos.
- Rango dinámico (más de 8 órdenes de magnitud).
- Requiere menos RNA (hasta 3 picogramos!

  → biopsias, material microdisecado ….
- Menor riesgo de contaminación.
- Se pueden detectar reacciones con baja eficiencia.
- Mayor eficiencia de amplificación al usar amplicones pequeños (muestras degradadas).

- Falsos positivos (sensibilidad).
- Falsos negativos (transcritos raros/MUTADOS, muestra muy degradada).
- No es ideal para reacciones múltiples (necesidad ajuste de multiplex).
- La puesta a punto requiere destreza técnica y soporte.
- Equipos caros.
- El RNA es inestable.
- Influencia de la contaminación con DNA.

DETECCIÓN: LOS INSTRUMENTOS

Table 2 RQ-PCR instruments and their main characteristics[a]

	Light source	*Detection channels*[b]	*Sample format*	*Number of samples*	*Sample volume (μl)*	*Speed (50 cycles) (h)*
Lightcycler (Roche)	LED (450–490 nm)	3 filters (530/640/710 nm)	Capillary	32	20	<1
Smartcycler (Cepheid)	LED (450–490 nm)	4 filters (520/550/585/710 nm)	Tubes	16–96	25–100	<1
Abi prism 7000 (Appl. Biosyst.)	Tungsten–halogen	4 filters (500–660 nm)	96 well	96	25–100	2–3
Abi prism 7700 (Appl. Biosyst.)	Argon laser (488 nm)	500–660 nm	96 well	96	25–100	2–3
Abi prism 7900 (Appl. Biosyst.)	Argon laser (488 nm)	500–660 nm	96 well/384 well	96/384	5–100	2–3
i-Cycler (Biorad)	Tungsten–halogen	5 filters (flexible)	96 well	96	25–100	2–3
MX-4000 (Stratagene)	Tungsten–halogen	4 (350–830 nm)	96 well	96	10–50	2–3

[a]Data obtained from instrument information booklets and from the web-sites of the different suppliers: http://home.appliedbiosystems.com/; http://www.biochem.roche.com; www.biorad.com/iCycler ; www.stratagene.com; www.eurogentec.com. Other RQ-PCR instruments not listed in the table include the Rotor-Gene (www.ozyme.fr) and the DNA engine cycler (www.biozymtc.com).
[b]Emission maxima FAM: 530 nm; SYBR Green I: 520 nm; VIC: 550 nm; JOE: 550 nm; TET: 550 nm; TAMRA: 585 nm; Cy3: 585 nm; LC Red 640: 640 nm; LC Red 705: 705 nm; ROX: 710 nm.

VHJ Van der Velden et al. Leukemia (2003)

DISEÑO DE PRIMERS Y SONDAS

Estandarización de procesos, sondas estandarizadas → BIOMED 1

Leukemia (2003) 17, 2318-2357

www.nature.com/leu

LEADING ARTICLE

Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program

J Gabert[1,17,18,19], E Beillard[1,17], VHJ van der Velden[2], W Bi[3], D Grimwade[4], N Pallisgaard[5], G Barbany[6,20], G Cazzaniga[7], JM Cayuela[8], H Cavé[9], F Pane[10], JLE Aerts[11], D De Micheli[12], X Thirion[13], V Pradel[13], M González[14], S Viehmann[15], M Malec[16], G Saglio[12] and JJM van Dongen[2]

[1]Department of Hematology Biology, Institut Paoli Calmettes, France; [2]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; [3]Applied Biosystems, Foster City, CA, USA; [4]Department of Haematology, Division of Medical and Molecular Genetics, Guy's, King's & St Thomas' School of Medicine, University College London Hospitals, London, UK; [5]Department of Immuno-haematology, Aarhus University Hospital, Aarhus, Denmark; [6]Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden; [7]Pediatric Clinic, University of Milan Bicocca, San Gerardo Hospital, Monza, Italy; [8]Central Laboratory of Hematology and INSERM U462, Hôpital Saint Louis, Paris, France; [9]Department of Genetic Biochemistry, Hôpital Robert Debré, Paris, France; [10]Department of CEINGE & Department of Biochemistry & Medical Biotechnology, University Hospital 'Federico II', Naples, Italy; [11]Department of Haematology, University Hospital, Leuven, Belgium; [12]Clinical and Biological Science, University of Turin, Ospedale San Luigi Gonzaga, Orbassano-Torino, Italy; [13]Department of Medical Information, University Hôpital Sainte Marguerite/Biostatistics Laboratory Timone, Université de la Méditerranée, Marseille, France; [14]Department of Hematology, Hospital Clínico Universitario, Salamanca, Spain; [15]Biogenetics Laboratory, Children's University Hospital, Giessen, Germany; and [16]Department of Hematology, Karolinska, Stockholm, Sweden

Detection of minimal residual disease (MRD) has proven to provide independent prognostic information for treatment stratification in several types of leukemias such as childhood acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and acute promyelocytc leukemia. This report focuses on the accurate quantitative measurement of fusion gene (FG) transcripts as can be applied in 35–45% of ALL and acute myeloid leukemia, and in more than 90% of CML. A total of 26 European university laboratories from 10 countries have collaborated to establish a standardized protocol for TaqMan-based real-time quantitative PCR (RQ-PCR) analysis of the main leukemia-associated FGs within the Europe Against Cancer (EAC) program. Four phases were scheduled: (1) training, (2) optimization, (3) sensitivity testing and (4) patient sample testing. During our program, three quality control rounds on a large series of coded RNA samples were performed including a balanced randomized assay, which enabled final validation of the EAC primer and probe sets. The expression level of the nine major FG transcripts in a large series of stored diagnostic leukemia samples ($n=278$) was evaluated. After normalization, no statistically significant difference in expression level was observed between bone marrow and peripheral blood on paired samples at diagnosis. However, RQ-PCR revealed marked differences in FG expression between transcripts in leukemic samples at diagnosis that could account for differential assay sensitivity. The development of standardized protocols for RQ-PCR analysis of FG transcripts provides a milestone for molecular determination of MRD levels. This is likely to prove invaluable to the management of patients entered into multi-center therapeutic trials.

Leukemia (2003) **17**, 2318–2357. doi:10.1038/sj.leu.2403135
Published online 9 October 2003
Keywords: real-time quantitative PCR; leukemia; standardization; fusion gene transcript

ANÁLISIS DE RESULTADOS

Cuantificación relativa.

Analiza cambios en la expresión de un gen en una muestra en relación a la expresión de ese gen en una muestra control (p.ej. Muestra sin tratamiento). Se pueden emplear varios métodos:

- Método de curva estándar
- Métodos de comparación de Ct: ΔΔCt y PFAFFL.

Cuantificación absoluta.

Cuando se requiere saber el número exacto de copias. Emplea una curva estándar sobre la que se INTRApola el Ct de las muestras para obtener una cantidad.

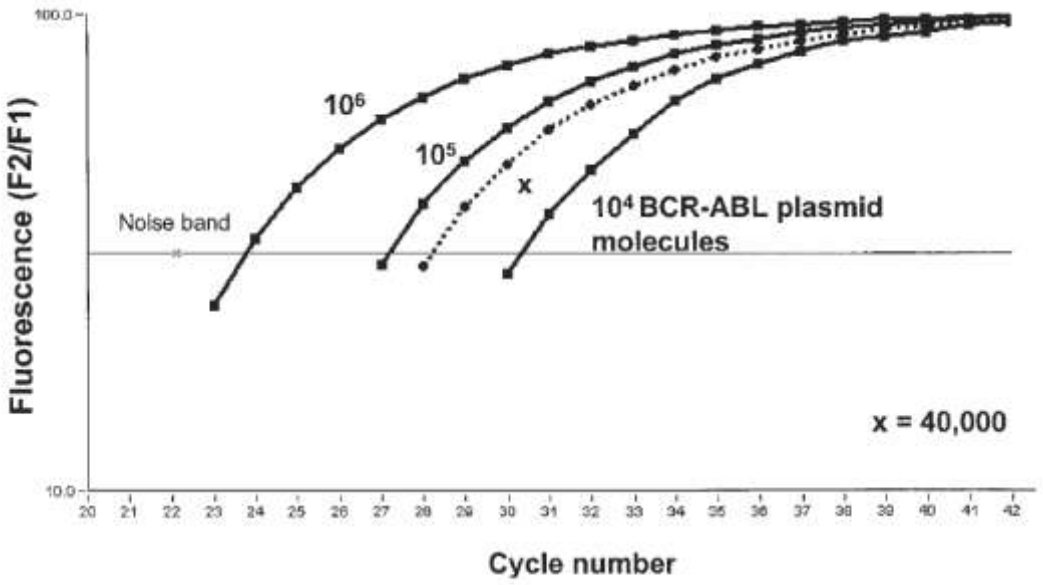


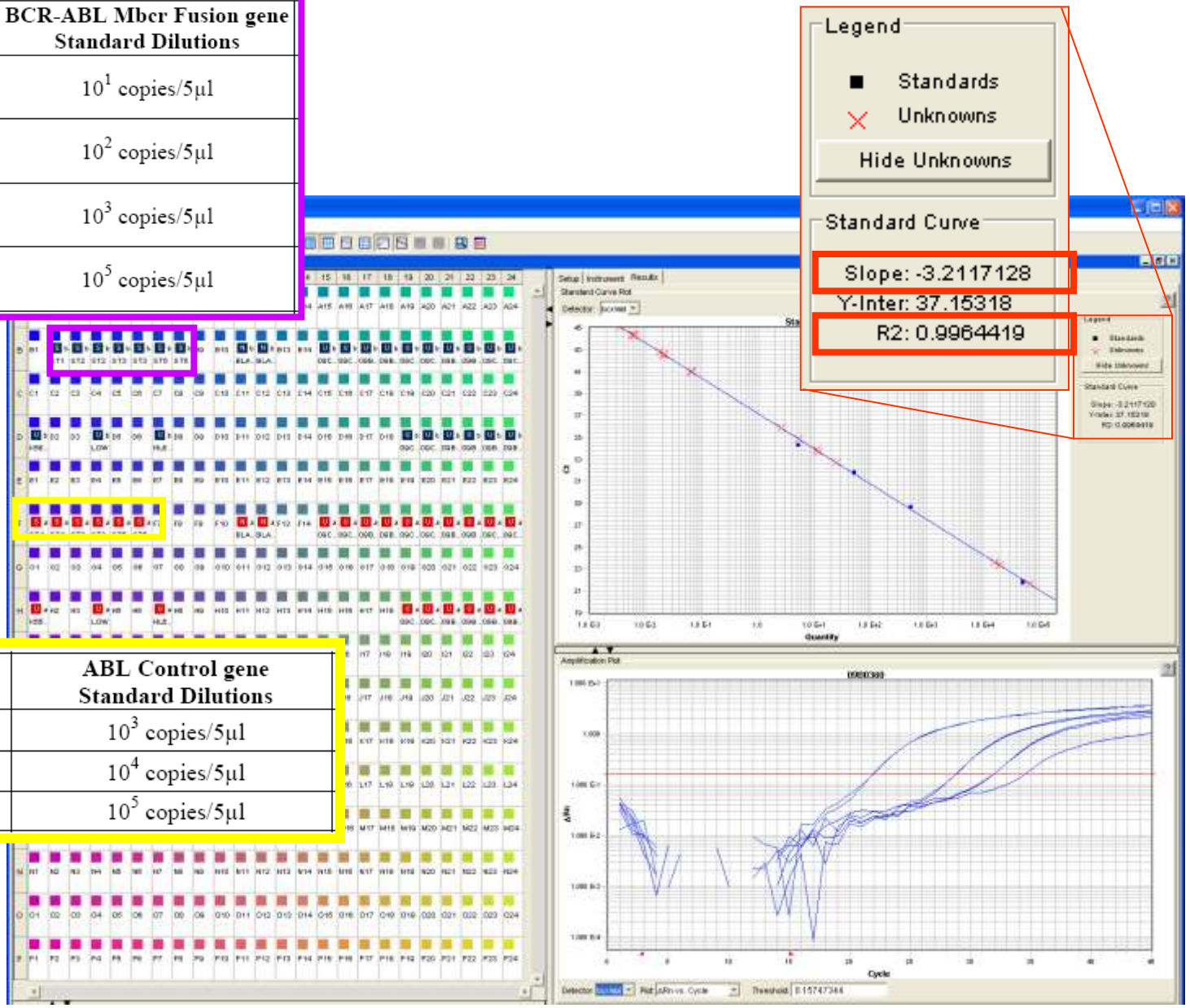
Fig. 13. Example of real-time PCR. Three standard dilutions of plasmid pGD210 ($b3a2^{BCR-ABL}$) were compared with a patient's sample of unknown BCR-ABL concentration. Each point represents the fluorescence intensity (F2/F1) measured after each PCR cycle. Plotting the cycle threshold of the unknown sample on the standard curve revealed that 40,000 BCR-ABL transcripts were present at the start of the reaction.

CURVA ESTÁNDAR PARA ABSOLUTA

Vial	BCR-ABL Mbcr Fusion gene Standard Dilutions
F1-BCR-ABL Mbcr	10^1 copies/5µl
F2-BCR-ABL Mbcr	10^2 copies/5µl
F3-BCR-ABL Mbcr	10^3 copies/5µl
F4-BCR-ABL Mbcr	10^5 copies/5µl

Ipso gen
CancerProfiler
FusionQuant® Standards

Vial	ABL Control gene Standard Dilutions
C1-ABL FusionQuant	10^3 copies/5µl
C2-ABL FusionQuant	10^4 copies/5µl
C3-ABL FusionQuant	10^5 copies/5µl



Ejemplo 1: EMR EN NEOPLASIAS MIELOIDES: LEUCEMIA MIELOÏDE CRÓNICA (LMC)

Considerado como el estándar para pacientes con LMC tratados con mesilato de imatinib (Gleevec/Glivec).

La LMC se caracteriza por la presencia del cromosoma de Filadelfia

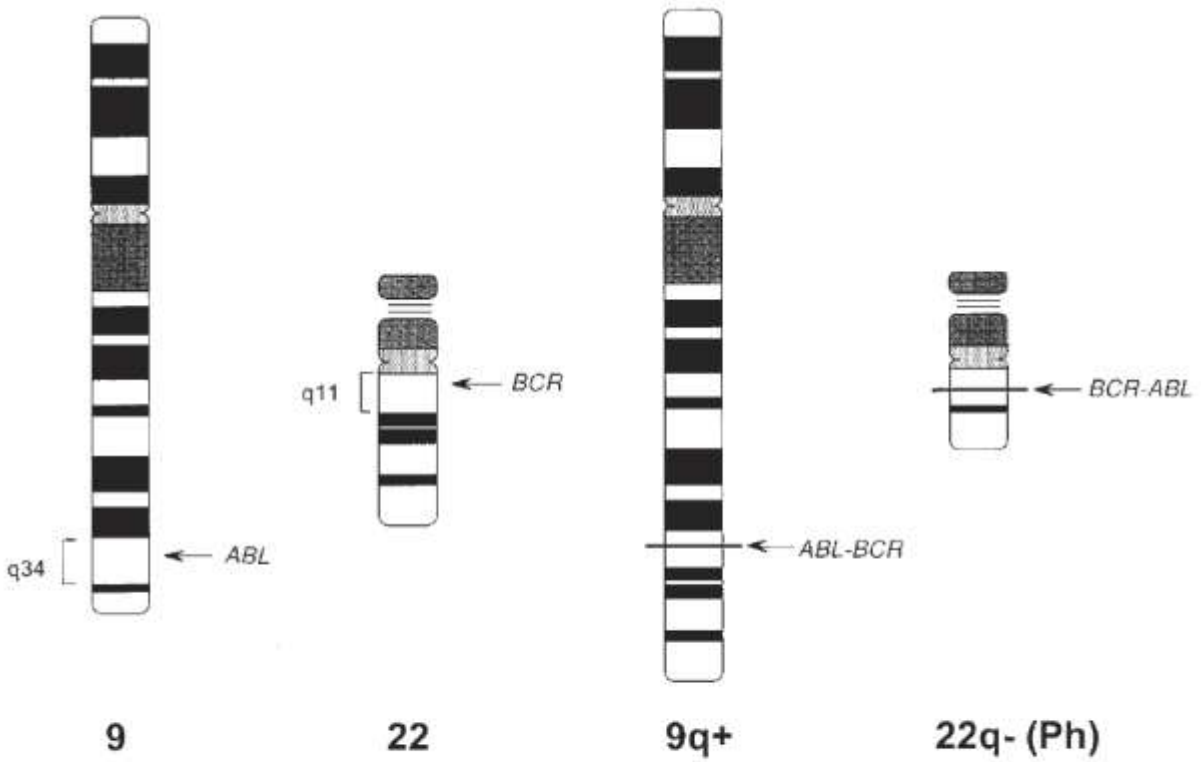
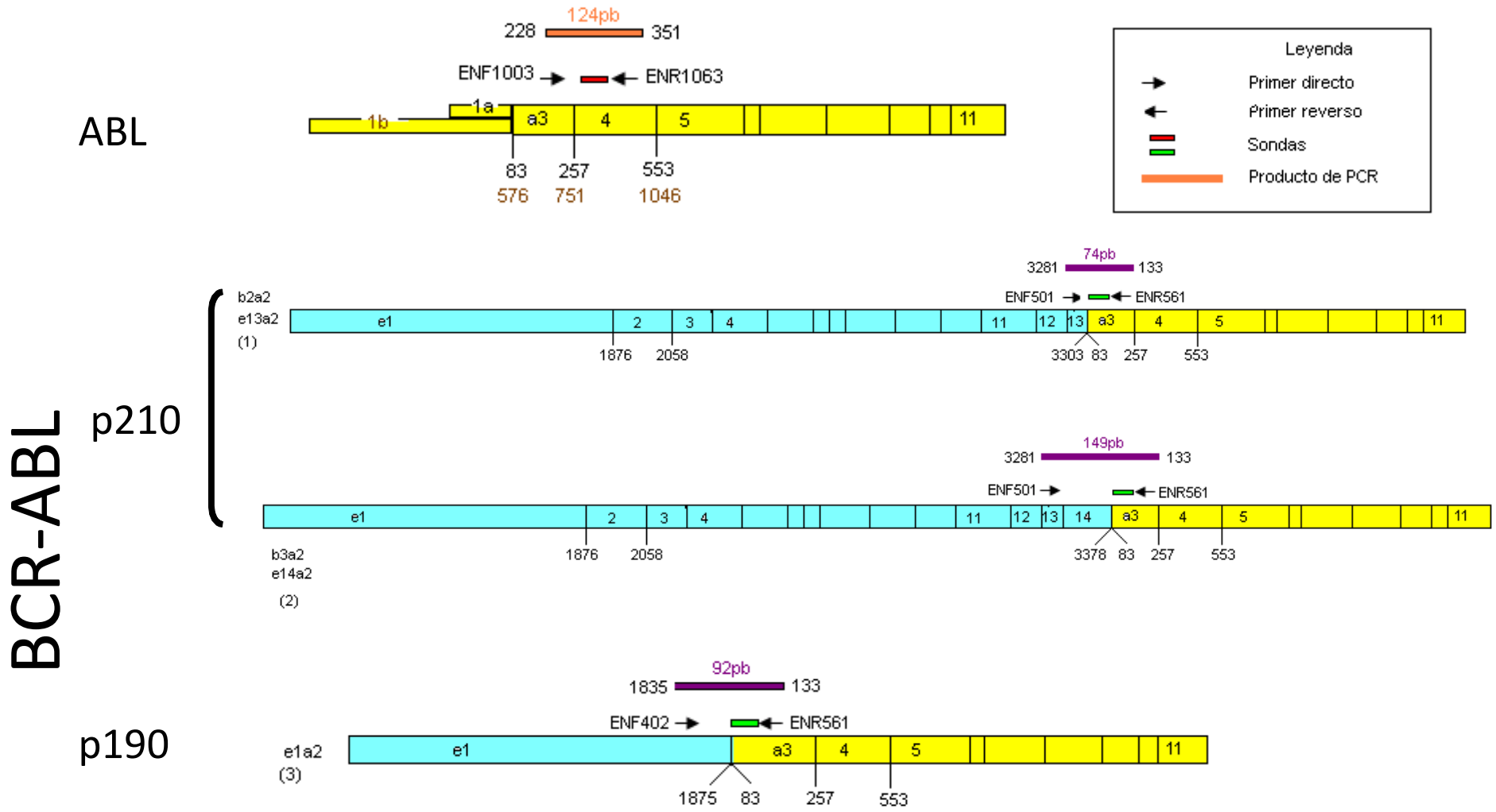


Fig. 1. Philadelphia (Ph) translocation. Reciprocal translocation of genomic material from the long arms of chromosomes 9 and 22. The resulting fusion genes are *ABL-BCR* on chromosome 9q+ and *BCR-ABL* on chromosome 22q−.

EMR EN LEUCEMIA MIELOÏDE CRÓNICA (LMC)

También denominada translocación t(9;22) ó gen de fusión BCR-ABL.



Gabert J. *Leukemia,* (2003).

EMR EN LEUCEMIA MIELOÏDE CRÓNICA (LMC)

Existen otras variantes de la translocación t(9;22) pero con una tasa de incidencia menor en LMC.

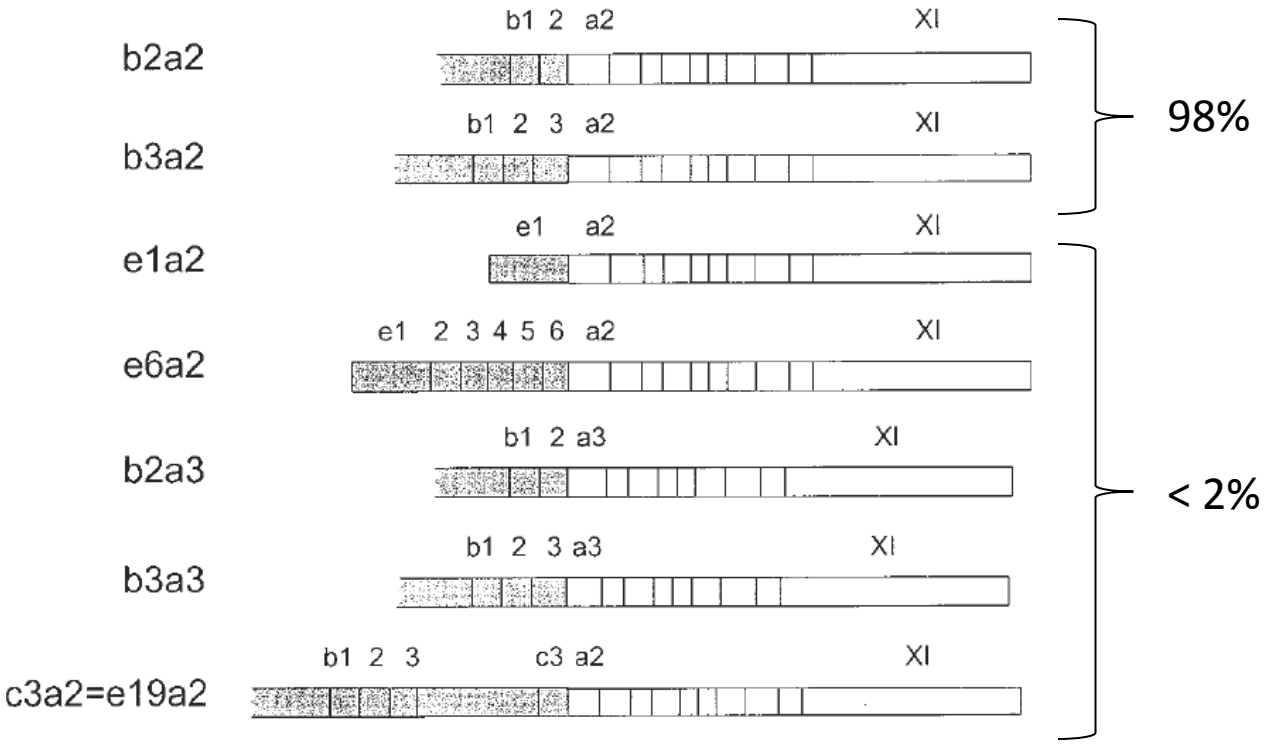


Fig. 6. Types of *BCR-ABL* fusion transcripts. Ninety-eight percent of patients show the typical b2a2 or b3a2 *BCR-ABL* fusion transcripts. Two percent of cases show rare transcripts resulting from genomic breakpoints outside M-bcr (e1a2, e6a2, e19a2) or *BCR-ABL* transcripts lacking *ABL* exon a2 (b2a3 or b3a3).

Molecular Analysis of Cancer. Methods in molecular medicine – p69

EMR en LMC vía RT-PCR

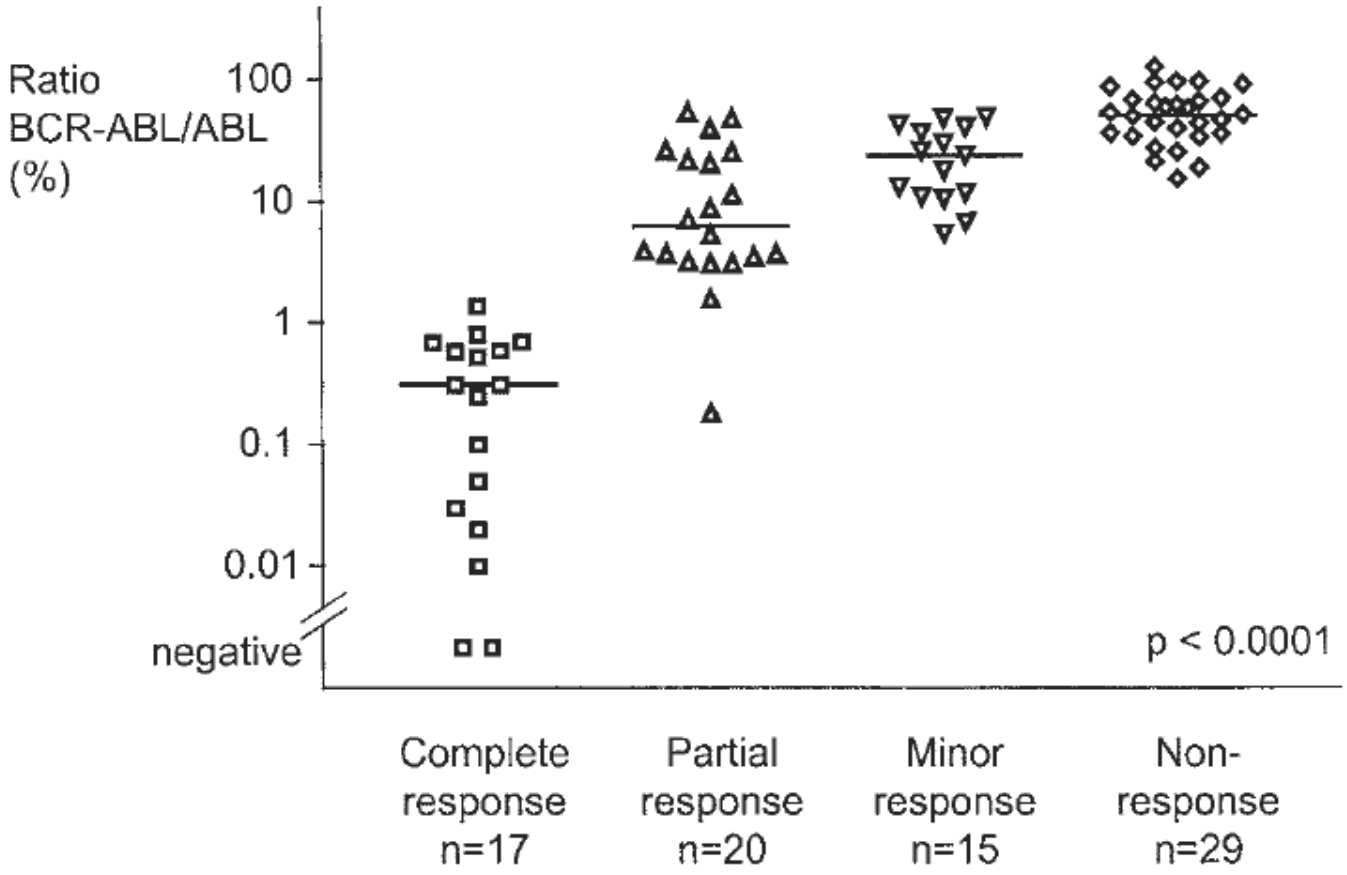


Fig. 15. Ratios of BCR-ABL/ABL derived from real-time PCR according to contemporaneous cytogenetic response. The ratios of BCR-ABL/ABL are significantly different among complete, partial, minor, and nonresponders ($p < 0.0001$) ***(92)***.

EMR en LMC vía RT-PCR

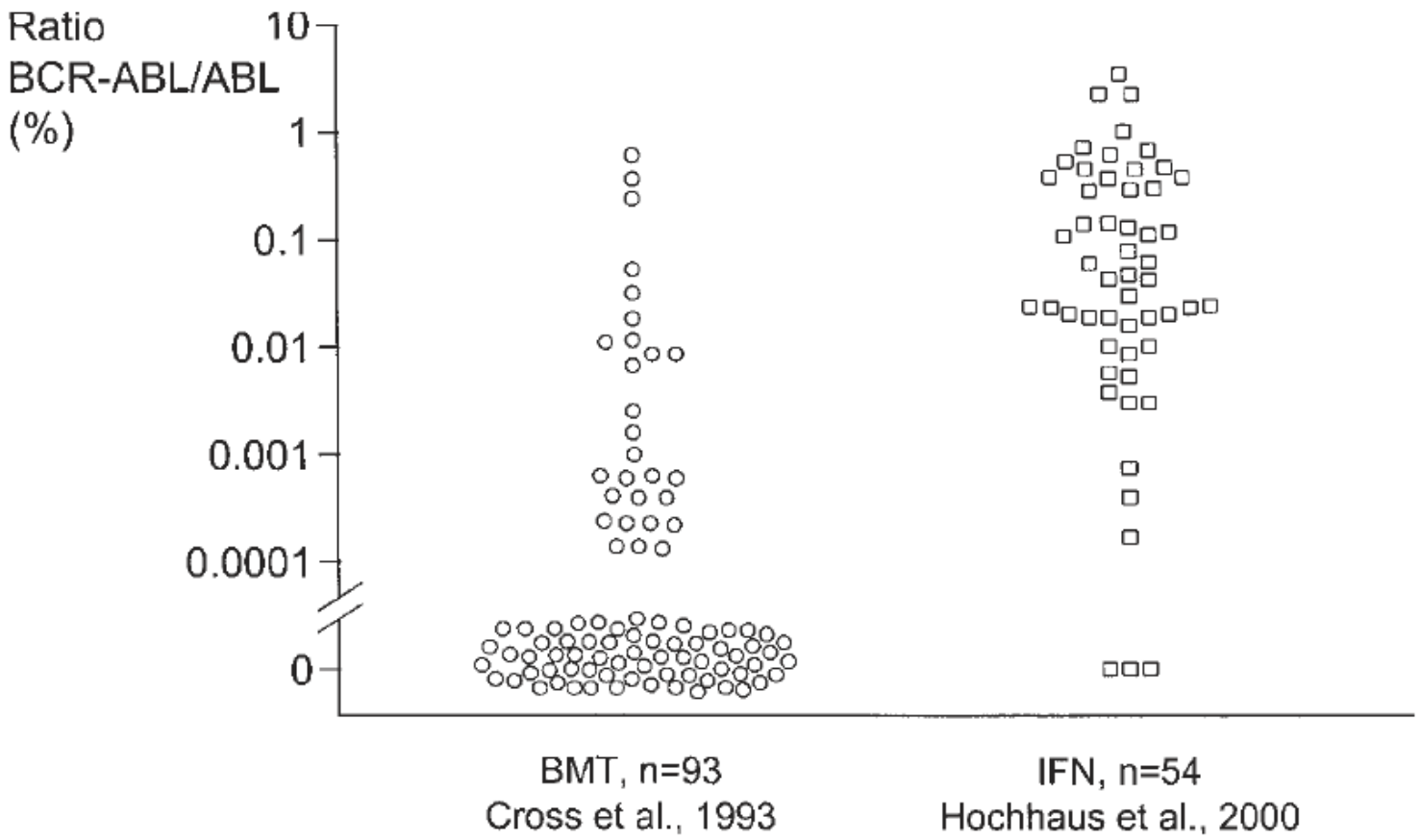


Fig. 10. Quantitative PCR analysis for the ratio to BCR-ABL/ABL in complete cytogenetic responders after allogeneic BMT and IFN therapy. Two-thirds of patients after allogeneic BMT are after 6 mo RT-PCR negative ***(56)***, whereas almost all patients after IFN therapy are persistently RT-PCR positive. Only 3 of 54 patients showed transient PCR negativity ***(81)***.

SEGUIMIENTO DE LOS PACIENTES CON LMC

VOLUME 27 · NUMBER 35 · DECEMBER 10 2009

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

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Chronic Myeloid Leukemia: An Update of Concepts and Management Recommendations of European LeukemiaNet

Michele Baccarani, Jorge Cortes, Fabrizio Pane, Dietger Niederwieser, Giuseppe Saglio, Jane Apperley, Francisco Cervantes, Michael Deininger, Alois Gratwohl, François Guilhot, Andreas Hochhaus, Mary Horowitz, Timothy Hughes, Hagop Kantarjian, Richard Larson, Jerald Radich, Bengt Simonsson, Richard T. Silver, John Goldman, and Rudiger Hehlmann

A B S T R A C T

Purpose
To review and update the European LeukemiaNet (ELN) recommendations for the management of chronic myeloid leukemia with imatinib and second-generation tyrosine kinase inhibitors (TKIs), including monitoring, response definition, and first- and second-line therapy.

Methods
These recommendations are based on a critical and comprehensive review of the relevant papers up to February 2009 and the results of four consensus conferences held by the panel of experts appointed by ELN in 2008.

Results
Cytogenetic monitoring was required at 3, 6, 12, and 18 months. Molecular monitoring was required every 3 months. On the basis of the degree and the timing of hematologic, cytogenetic, and molecular results, the response to first-line imatinib was defined as optimal, suboptimal, or failure, and the response to second-generation TKIs was defined as suboptimal or failure.

Conclusion
Initial treatment was confirmed as imatinib 400 mg daily. Imatinib should be continued indefinitely in optimal responders. Suboptimal responders may continue on imatinb, at the same or higher dose, or may be eligible for investigational therapy with second-generation TKIs. In instances of imatinib failure, second-generation TKIs are recommended, followed by allogeneic hematopoietic stem-cell transplantation only in instances of failure and, sometimes, suboptimal response, depending on transplantation risk.

J Clin Oncol 27:6041-6051. © 2009 by American Society of Clinical Oncology

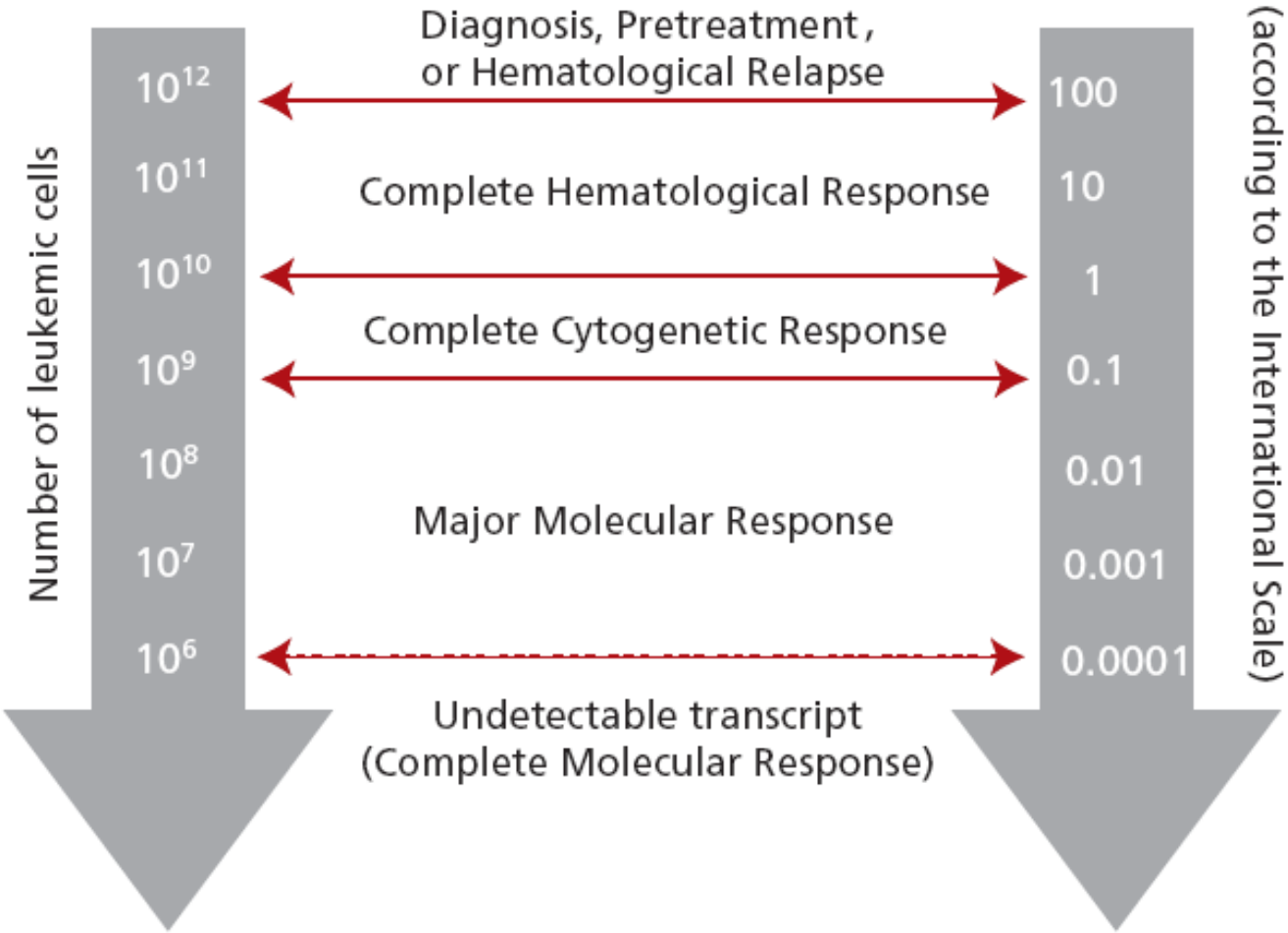
PRESENTACIÓN CLÍNICA DE LOS PACIENTES CON LMC

Parameter	Phase of Ph+ CML		
	Chronic	Accelerated	Blast
Median duration prior to availability of imatinib therapy	5-6 years	6-9 months	3-6 months
WBC count	≥20 × 10^9/L	–	–
Blasts	0%	≥10%	≥30%
Basophils	↑	≥20%	–
Platelets	↑ or normal	↑ or ↓	↓
Bone marrow	Myeloid hyperplasia →		
Cytogenetics	Ph+ →		
BCR-ABL	+	+	+

Ph+, Philadelphia chromosome positive.

1. Faderl S et al. *Oncology*. 1999;13:169. 2. Kantarjian HM et al. *Biol Ther Chronic Myelogenous Leukemia*. 1998;12:31-80. 3. Spiers AS. *Semin Oncol*. 1995;22:380.

SEGUIMIENTO: LAS DISTINTAS RESPUESTAS



Imatinib
PCR testing for CML

Setting the standards for the future of oncology

EUTOS for CML

European Treatment and Outcome Study

http://www.eutos.org/content/molecular_monitoring/documents/e133/infoboxContent786/PCR_Backgrounder_Booklet.pdf

SEGUIMIENTO: LAS DISTINTAS RESPUESTAS

Table 1. Definitions of Hematologic, Cytogenetic, and Molecular Response

Response by Type	Definitions
Hematologic	
Complete (CHR)	WBC $< 10 \times 10^9$/L
	Basophils $< 5\%$
	No myelocytes, promyelocytes, myeloblasts in the differential
	Platelet count $< 450 \times 10^9$/L
	Spleen nonpalpable
Cytogenetic*	
Complete (CCgR)	No Ph+ metaphases
Partial (PCgR)	1% to 35% Ph+ metaphases
Minor (mCgR)	36% to 65% Ph+ metaphases
Minimal (minCgR)	66% to 95% Ph+ metaphases
None (noCgR)	$> 95\%$ Ph+ metaphases
Molecular†	
Complete (CMolR)	Undetectable *BCR-ABL* mRNA transcripts by real time quantitative and/or nested PCR in two consecutive blood samples of adequate quality (sensitivity $> 10^4$)
Major (MMolR)	Ratio of *BCR-ABL* to *ABL* (or other housekeeping genes) $\leq 0.1\%$ on the international scale

Baccarani et al., 2009

LAS DISTINTAS RESPUESTAS Y SU SEGUIMIENTO

	Hematological Response (HR)	Cytogenetic Response (CyR)	Molecular Response (MR)*
Monitoring Frequency	■ **Every 2 weeks** until a complete response has been achieved and confirmed ■ **Every 3 months** unless otherwise required	■ **Every 6 months** until a complete response has been achieved and confirmed ■ **Then every 12 months**	■ **Every 3 months**
Monitoring Methods	■ Complete blood count (CBC) with differential	■ Conventional cytogenetic examination ■ FISH (only before treatment)	■ **RQ-PCR**

*Ratio of BCR-ABL to control gene transcripts according to International Scale.[14]
†Level of BCR-ABL transcripts according to the proposed International Scale.[14]
FISH, fluorescence in situ hybridization; RQ-PCR, real-time quantitative polymerase chain reaction; WBC, white blood cell.
Based on recommendations from European LeukemiaNet.[10]

http://www.eutos.org/content/molecular_monitoring/e132/e133/infoboxContent787/EUTOS_PCR_Casemono.pdf

EUTOS for CML
European Treatment and Outcome Study

EVALUACIÓN DE LA RESPUESTA AL TRATAMIENTO EN 1ª LÍNEA CON IMATINIB.

Recomendaciones 2006 actualizadas (2009) del ELN (European Leukemia Net)

		MESES DE TRATAMIENTO				
RESPUESTA AL TRATAMIENTO	**DIAGNÓSTICO**	**3**	**6**	**12**	**18**	**Siempre**
ÓPTIMA	-	RHC y al menos RCm	Al menos RCP	Al menos RCC	RMM	RMM estable o RMC
SUB-ÓPTIMA	-	Sin RC	<RCP	<RCC	<RMM	Pérdida RMM, mutaciones (Sensibilidad a IM)
FALLO	-	<RHC	Sin RC	<RCP	<RCC	Pérdida RHC y/o RCC, mutaciones (Resitencia a IM), ACA
ALERTAS	Alto riesgo (indice SOKAL/Hasford), del 9q+, ACA.	-	-	<RMM	-	Aumento del nivel de tránscritos

ACA: Anormalidades Cromosómicas Adicionales.

RH: Respuesta Hematológica.

RC: Respuesta Citogenética (**C**: Completa; **P**: Parcial; **m**: menor; **min**: mínima).

RM: Respuesta Molecular (**C**: Completa; **M**: Mayor).

RECOMENDACIONES PARA EL TRATAMIENTO

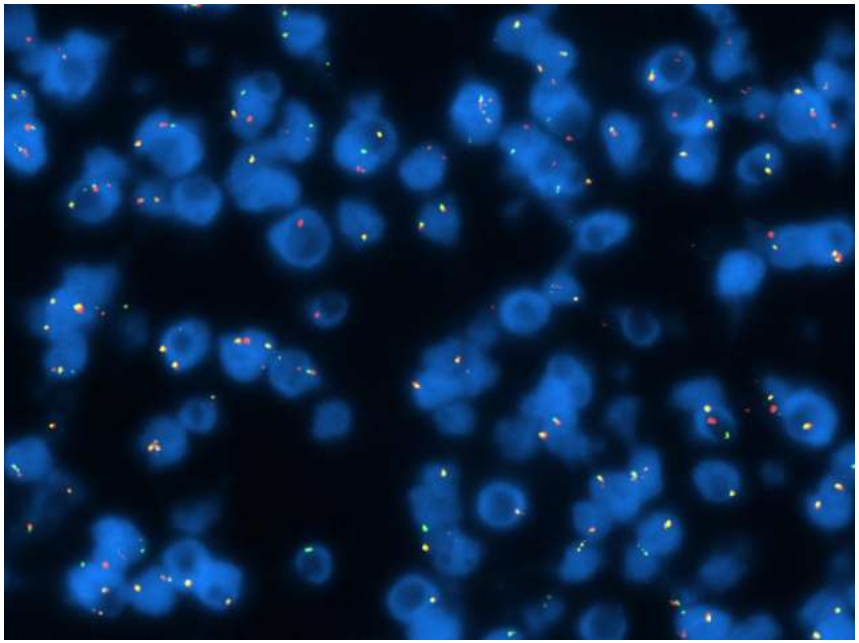
RESPUESTA AL TRATAMIENTO	TRATAMIENTO
ÓPTIMA	Imatinib 400 mg diarios.
SUB-ÓPTIMA	Imatinib 600 to 800 mg diarios. Trasplante alogénico (MO, CT).
FALLO	Imatinib 800 mg diarios. Trasplante alogénico (MO, CT).
ALERTAS	Imatinib 400 mg diarios. Trasplante alogénico (MO, CT). Ensayo clínico.
INTOLERANCIA o TOXICIDAD	Trasplante alogénico (MO, CTH). rIFN.

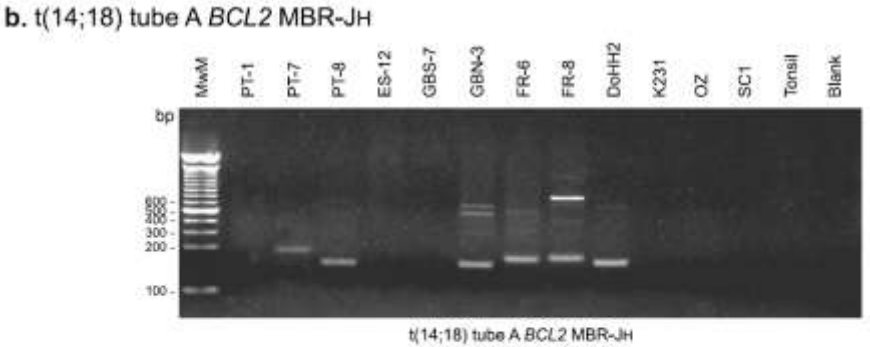
MO: Médula Ósea

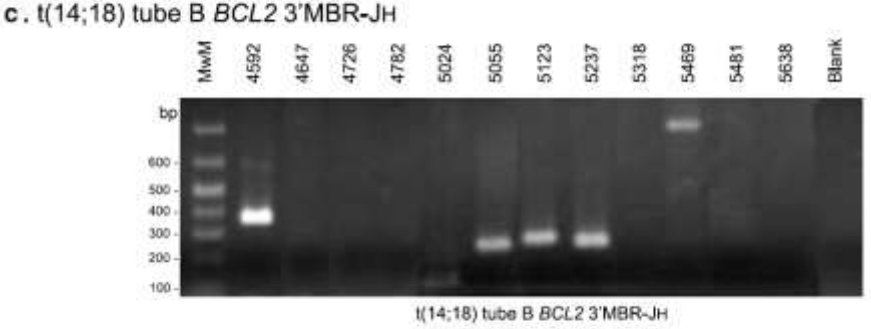
CT: Células Troncales Hematopoyéticas.

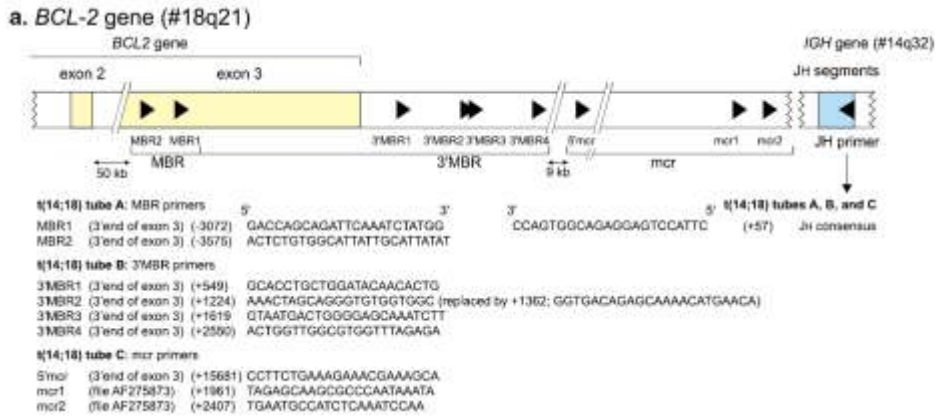
rIFN: Interferon recombinante.

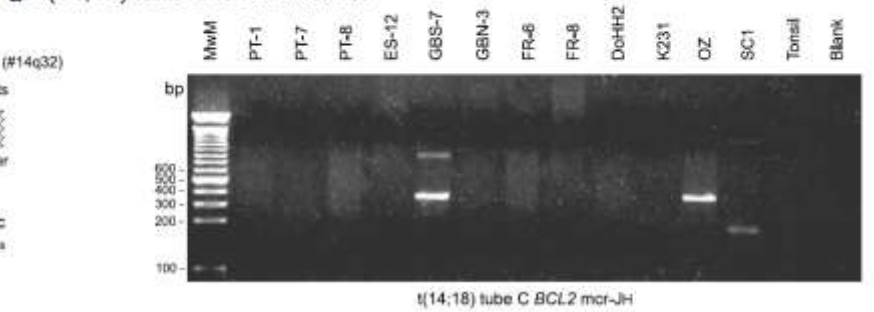
Ejemplo 2: EMR EN NEOPLASIAS LINFOIDES: LINFOMA FOLICULAR











PCR-based clonality studies in lymphoproliferations
JJM van Dongen *et al*

High Molecular Response Rate and Clinical Correlation in Patients with Follicular Lymphoma Treated with Cyclophosphamide-Vincristine-Prednisone plus Interferon α 2b[1]

Table 1 Patient's characteristics

a. Clinical data	
No. of patients	35
Age (median, range)	57 (26–79 years)
Sex (male/female)	14/21
Ann Arbor stage	
II	5
III–IV	30
IPI	
0–1	19
≥2	16
b. Molecular data	
Histological BM infiltration (n = 26)	
Bcl-2 (+)	15 (58%)
IgH rearrangement (+)	8 (31%)
Undetectable molecular marker	3 (11%)
Histological BM not infiltrated (n = 9)	
Bcl-2 (+)	5 (56%)
IgH rearrangement (+)	1 (11%)
Undetectable molecular marker	3 (33%)

Table 2 Therapy and clinical response of the patients

No. of patients	35
Treatment (CVP + IFN)	
No. of cycles (median, range)	8 (5–11)
Maintenance IFN	
Yes	10
No	25
Time between diagnosis and treatment (median, range)	33 (15–110 days)
Clinical response after treatment induction	
Complete	28 (80%)
Partial	6 (17%)
Progressive disease	1 (3%)
PFS	
At 2 years	90.5 ± 5% [95% (CI) 80.2–100%]
At 5 years	78.1 ± 8% [95% (CI) 62.3–93.9]
Follow-up (median, range)	37 (13–59 months)

Elena Fernandez-Ruiz et al (HUP)

Table 3 Correlation between clinical and molecular findings in 24 patients with positive markers found in BM at diagnosis and observed for >2 years[a]

	Clinical response (%)	Relapse/Prog (%)
Molecular response		
PCR− ($n = 16$)	15 (94%)	1 (6%)
PCR+ ($n = 8$)	4[b] (50%)	4 (50%)

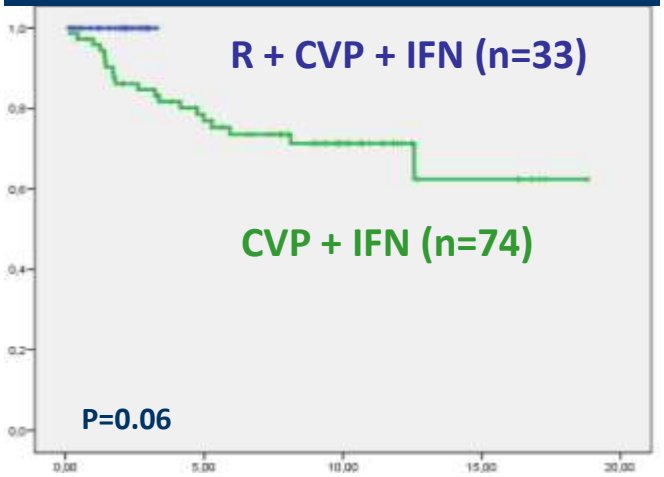
[a] $P < 0.05$; S, (sensitivity): 79%; E, (specificity): 80%; PPV, (positive predictive value): 94%; NPV, (negative predictive value): 50%.

[b] Three patients became once positive in the last follow-up, and 1 patient fluctuated between negative and positive results.

LNH- PRO-05: Rituximab + CVP + 12 Semanas de IFN-α2b en pacientes con LNH Folicular de Intermedio-Alto Riesgo (FLIPI≥2)

CARACTERISTICAS	N=33 (%)
Edad (mediana)	53 años
Sexo H / M	42% / 58%
FLIPI 2 3-4	11 (58%) 8 (42%)
Ann Arbor ...III ...IV	12% 88%
Extranodal	88%
Afectación Medular	72%
Masa Voluminosa	30%
LDH elevada	18%
Molecular positiva DXO …*Bcl2-IgH* (cuali/RT-PCR/FISH) …Reordenamiento *IgH*	17/23 (74%) 15/23 (65%) 4/23 (17%)

OS Y PFS



RESPUESTA MOLECULAR

- Evaluada (hasta 8º ciclo) en 18 pacientes
  - *Bcl-2/IgH (RT-PCR) negativos* tras 4º ciclo.....13/18 (72%)
  - Oscilacion de la PCR cuantitativa en 5 pacientes sin significación clínica (nivel ≤ 0.05% copias)

R Arranz, J Cannata, S Montes-Moreno (HUP, CNIO)

CONCLUSIONES

La aplicación de técnicas moleculares para deteccion de EMR precisa de varias etapas de estandarización necesarias a la aplicación clínica (estandarización técnica/estandarización en la interpretación).

La infraestructura y personal implicados deben tener preparacion específica (biologos moleculares y técnicas especialistas en molecular).

El impacto clínico de el resultado específico depende de forma critica del contexto patológico y clínico. El patólogo molecular es la figura capaz de interpreta e integrar la informacion molecular, patológica y clínica y emitir un **informe diagnóstico integrado.**

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