NOVARTIS ROUND TABLE ON MOLECULAR MONITORING IN CML

THURSDAY FEBRUARY 4TH 2016

DR FRÉDÉRIC LAMBERT

UNITÉ DE GÉNÉTIQUE MOLÉCULAIRE HÉMATO-ONCOLOGIQUE

UNILAB LG / CHU DE LIÈGE

AGENDA OF THE MEETING

Introduction – Karen

- Evolution of the CML treatment, and the fact that deep molecular responses are becoming the goal of current treatments: what molecular monitoring is and its rational ?

Part 1: Molecular Monitoring in CML at Unilab Lg in practice

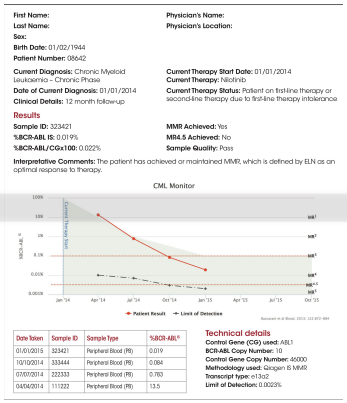
1. How is quantitative PCR monitoring of *BCR-ABL* mRNA performed ?
2. How is % IS *BCR-ABL* calculated and why the number of Housekeeping gene transcripts is so important ?
3. Interactive discussion on the lab report(s)
4. Difference between accredited lab and standardized lab
5. Explanation of the Belgian Standardization Project

Part 2: Monitor the Milestones: clinical implications – Karen

- The importance and prognostic value of EMR, MMR, MR4.5 and why it's so important for the future if patients want to stop TKI therapy

1. HOW IS *BCR-ABL1* mRNA MOLECULAR MONITORING PERFORMED AT UNILAB LG *IN PRACTICE ?*

« MR3 »



First Name:
Last Name:
Sex:
Birth Date: 01/02/1944
Patient Number: 08642

Physician's Name:
Physician's Location:

Current Diagnosis: Chronic Myeloid Leukaemia – Chronic Phase
Date of Current Diagnosis: 01/01/2014
Clinical Details: 12 month follow-up

Current Therapy Start Date: 01/01/2014
Current Therapy: Nilotinib
Current Therapy Status: Patient on first-line therapy or second-line therapy due to first-line therapy intolerance

Results

Sample ID: 323421
%BCR-ABL IS: 0.019%
%BCR-ABL/CGx100: 0.022%

MMR Achieved: Yes
MR4.5 Achieved: No
Sample Quality: Pass

Interpretative Comments: The patient has achieved or maintained MMR, which is defined by ELN as an optimal response to therapy.

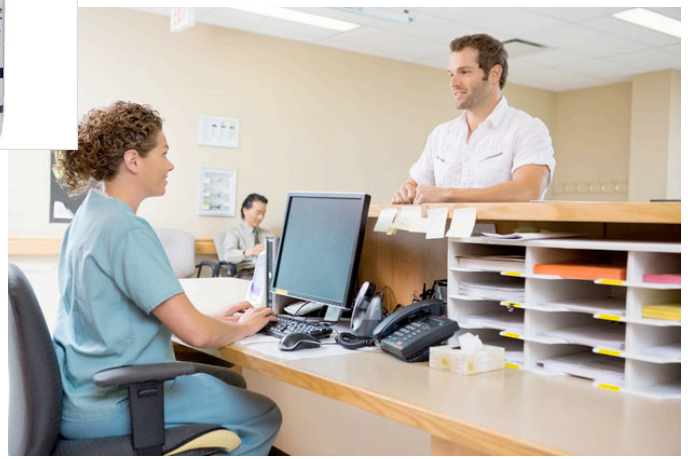
CML Monitor

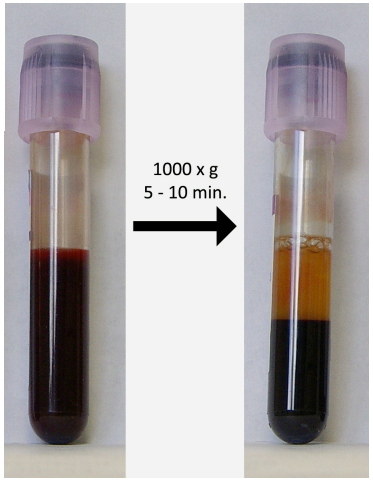
Date Taken	Sample ID	Sample Type	%BCR-ABL^{IS}
01/01/2015	323421	Peripheral Blood (PB)	0.019
10/10/2014	333444	Peripheral Blood (PB)	0.084
07/07/2014	222333	Peripheral Blood (PB)	0.760
04/04/2014	111222	Peripheral Blood (PB)	13.5

Technical details
Control Gene (CG) used: ABL1
BCR-ABL Copy Number: 10
Control Gene Copy Number: 46000
Methodology used: Qiagen IS MMR
Transcript type: e13a2
Limit of Detection: 0.0023%

Temps de livraison (rêvé): 2 H

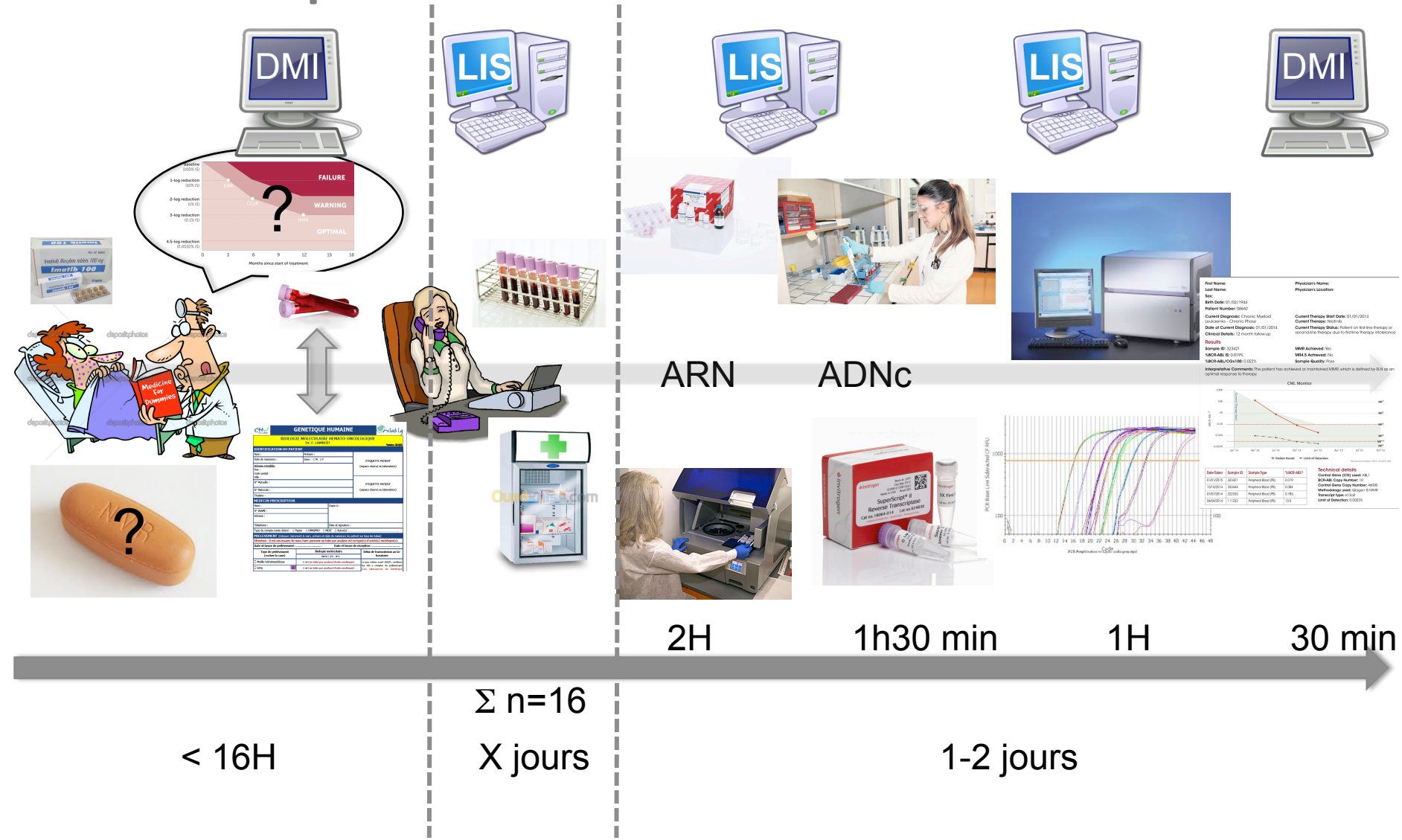
The journey from the consultation of the patient until the lab report





Signalétique patient
Diagnostic – thérapie
Prélèvement – conditionnement (tube)
Délais d'acheminement

The journey from the consultation of the patient until the lab report



Temps de livraison moyen (2015): 16,11j (médian; 14)

The journey from the consultation of the patient until the lab report

CHU de Liège
Chilab Lg.

Centre Hospitalier Universitaire de Liège
Domaine Universitaire du Sart Tilman - B35 - 4000 LIEGE 1
www.chuliege.be

Agréation :
N° 8.62700.18.998

Prescrit par DR DE PASQUAL AURELIE

CENTRE DE GENETIQUE
Agréation : 8.62990.19.996 art.33 & bis
Génétique clinique - 04/366.71.24
Biochimie génétique - 04/366.76.95 - fax 04/366.84.74
Cytogénétique - 04/366.25.61 - fax 04/366.29.74
Génétique moléculaire - 04/366.24.78
Biologie moléculaire hématologique - 04/366.25.61

16841574598 LHCV

Impression du: **01/02/2016 à 18:56**

Réf du labo: 14-160119-0087
Votre Réf: 248518

Protocole DUPLICATA

C.H PELTZER - LA TOURELLE
Laboratoire d'Analyses Médicale
rue du Parc 29
4800 VERVIERS - BELGIQUE

Nom, prénom: **WILK**
Né(e) le 29/03/1937
Sexe:Masculin
Code Patient: 3469520B
N° Traitement: 116015621F

Date du prélèvement: **19/01/2016 10:40**
Date de réception: **19/01/2016 15:55**
Date de validation: **28/01/2016 10:27**

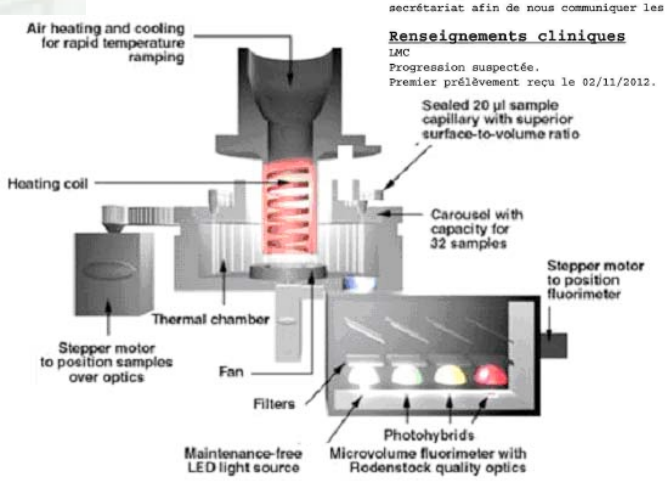
1/3

BIOLOGIE MOLECULAIRE HEMATOLOGIQUE

Echantillon
Moelle

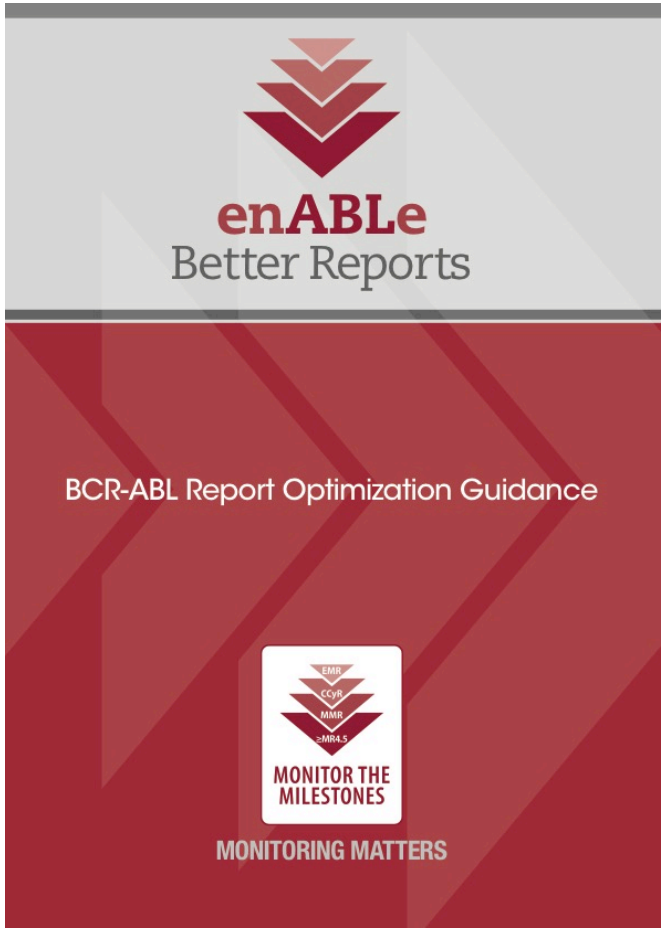
La prescription reçue n'était pas cochée. Pourriez vous prendre contact rapidement avec notre secrétariat afin de nous communiquer les analyses à réaliser. Merci

Renseignements cliniques
LMC
Progression suspectée.
Premier prélèvement reçu le 02/11/2012.



PCR quantitative *BCR-ABL1*: quels renseignements cliniques transmettre au labo ?

Ceux qui sont indispensables à l'interprétation et la représentation graphique optimales des résultats quantifiés sur l'échelle internationale (I.S scale)



GUIDANCE ON THE DATA FIELDS THAT SHOULD BE INCLUDED IN THE CLINICAL REPORT

Report Field	Implementation Guidance
Current Diagnosis	This field gives clinical context to the result and can be one of three options; 1. CML Chronic Phase 2. CML Accelerated Phase 3. CML Blast Phase Importantly, only a diagnosis of CML with a p210 transcripts can be reported on the IS.
Date of Current Diagnosis	This field allows key milestones to be observed. + TKI initiation This field is commonly excluded from clinical reports.
Clinical Details	This is the patient's clinical details as provided on the test request form by the referring physician giving clinical information to the laboratory to aid in the interpretation of the result. Example: *Patient not tolerating therapy. Query: progression?* This field is commonly excluded from clinical reports.
Current Therapy Start Date	This vital piece of information is very often not included in clinical reports. Without this information interpretation of the ELN recommendations cannot be achieved.
Current Therapy	This is the patient's therapy regime at the time this sample was taken. Options are: • Imatinib • Nilotinib • Dasatinib • Ponatinib • Bosutinib • Not currently using TKI therapy • Other

http://www.chu.ulg.ac.be/jcms/c_1305793/formulaires-utiles

GENETIQUE HUMAINE

BIOLOGIE MOLECULAIRE HEMATO-ONCOLOGIQUE

Dr. F. LAMBERT

Version 2015/2

IDENTIFICATION DU PATIENT

Nom :	Prénom :
Date de naissance :	Sexe : ☐ M ☐ F
Adresse complète Rue : Code postal : Ville :	ETIQUETTE PATIENT (espace réservé au laboratoire)
N° Mutuelle :	
N° Matricule :	ETIQUETTE PATIENT (espace réservé au laboratoire)
Titulaire :	

MEDECIN PRESCRIPTEUR

Nom :	Copie à :
N° INAMI :	
Adresse :	
Téléphone :	Date et signature :

Type de compte-rendu désiré : ☐ Papier ☐ OMNIPRO ☐ MEXI ☐ Autre(s) :.....

PRELEVEMENT (indiquer clairement le nom, prénom et date de naissance du patient sur tous les tubes)

Attention : il est nécessaire de nous faire parvenir un tube par analyse et/ou type(s) d'acide(s) nucléique(s).

Date et heure de prélèvement : ..... **Date et heure de réception : .....**

Type de prélèvement (cocher la case)	Biologie moléculaire EDTA / 2°C - 8°C	Délai de transmission au laboratoire
☐ Moëlle hématopoïétique	3 ml (un tube par analyse/Acide nucléique)	Le jour même avant 16h30 ; endéans les 16h à compter du prélèvement (Les laboratoires de Génétique
☐ Sang E	3 ml (un tube par analyse/Acide nucléique)	

☐ **Suivi**
☐ **Rechute**
☐ **Prégreffe / Postgreffe**
☐ **Traitement par : .....** **Date : J+.....** ☐ **1ère ligne** ☐ **2ème ligne**

CONTACTS dispa.genetique@chu.ulg.ac.be

Biologie Moléculaire Hématologique	Oncogénétique Moléculaire	Génétique clinique
Dr F.LAMBERT / Dr Sc. B.KOOPMANSCH / Secrétariat : 04/366.24.78	Dr Sc. K.SEGERS / Secrétariat : 04/366.24.78	Dr V.BOURS / Dr S.GAILLEZ / Secrétariat : 04/366.71.24

Fichier téléchargeable à l'adresse : http://www.chuliege.be/unilab/formulaires

MQ.A11.26 – version 10 - Page 1/4

A. AFFECTIONS HEMATOLOGIQUES ET ONCOLOGIQUES (ACQUIS)

SUIVI / MALADIE RESIDUELLE

Ces analyses sont facturées selon l'Arrêté Royal du 07 juin 2007, dit "article 33bis", modifiant l'annexe à l'Arrêté Royal du 14 septembre 1984, établissant la nomenclature des prestations de santé en matière d'assurance soins de santé et indemnités. Si marqueur préalablement identifié au diagnostic : règle INAMI 33 bis : suivi de maximum 1 marqueur si "positif" au diagnostic, maximum 4x/année de suivi, ensuite à charge du patient moyennant consentement signé.

Menu des analyses	Type de prélèvement	Type d'acide nucléique
☐ RT-PCR quantitative *BCR-ABL1*, t(9;22)(q34;q11) **(Pas au diagnostic)**	Sang	ARN
☐ RT-PCR quantitative *WT1* **(Si surexprimé au diagnostic)**	Moelle/Sang	ARN
☐ RT-PCR quantitative *NPM1* **(Effectué en sous-traitance)**	Moelle/Sang	ARN
☐ RT-PCR quantitative *ETV6-AML1* (RUNX1), t(12;21)(q13;q22)	Moelle/Sang	ARN
☐ RT-PCR quantitative *E2A-PBX1*, t(1;19)(q23;q13)	Moelle/Sang	ARN
☐ RT-PCR quantitative *MLL-AF4*, t(4;11)(q21;q23)	Moelle/Sang	ARN
☐ RT-PCR quantitative *HOX11 (TLX1), HOX11L2 (TLX3)*, t(5;14)(q35;q32)	Moelle/Sang	ARN
☐ RT-PCR quantitative *SIL-TAL1*, t(1;14)(q32;q11)	Moelle/Sang	ARN
☐ RT-PCR quantitative *PML-RARAs*, t(15;17)	Moelle/Sang	ARN
☐ RT-PCR quantitative *MYH11CBFb*,inv(16)(p13q23),t(16;16)(p13;q23)	Moelle/Sang	ARN
☐ RT-PCR quantitative *MLL-AF4*, t(4;11)(q21;q23)	Moelle/Sang	ARN
☐ RT-PCR quantitative *MLL-AF9*, t(9;11)(q22;q23)	Moelle/Sang	ARN
☐ RT-PCR qualitative *FIP1L1-PDGFRA (del 4q12)*	Moelle/Sang	ARN
☐ Réarrangement du locus CDR I, II, III, DH-JH des IgH et/ou Kappa de l'IgL	Moelle/Sang	ADN
☐ Réarrangement du locus TCR γ, β ou δ	Moelle/Sang	ADN
☐ PCR quantitative V617F de *JAK2* **(Non remboursé par l'INAMI)**	Moelle/Sang	ADN
☐ Mutation de résistance aux TKI BCR-ABL1 si absence de MR3 **(effectué en sous-traitance)**	Moelle/Sang	ARN

LES DONNÉES CLINIQUES A PRÉSENTER DANS LE RAPPORT:

Report Field	Implementation Guidance
Current Diagnosis	This field gives clinical context to the result and can be one of three options; 1. CML Chronic Phase 2. CML Accelerated Phase 3. CML Blast Phase Importantly, only a diagnosis of CML with a p210 transcripts can be reported on the IS.
Date of Current Diagnosis	This field allows key milestones to be observed. This field is commonly excluded from clinical reports.
Clinical Details	This is the patient's clinical details as provided on the test request form by the referring physician giving clinical information to the laboratory to aid in the interpretation of the result. Example: *Patient not tolerating therapy. Query: progression?* This field is commonly excluded from clinical reports.
Current Therapy Start Date	This vital piece of information is very often not included in clinical reports. Without this information interpretation of the ELN recommendations cannot be achieved.
Current Therapy	This is the patient's therapy regime at the time this sample was taken. Options are: • Imatinib • Nilotinib • Dasatinib • Ponatinib • Bosutinib • Not currently using TKI therapy • Other

A. AFFECTIONS HEMATOLOGIQUES ET ONCOLOGIQUES (ACQUIS)

DIAGNOSTIC / MISE AU POINT INITIALE

Si le diagnostic n'est pas connu lors de la prescription, merci de transmettre au secrétariat une copie du médullogramme, de l'histologie médullaire et du typage lymphocytaire lorsqu'ils sont disponibles (dispa.genetique@chu.ulg.ac.be).

☐ LEUCEMIE LYMPHOBLASTIQUE AIGUE (LLA)

☐ B ☐ T ☐ Lignée ambigue ☐ Non connu

(Règles INAMI : maximum 2 tests IgH/TCR + 5 tests non IgH/TCR)

	Type de prélèvement	Type d'acide nucléique
☐ Réarrangement des CDRs I, II, III et/ou DH-JH de l'IgH et/ou IgK	Moelle	ADN
☐ Réarrangement des TCR δ, γ et /ou β	Moelle	ADN
☐ RT-PCR **qualitative :**		
☐ **LLA-B :** *BCR-ABL1, ETV6-RUN…CF3-PBX1, MLL-X, …*	Moelle	ARN
☐ **LLA-T** : *SIL-TAL1, TLX1, T…*		
☐ Mutation du gène : ☐ *J…R)* ☐ *NOTCH1* **(LLA-T)**	Moelle	ADN

☐ LEUCEMIE M… …UE (LMA)

☐ *de novo* ☐ …ted ☐ Phén… ☐ Non connu

…test

	Type de prélèvement	Type d'acide nucléique
☐ 1ère **Ligne**	…elle	ADN
☐ Mutation somat…		
☐ 2ème **Ligne**	Moelle	ADN
☐ Mutation des gènes : …		
☐ RT-PCR **qualitative :** *RUNX1-…*	Moelle/Sang	ARN
☐ RT-PCR **quantitative** *WT…*	Moelle/Sang	ARN

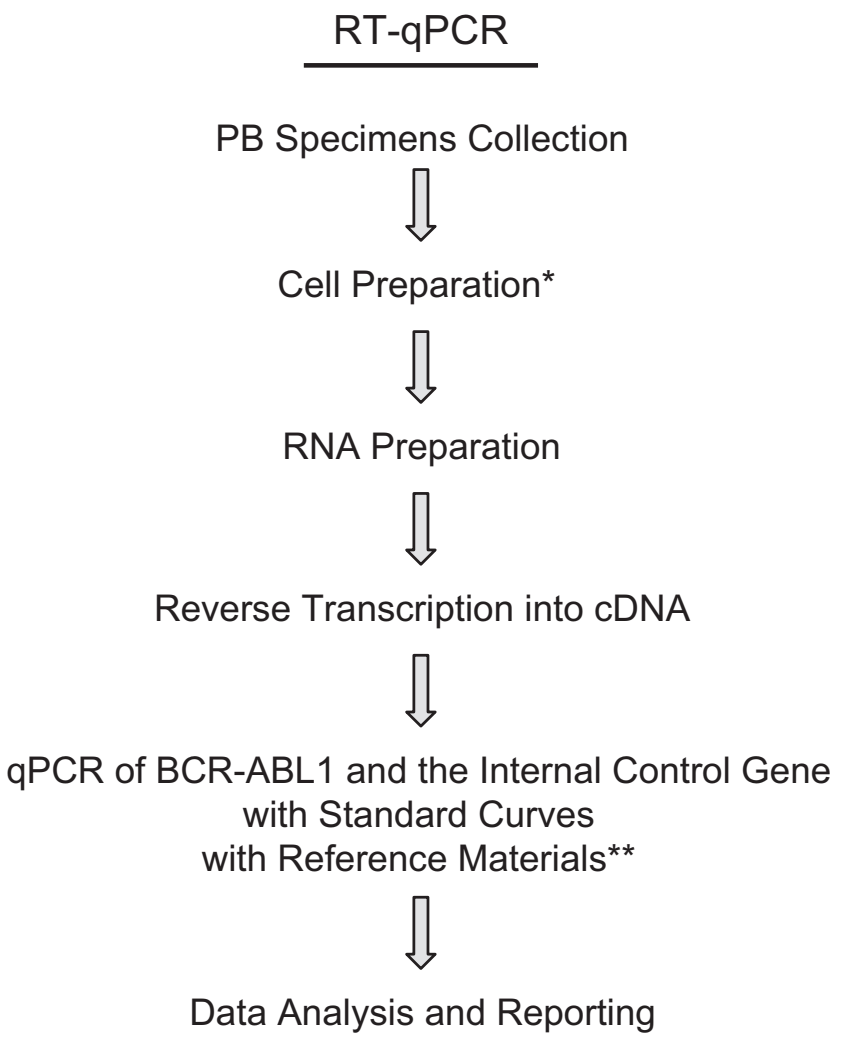
☐ NEOPLASIE MYEL…

☐ …eucémie myéloïde … …tique SVP)
☐ L…cémie chroni…
☐ Po…ycythémie …
☐ T…rombo…
☐ …yélofibr… …gie me…
☐ Syndrome d'… …nique à Eosinop…
☐ Mastocytose syst… …d'histologie médulla…

(Règles INAMI : ces … …nulables entre eux sur la m…

	Type de prélèvement	Type d'acide nucléique
☐ PV/TE ou PMF : Mutation p.Va… …dans l'exon 14 du gène *JAK2*	Sang	ADN
☐ PV « *JAK2* V617F-neg » : Mutations de l'exon 12 de *JAK2* **(Fournir dosage EPO sérique)**	Moelle	ADN
☐ TE ou PMF (Quand *JAK2* V617F négatif)		
☐ Mutation exon 9 du gène *CALR*	Moelle	ADN
☐ Mutation MPLW515 K/L du gène *MPL*		
☐ MS : Mutation p.Asp816Val du gène *c-KIT* **(SI infiltration démontrée par histologie médullaire et IHC spécifique anti-tryptase)**	Moelle	ADN/ARN
☐ SHE/LCE : RT-PCR *FIP1L1-PDGFRA* ; ***PDGFRB*** : caryotype à effectuer en Cytogénétique.	Moelle	ARN
☐ LCN : Mutation *CSF3R/SETBP1*	Moelle	ADN

FLUX SCHÉMATIQUE DE L'ANALYSE *BCR-ABL1 PAR* RT-PCR QUANTITATIVE (qPCR)



ChaoJie Zhen, J Mol Diagn 2013, 15: 556e564; http://dx.doi.org/10.1016/j.jmoldx.2013.05.010

PCR quantitative *BCR-ABL1*: pour qui et quand ?

Review Article

European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013

Michele Baccarani,[1] Michael W. Deininger,[2] Gianantonio Rosti,[3] Andreas Hochhaus,[4] Simona Soverini,[3] Jane F. Apperley,[5] Francisco Cervantes,[6] Richard E. Clark,[7] Jorge E. Cortes,[8] François Guilhot,[9] Henrik Hjorth-Hansen,[10] Timothy P. Hughes,[11] Hagop M. Kantarjian,[8] Dong-Wook Kim,[12] Richard A. Larson,[13] Jeffrey H. Lipton,[14] François-Xavier Mahon,[15] Giovanni Martinelli,[3] Jiri Mayer,[16] Martin C. Müller,[17] Dietger Niederwieser,[18] Fabrizio Pane,[19] Jerald P. Radich,[20] Philippe Rousselot,[21] Giuseppe Saglio,[22] Susanne Saußele,[17] Charles Schiffer,[23] Richard Silver,[24] Bengt Simonsson,[25] Juan-Luis Steegmann,[26] John M. Goldman,[27] and Rüdiger Hehlmann[17]

BLOOD, 8 AUGUST 2013 • VOLUME 122, NUMBER 6

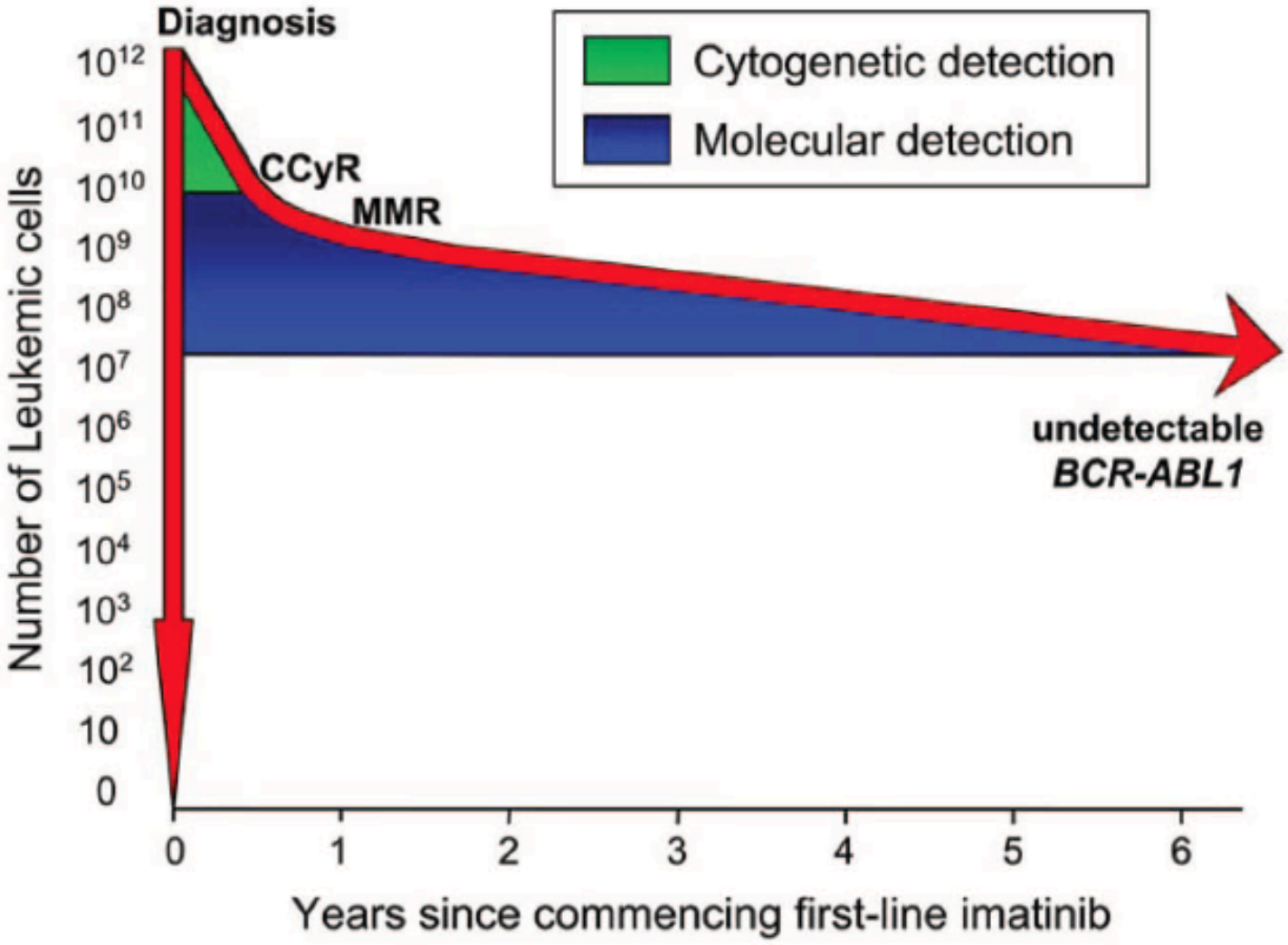
Table 9. Recommendations for cytogenetic and molecular monitoring

At diagnosis	Chromosome banding analysis (CBA) of marrow cell metaphases FISH in case of Ph negativity to identify variant, cryptic translocations Qualitative PCR (identification of transcript type)
During treatment	Quantitative real-time PCR (RQ-PCR) for the determination of *BCR-ABL1* transcripts level on the international scale, to be performed every 3 months until an MMR (BCR-ABL ≤0.1%, or $MR^{3.0}$) has been achieved, then every 3 to 6 months and/or CBA of marrow cell metaphases (at least 20 banded metaphases), to be performed at 3, 6, and 12 months until a CCyR has been achieved, then every 12 months. Once a CCyR is achieved, FISH on blood cells can be done. If adequate molecular monitoring can be ensured, cytogenetics can be spared.
Failure, progression	RQ-PCR, mutational analysis, and CBA of marrow cell metaphases. Immunophenotyping in BP.
Warning	Molecular and cytogenetic tests to be performed more frequently. CBA of marrow cell metaphases recommended in case of myelodysplasia or CCA/Ph– with chromosome 7 involvement.

The responses can be assessed either with molecular tests alone or with cytogenetic tests alone, depending on the local laboratory facilities, but whenever possible, both cytogenetic and molecular tests are recommended until a CCyR and an MMR are achieved. Then RQ-PCR alone may be sufficient. Mutational analysis by conventional Sanger sequencing is recommended in case of progression, failure, and warning.[59] In case of failure, warning, and development of myelodysplastic features (unexpected leucopenia, thrombocytopenia, or anemia), CBA of marrow cell metaphases is recommended.

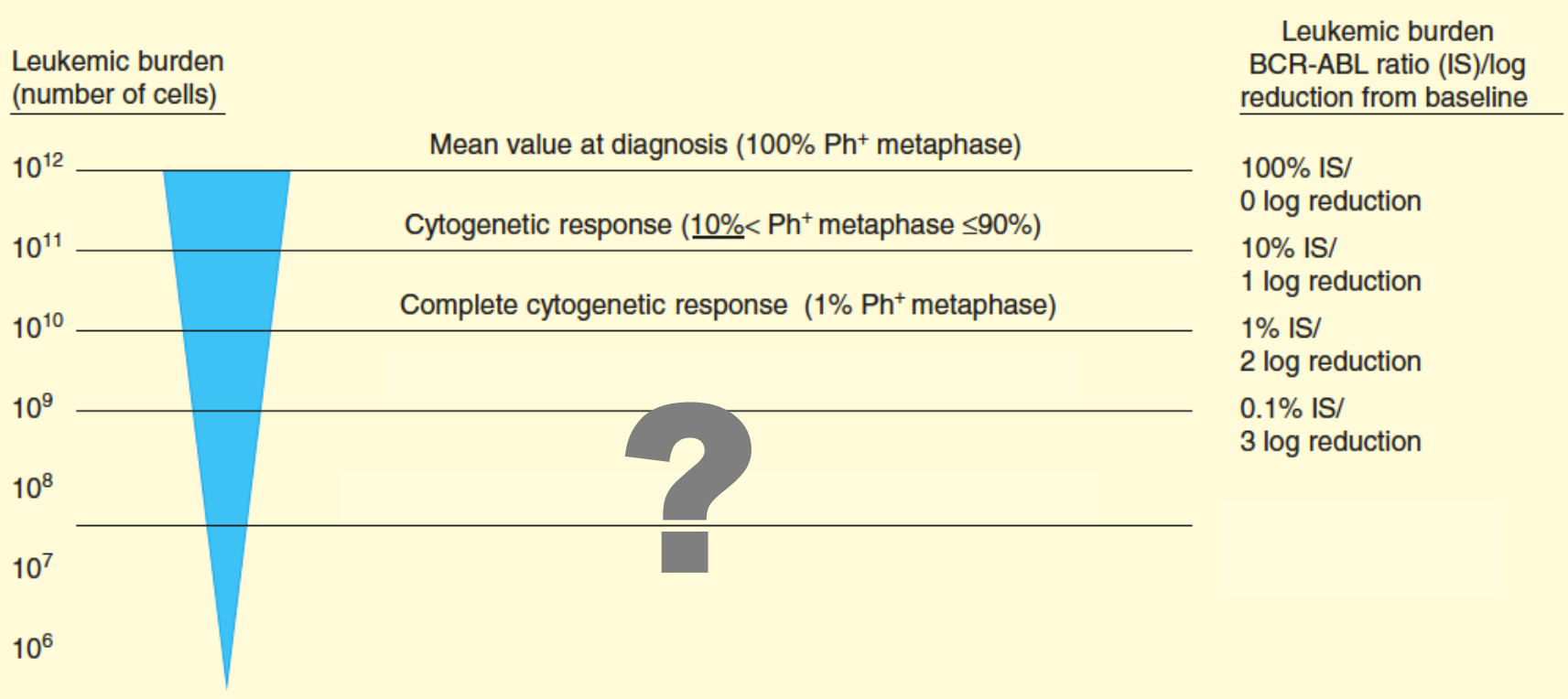
FISH, fluorescence in situ hybridization; CCA/Ph–,clonal chromosome abnormalities in Ph– cells.

PCR quantitative *BCR-ABL1*: pour qui et quand ?



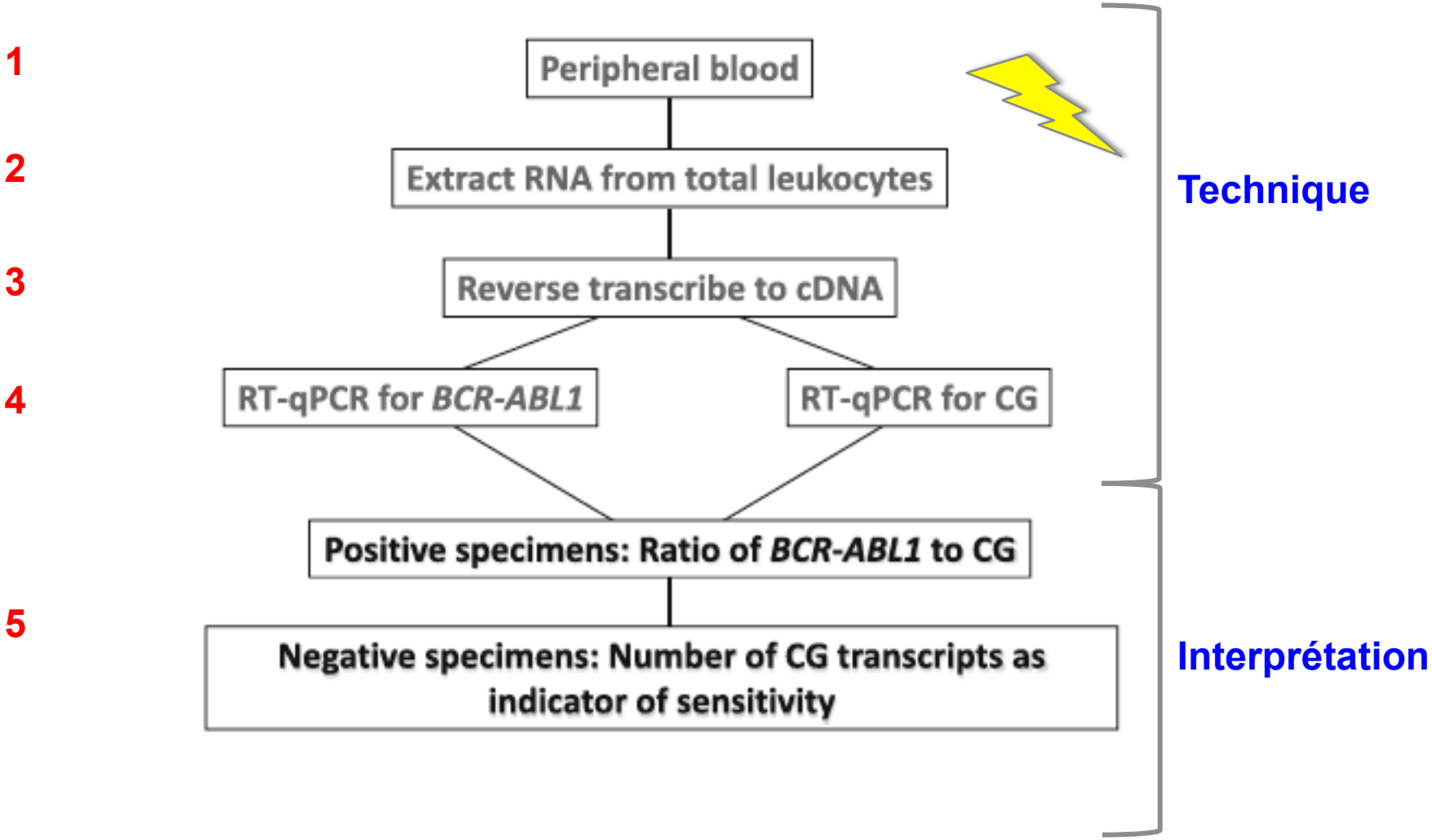
Susan Branford, Hematology 2012

PCR quantitative *BCR-ABL1*: pour qui et quand ?



Adapted from Martin Luu and Richard D, Expert Rev. Mol. Diagn. 13(7), 749–762 (2013)

FLUX SCHÉMATIQUE DE L'ANALYSE *BCR-ABL1 PAR* RT-PCR QUANTITATIVE (qPCR)

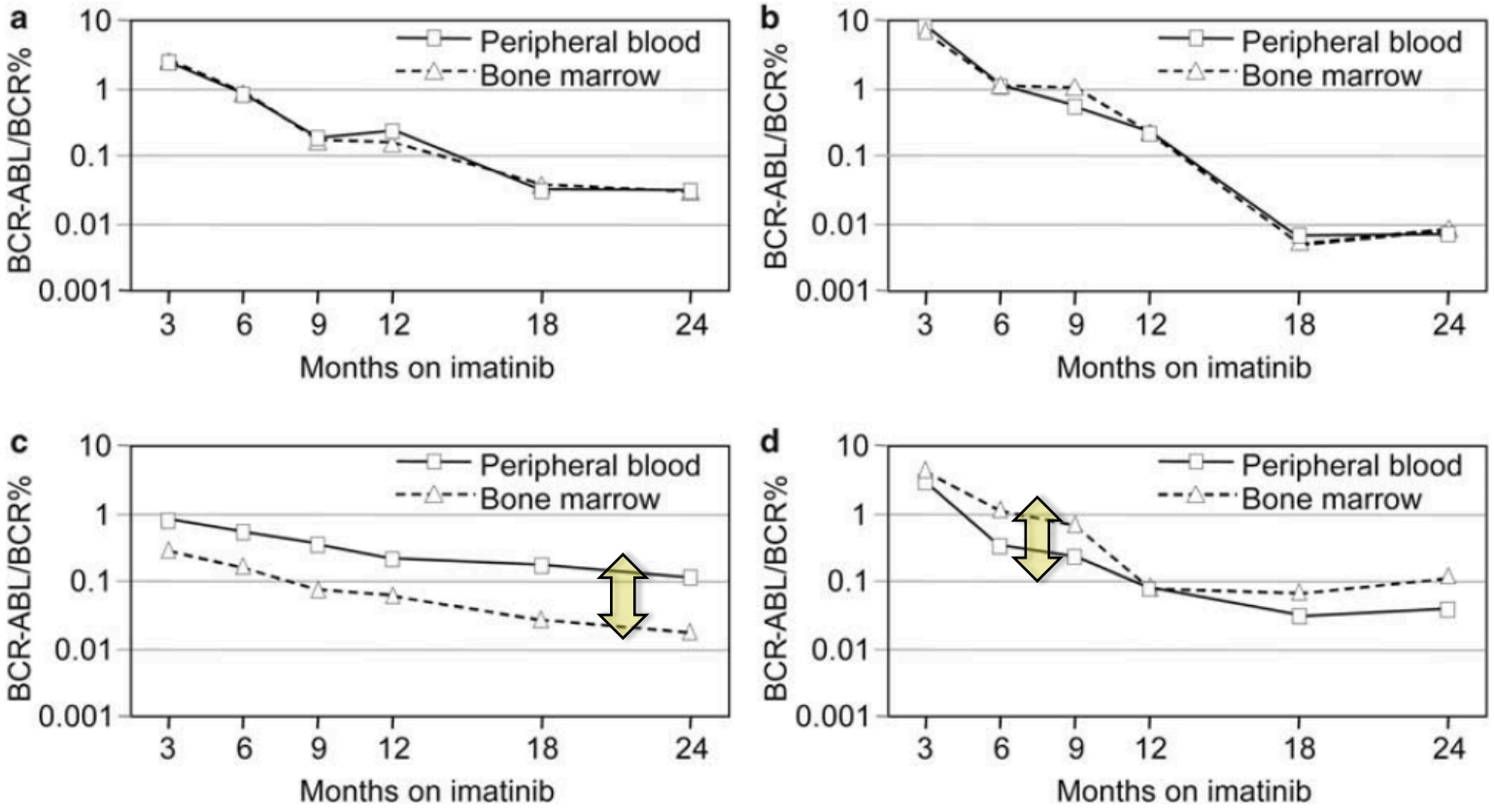


Nicholas C. P. Cross · Andreas Hochhaus · Martin C. Müller

Ann Hematol (2015) 94(Suppl 2):S219–S225
DOI 10.1007/s00277-015-2315-1

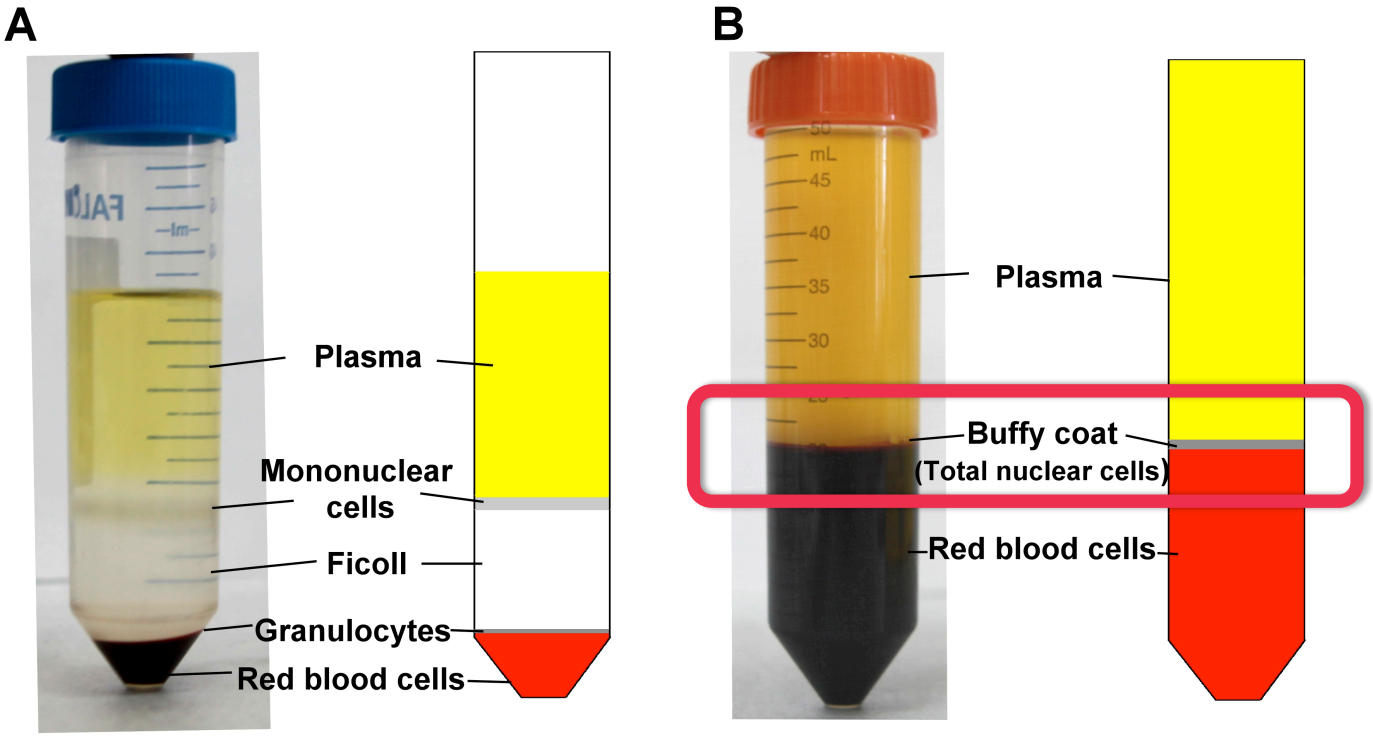
PCR quantitative *BCR-ABL1*: quel matériel utiliser pour le suivi quantitatif par PCR de *BCR-ABL1*, sang ou moelle ?

S Branford, Leukemia (2006) 20, 1925–1930



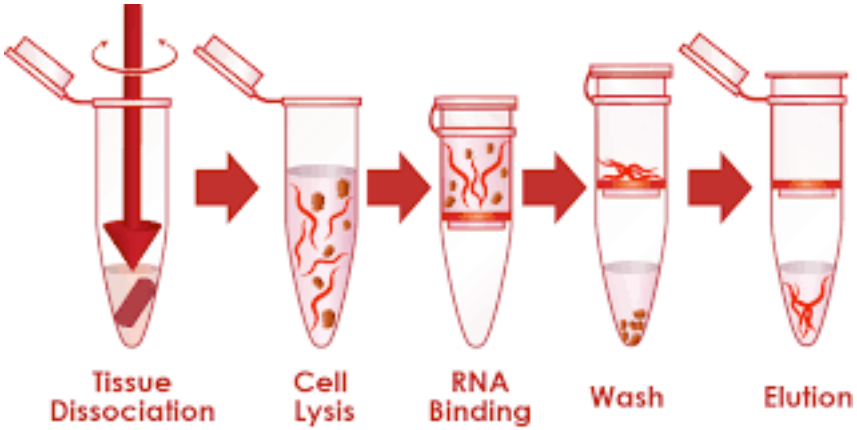
- Privilégier sang pour des raisons pratiques (accès, répétabilité prlvmt si confirmation du résultat requise);
- Ne pas comparer valeurs obtenues au départ de sang et moelle

SÉPARATION DES FRACTIONS CELLULAIRES SANGUINES, UNE PREMIÈRE SOURCE DE VARIABILITÉ DES RÉSULTATS INTERLABORATOIRES….



Quelles fractions cellulaires garder,
cellules mononuclées (PBMNC) versus leucocytes totaux?

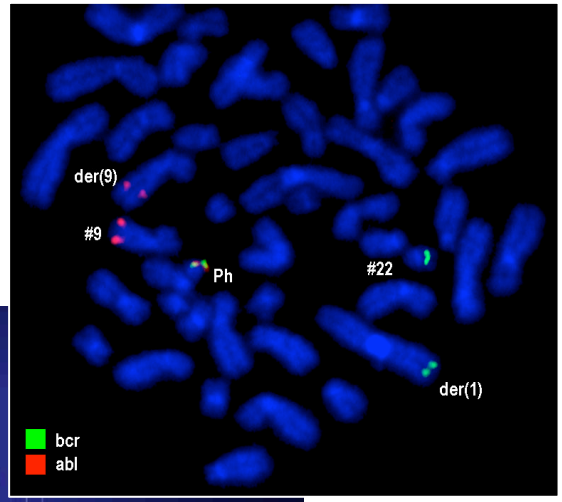
EXTRACTION D'ARN, UN MOMENT CRUCIAL POUR GARANTIR LA QUALITÉ DU RÉSULTAT

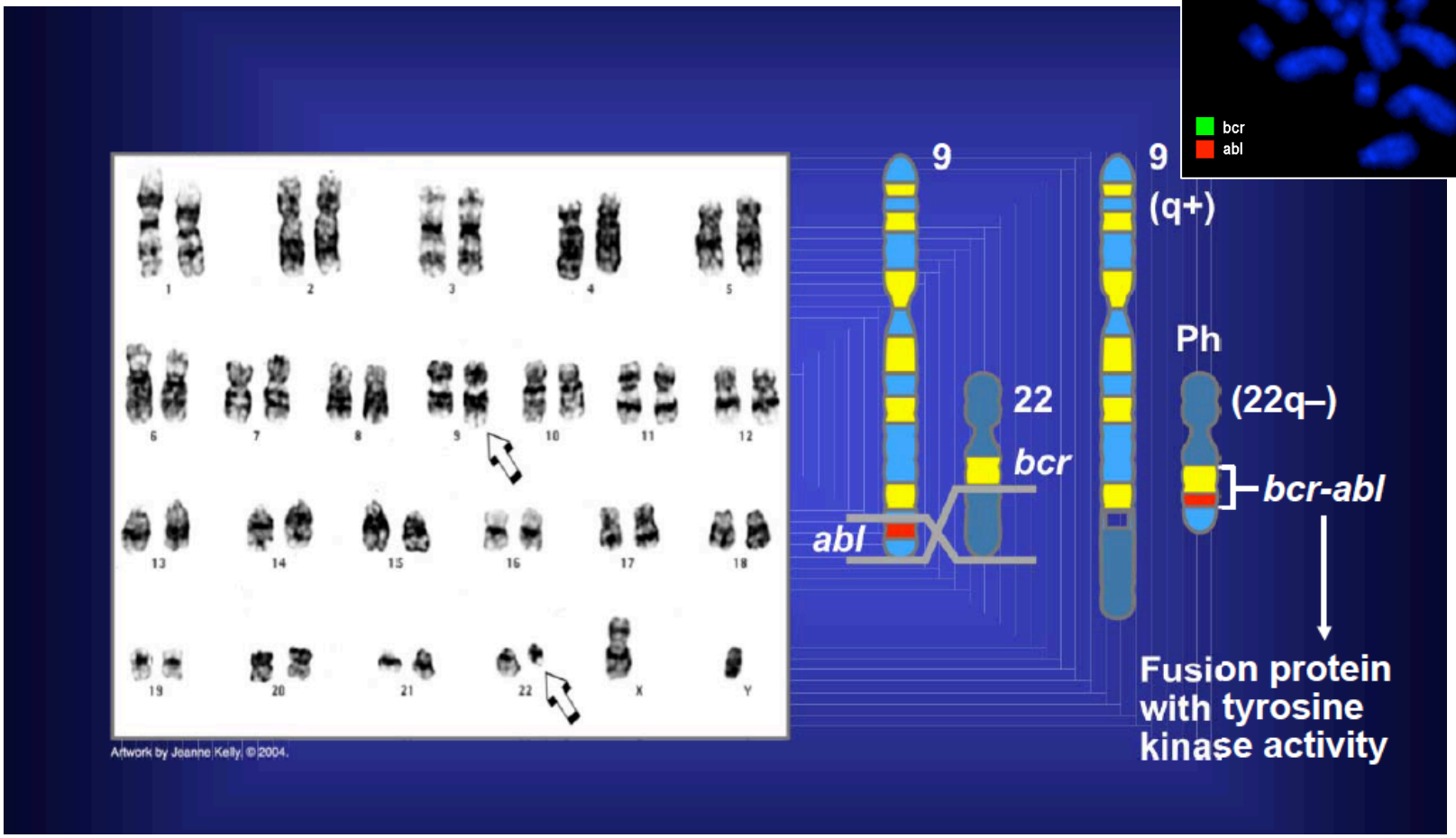


- **Susceptibilité à la dégradation -> délais d'acheminement < 24 (16H)**
- **ARN total ou ARN messager**

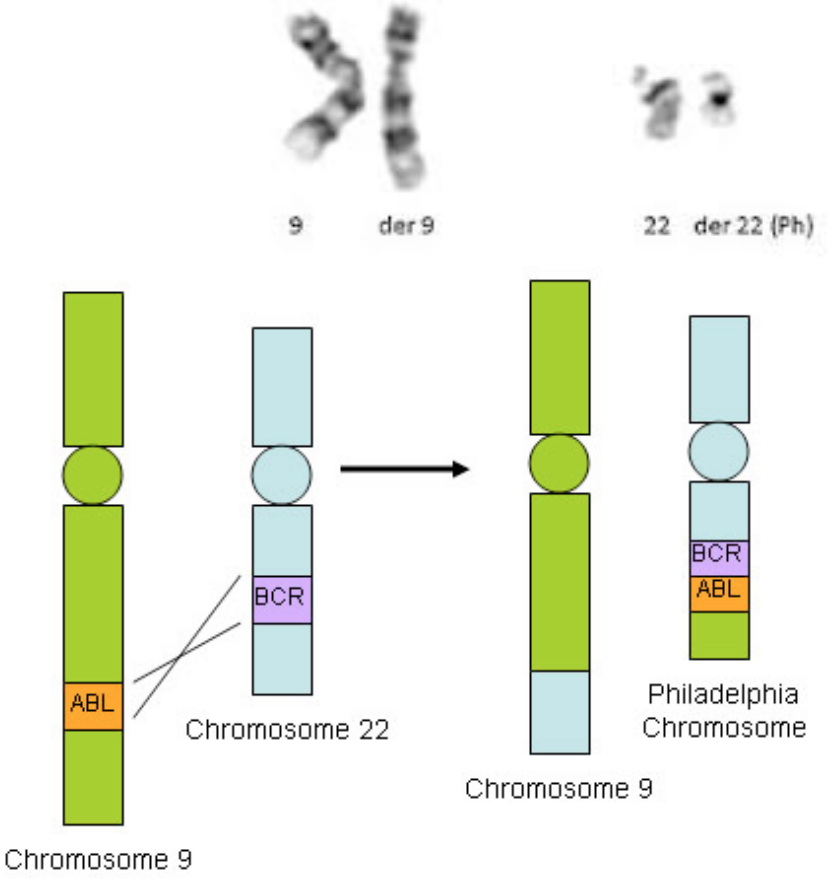
PCR quantitative *BCR-ABL1*: pourquoi utiliser l'ARN, un matériel délicat ?

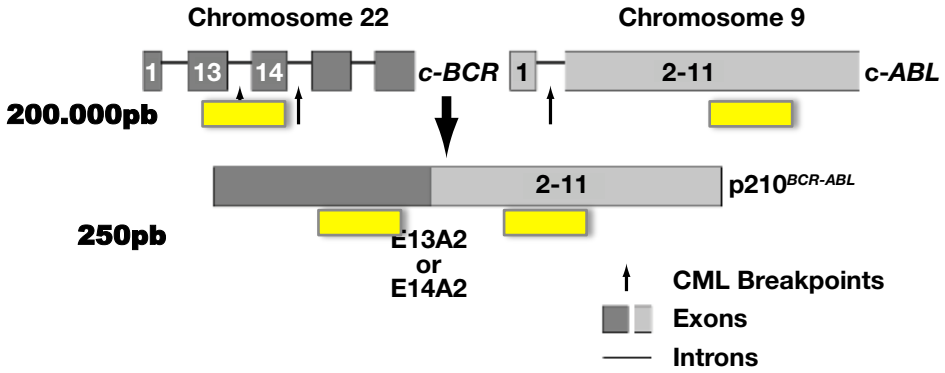
THE t(9;22)(q11; q34) IS THE HALLMARK OF CML,
SO WHY WORKING ON RNA INSTEAD OF DNA ?





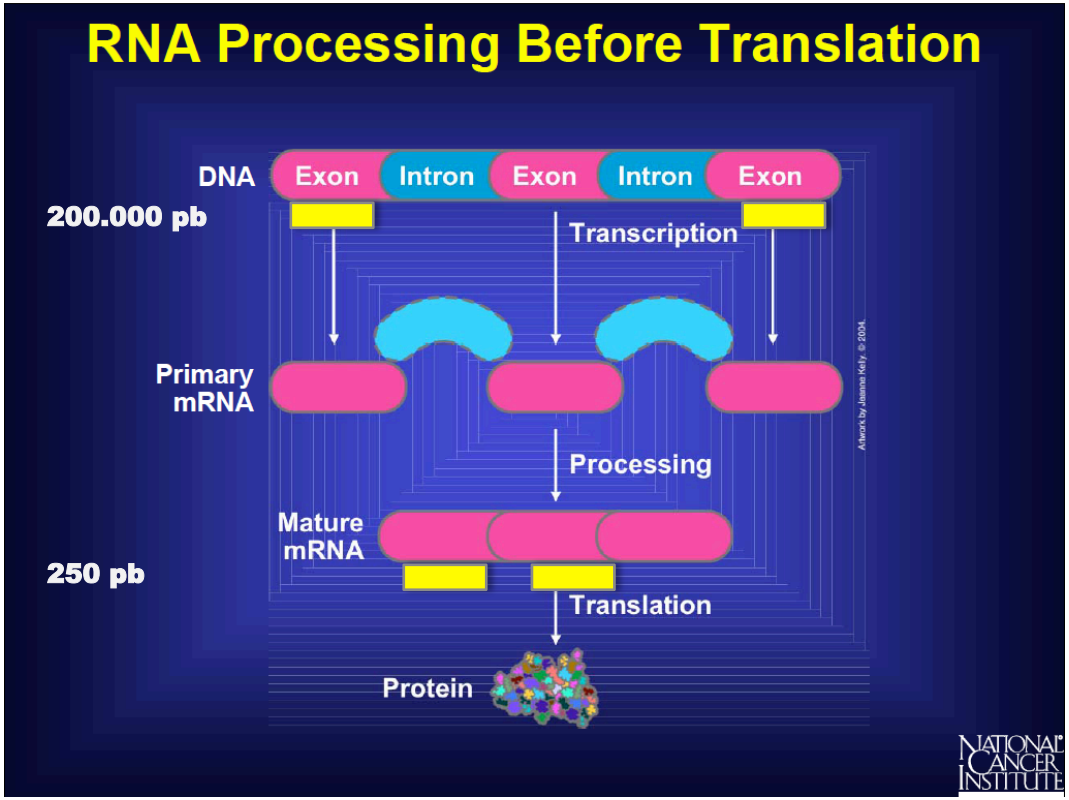
Pourquoi utiliser l'ARN, un matériel délicat ?: les points de cassures génomiques surviennent à des endroits distincts chez chaque patient





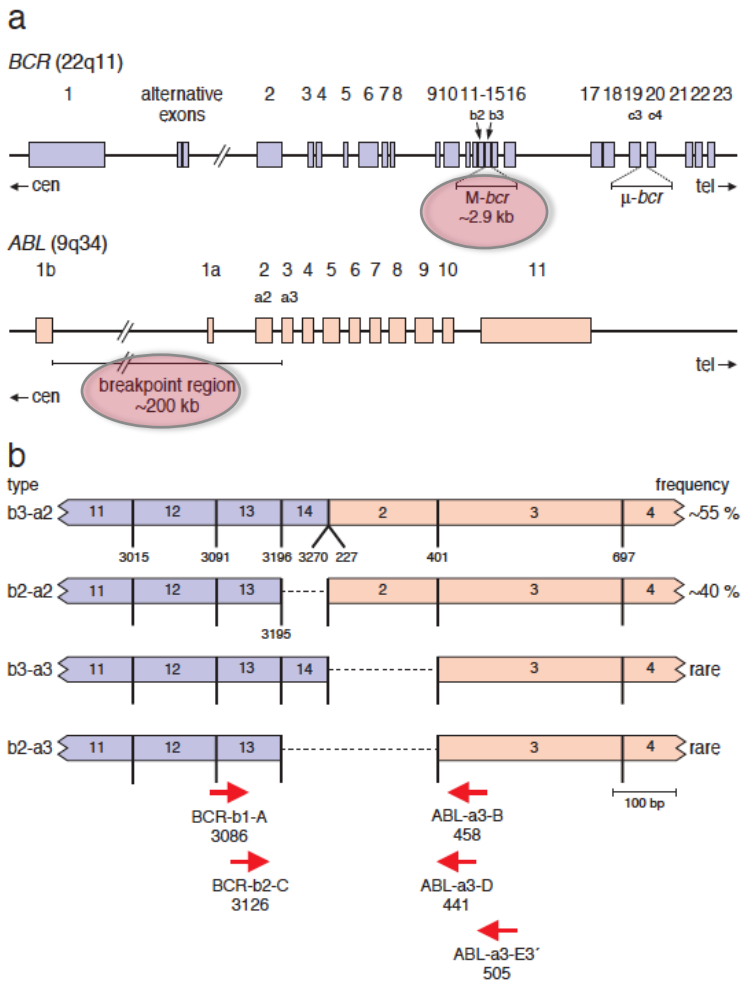
Le fusions *BCR-ABL1* sont difficilement amplifiables au départ de l'ADN

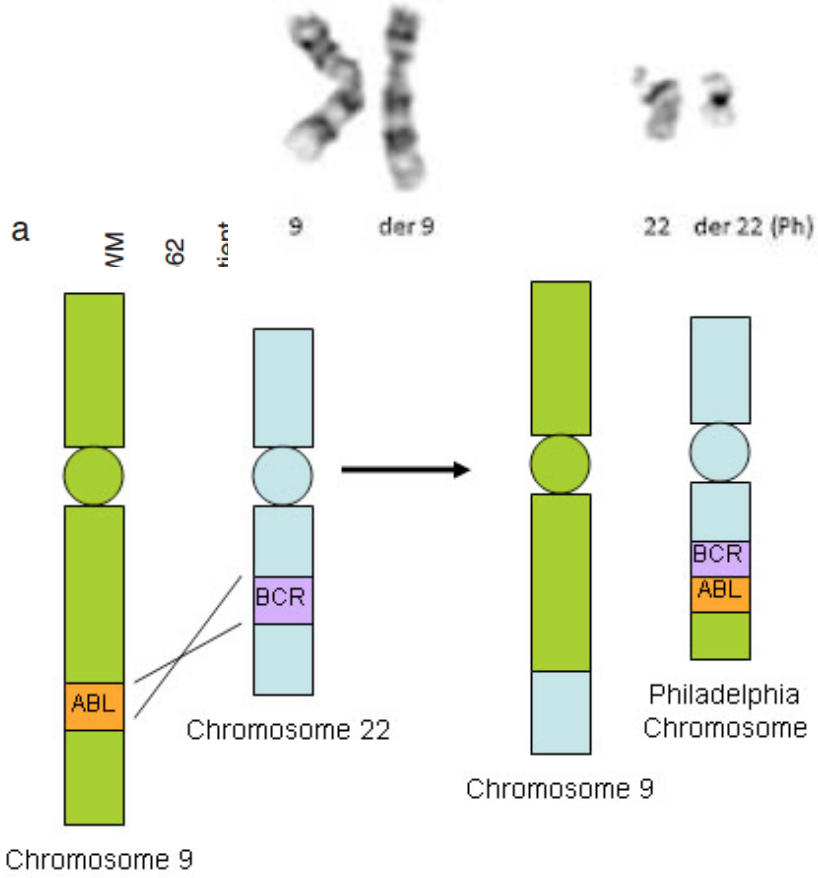
Transcription/Traduction (ADN->ARN->Protéine): Epissage des introns



Transcription/traduction
DNA -> mRNA -> Protéine

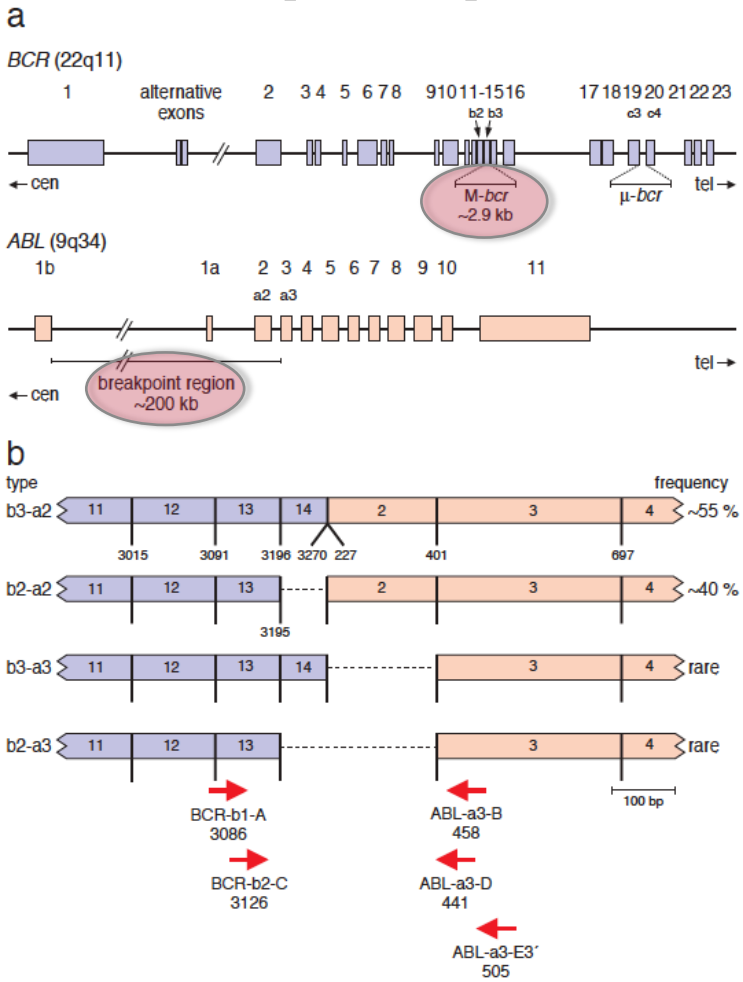
Pourquoi utiliser l'ARN, un matériel délicat ?: les points de cassures génomiques surviennent à des endroits distincts, générant des gènes de fusions spécifiques à chaque patient

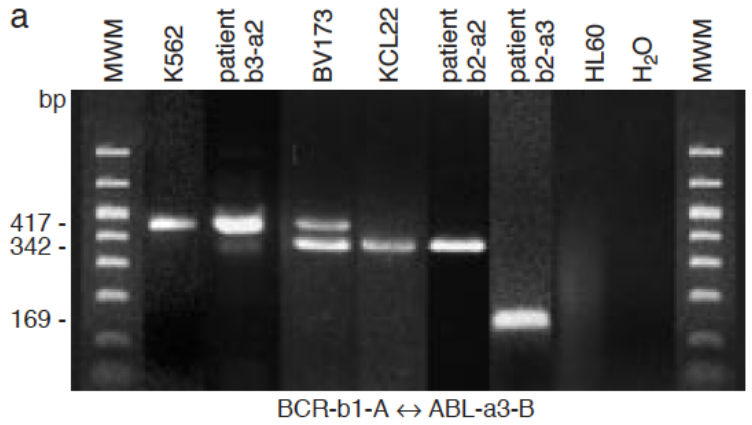




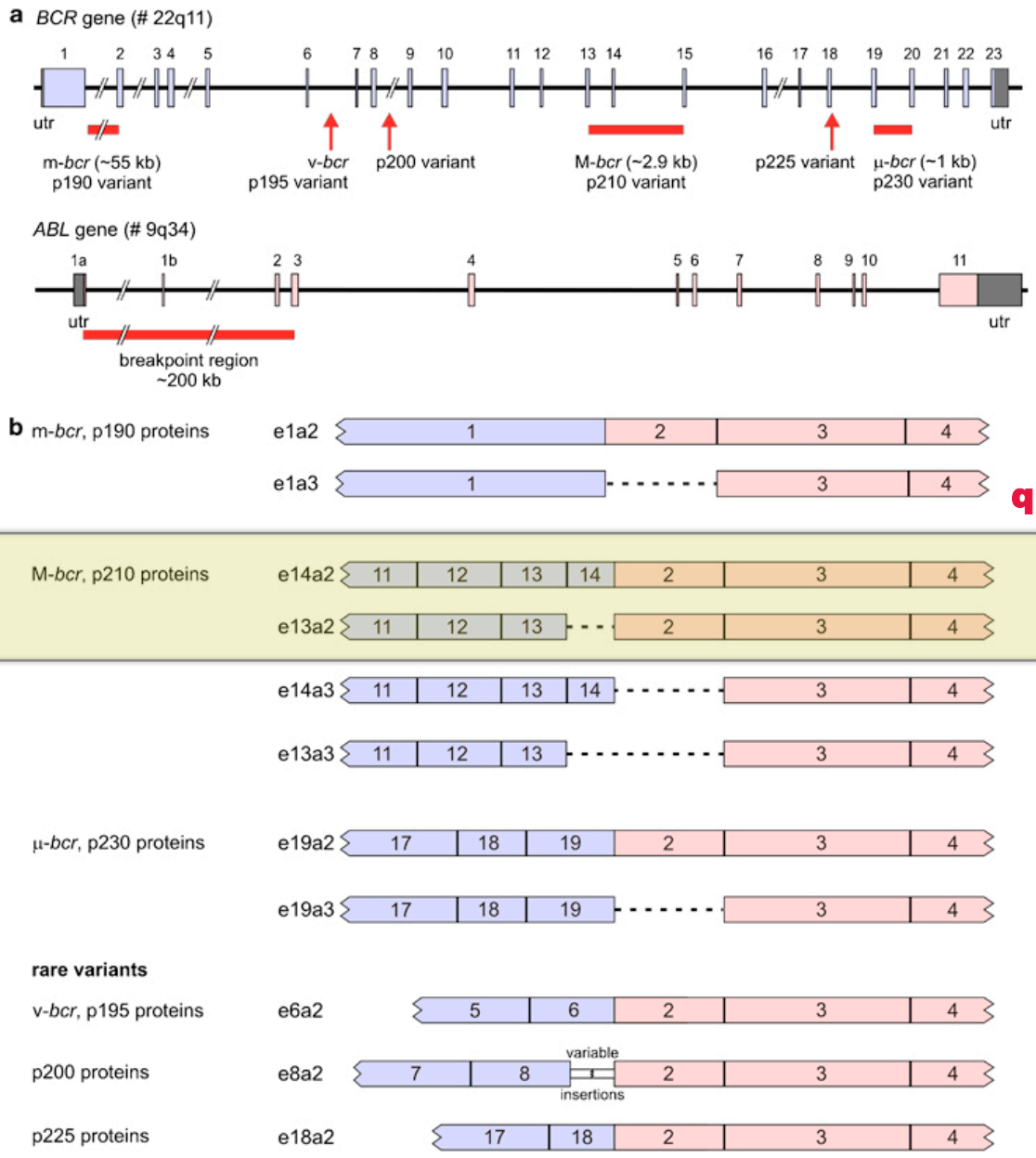
JJM van Dongen et al., Leukemia (1999) 13, 1901–1928

Pourquoi utiliser l'ARN, un matériel délicat ?: les points de cassures génomiques surviennent à des endroits distincts, générant des gènes de fusions spécifiques à chaque patient





JJM van Dongen et al., Leukemia (1999) 13, 1901–1928



< 1% CML

≈ 90% CML

< 5% CML

X% CNL

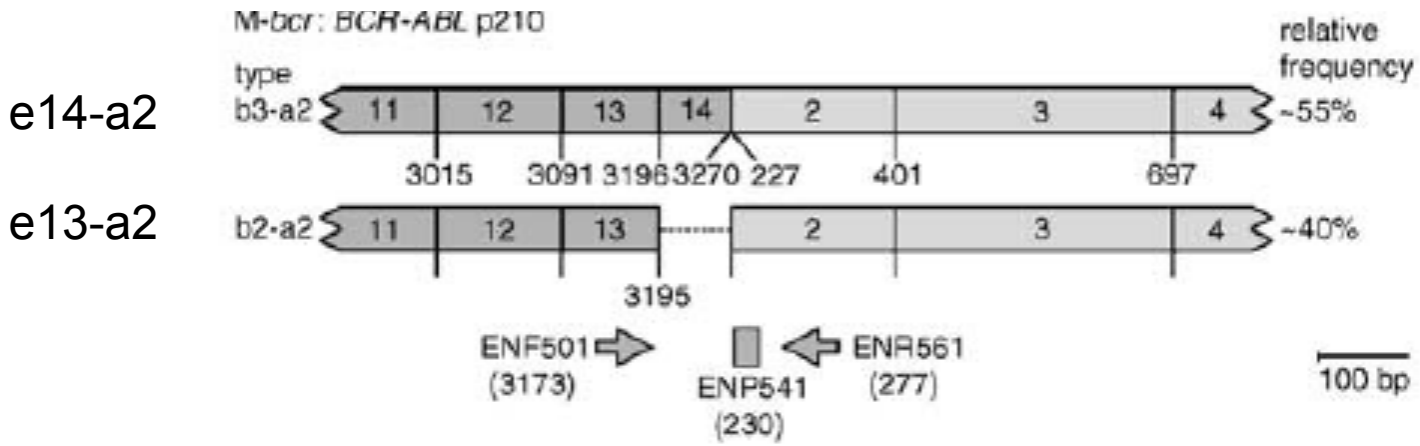
< 0,5% CML

qPCR BCR-ABL1 I.S

LES VARIANTS (TRÈS) RARES NE PEUVENT ÊTRE SUIVI PAR RT-qPCR

Foroni L., Am. J. Hematol. 84:517–522, 2009.

PCR quantitative *BCR-ABL1*: les sondes/amorces utilisées dans la PCR dictent la sensibilité clinique du test

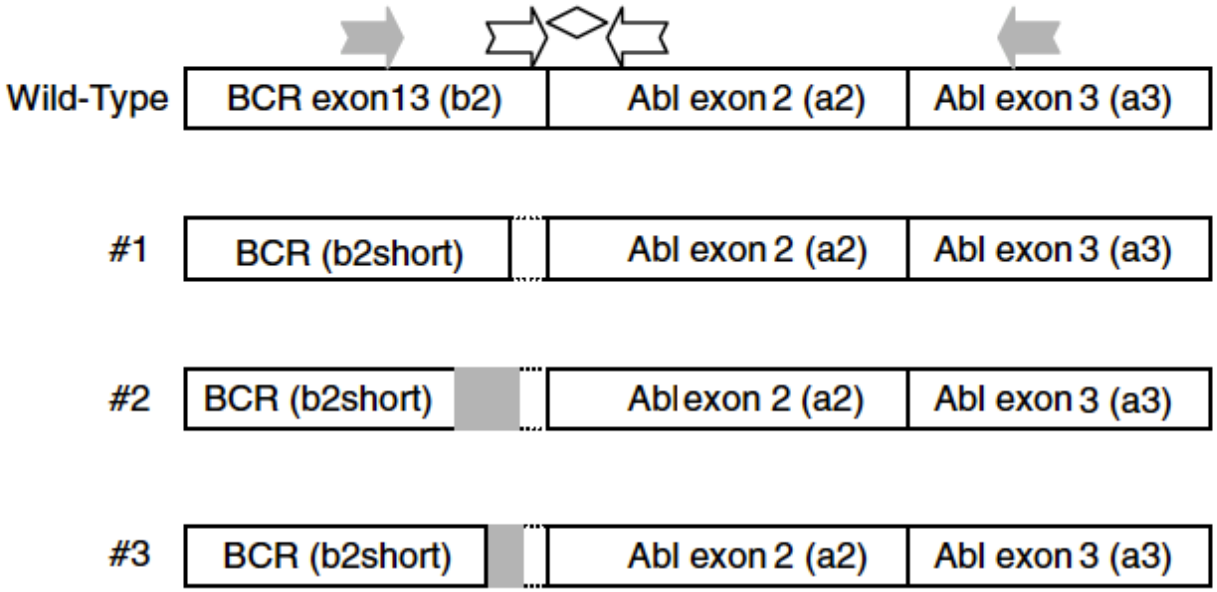


Sensibilité diagnostique (faux négatifs)

e14/e13-a2 = > 90%CML

Gabert et al., Leukemia (2003) 17, 2318–2357

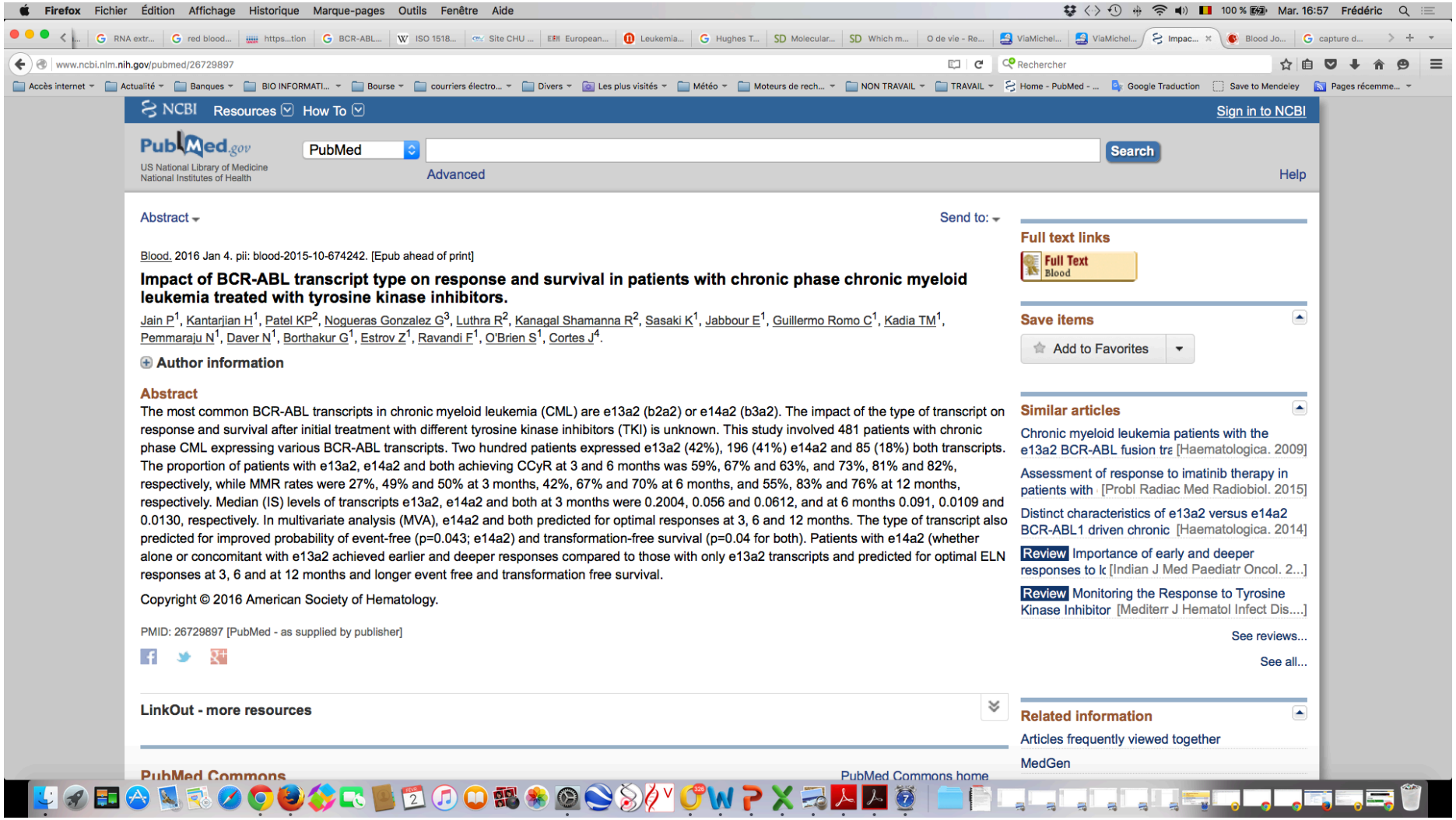
CERTAINS TRANSCRITS E13A2 VARIANTS NE PEUVENT ÊTRE SUIVI PAR RT-qPCR




```
    V   K   L   Q   T   V   H   S   I   P   L   N   I   N   K   E   E   A
WT GTG AAA CTC CAG ACT GTC CAC AGC ATT CCG CTG AAC ATC AAT AAG GAA GAA GCC
    V   K   L   Q   T   V   H   S   I                               K   A
#1 GTG AAA CTC CAG ACT GTC CAC AGC ATT A                           AA GCC
    V   N   Y   D   V   G   H   K   C   Q   Q                   E   E   A
#2 GTG AAC TAT GAT GTT GGG CAC AAG TGC CAG CAG                   GAA GAA GCC
    V   K   L   Q   T   P   L   S   L   Y                           K   A
#3 GTG AAA CTC CAG ACG CCT TTG TCG TTA TAC A                       AA GCC
```

Rational use of the EAC real-time quantitative PCR protocol in chronic myelogenous leukemia: report of three false-negative cases at diagnosis

Leukemia (2006) **20,** 886–888. doi:10.1038/sj.leu.2404174; published online 9 March 2006



Blood. 2016 Jan 4. pii: blood-2015-10-674242. [Epub ahead of print]

Impact of BCR-ABL transcript type on response and survival in patients with chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors.

Jain P[1], Kantarjian H[1], Patel KP[2], Nogueras Gonzalez G[3], Luthra R[2], Kanagal Shamanna R[2], Sasaki K[1], Jabbour E[1], Guillermo Romo C[1], Kadia TM[1], Pemmaraju N[1], Daver N[1], Borthakur G[1], Estrov Z[1], Ravandi F[1], O'Brien S[1], Cortes J[4].

Author information

Abstract

The most common BCR-ABL transcripts in chronic myeloid leukemia (CML) are e13a2 (b2a2) or e14a2 (b3a2). The impact of the type of transcript on response and survival after initial treatment with different tyrosine kinase inhibitors (TKI) is unknown. This study involved 481 patients with chronic phase CML expressing various BCR-ABL transcripts. Two hundred patients expressed e13a2 (42%), 196 (41%) e14a2 and 85 (18%) both transcripts. The proportion of patients with e13a2, e14a2 and both achieving CCyR at 3 and 6 months was 59%, 67% and 63%, and 73%, 81% and 82%, respectively, while MMR rates were 27%, 49% and 50% at 3 months, 42%, 67% and 70% at 6 months, and 55%, 83% and 76% at 12 months, respectively. Median (IS) levels of transcripts e13a2, e14a2 and both at 3 months were 0.2004, 0.056 and 0.0612, and at 6 months 0.091, 0.0109 and 0.0130, respectively. In multivariate analysis (MVA), e14a2 and both predicted for optimal responses at 3, 6 and 12 months. The type of transcript also predicted for improved probability of event-free (p=0.043; e14a2) and transformation-free survival (p=0.04 for both). Patients with e14a2 (whether alone or concomitant with e13a2 achieved earlier and deeper responses compared to those with only e13a2 transcripts and predicted for optimal ELN responses at 3, 6 and at 12 months and longer event free and transformation free survival.

PMID: 26729897 [PubMed - as supplied by publisher]

LES DIVERS TYPES DE VARIANTS BCR-ABL1 POURRAIENT ÊTRE ASSOCIÉS A UNE RÉPONSE ET UNE SURVIE DIFFÉRENTES SOUS TKI

PCR quantitative *BCR-ABL1*:

des PCR quantitatives génomiques pourraient être utilisées ...à l'avenir

The Journal of Molecular Diagnostics, Vol. 17, No. 2, March 2015

the Journal of Molecular Diagnostics
jmd.amjpathol.org

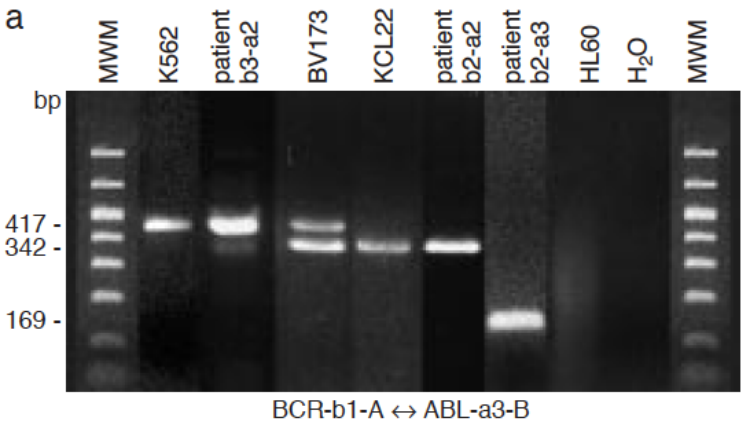
A DNA Real-Time Quantitative PCR Method Suitable for Routine Monitoring of Low Levels of Minimal Residual Disease in Chronic Myeloid Leukemia

Paul A. Bartley,* Susan Latham,* Bradley Budgen,* David M. Ross,*† Elizabeth Hughes,* Susan Branford,‡ Deborah White,§ Timothy P. Hughes,† and Alexander A. Morley*¶

Quelles RT-PCR *BCR-ABL1, pour quel usage ?*

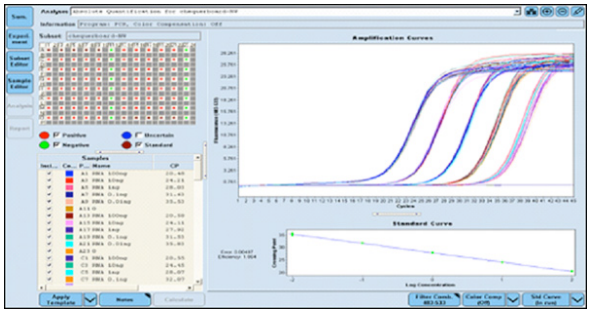
DIAGNOSTIC:

RT-PCR QUALITATIVE



SUIVI:

RT-PCR QUANTITATIVE



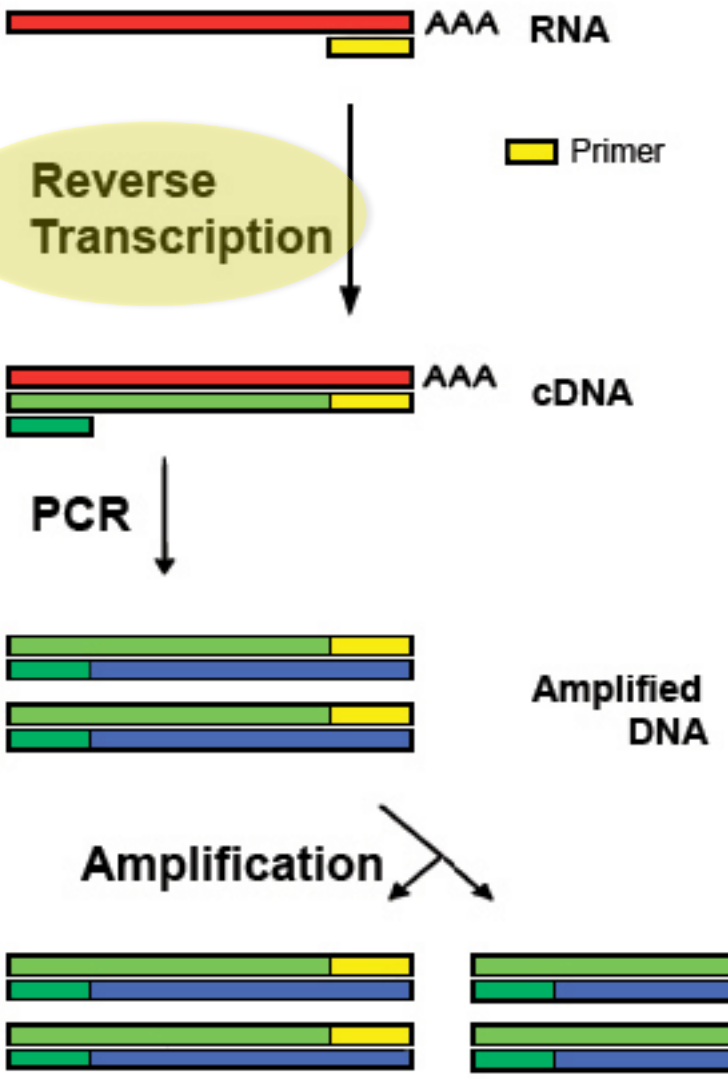
+ diagnostic si cinétique importante

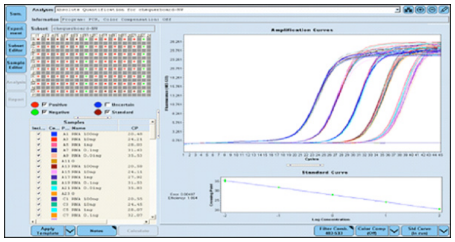
PCR quantitative *BCR-ABL1*: transcription reverse (ARN->ADN complémentaire), une nouvelle source de variabilité

Intégrité ARN

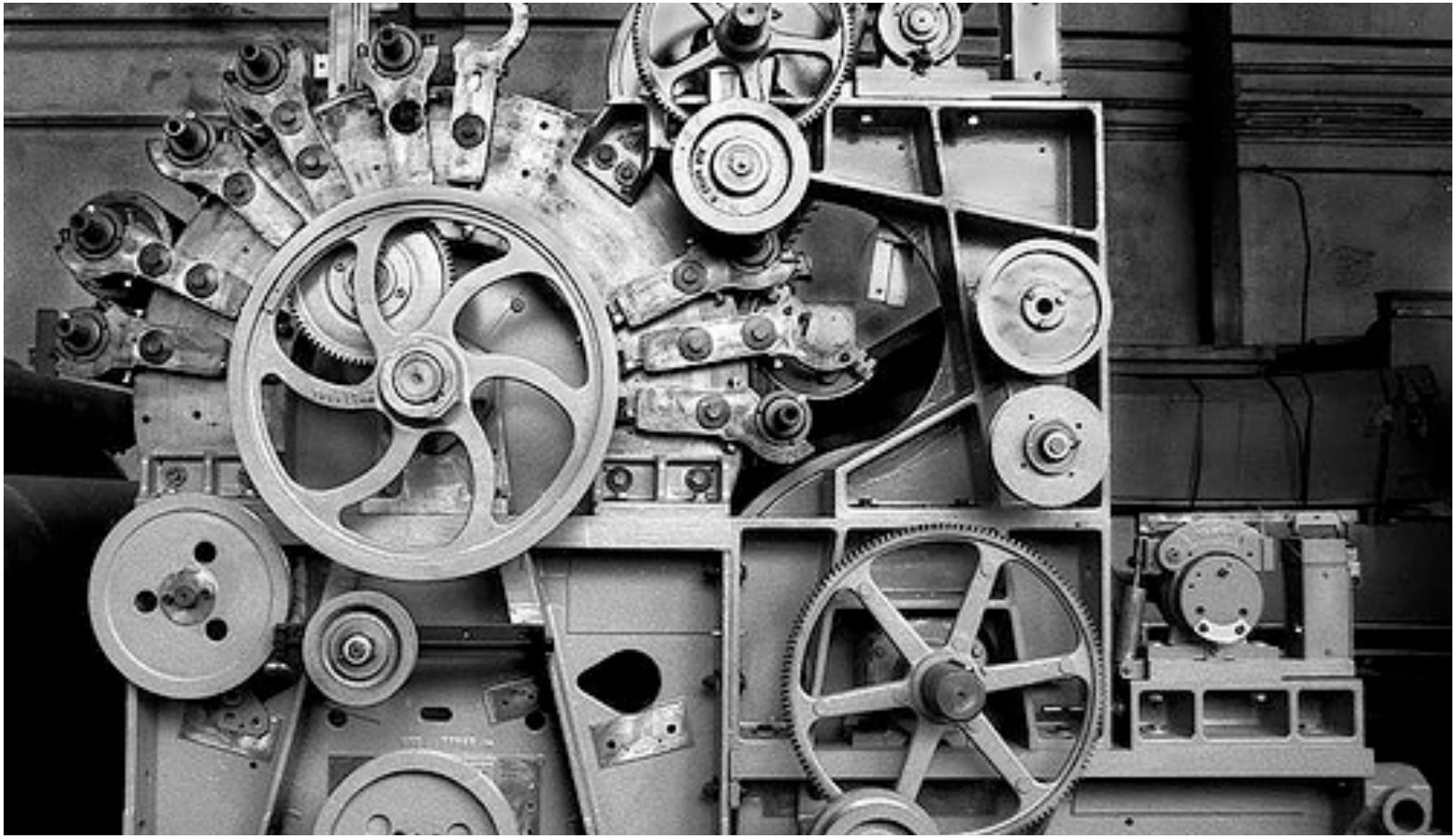
Efficacité Δ RT

Efficacité PCR
Spécificité
Sensibilité
Limite de détection

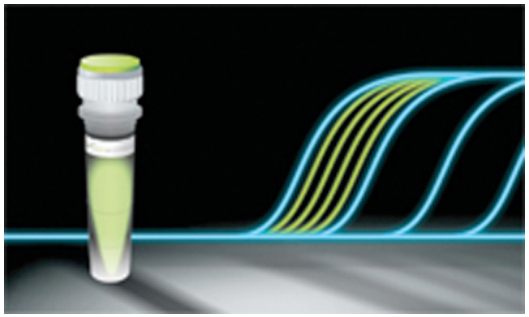




PCR QUANTITATIVE, COMMENT CELA FONCTIONNE (BRÈVE INTRODUCTION)?

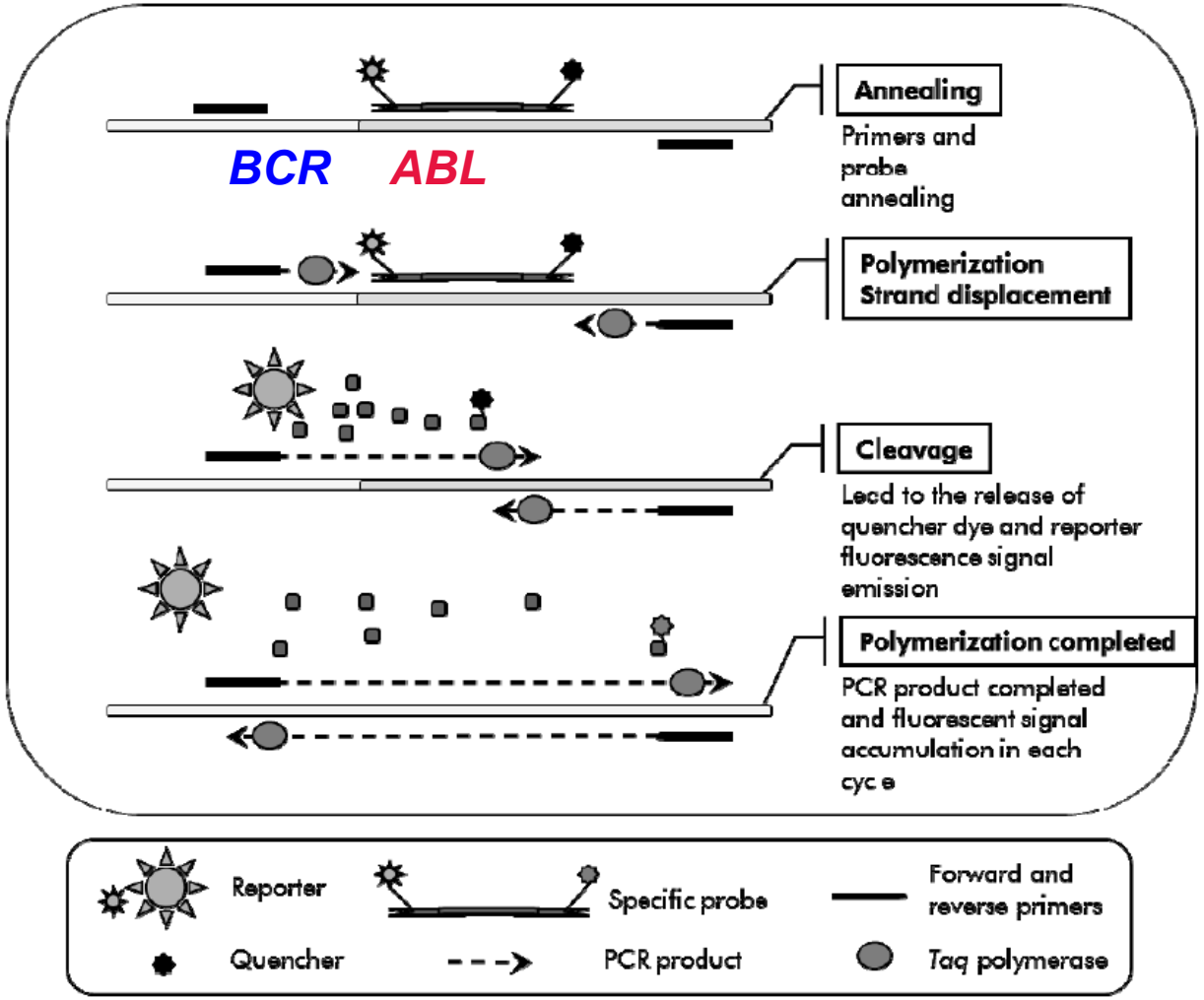


PRINCIPE DE BASE DE LA PCR QUANTITATIVE (qPCR)



Amplification spécifique

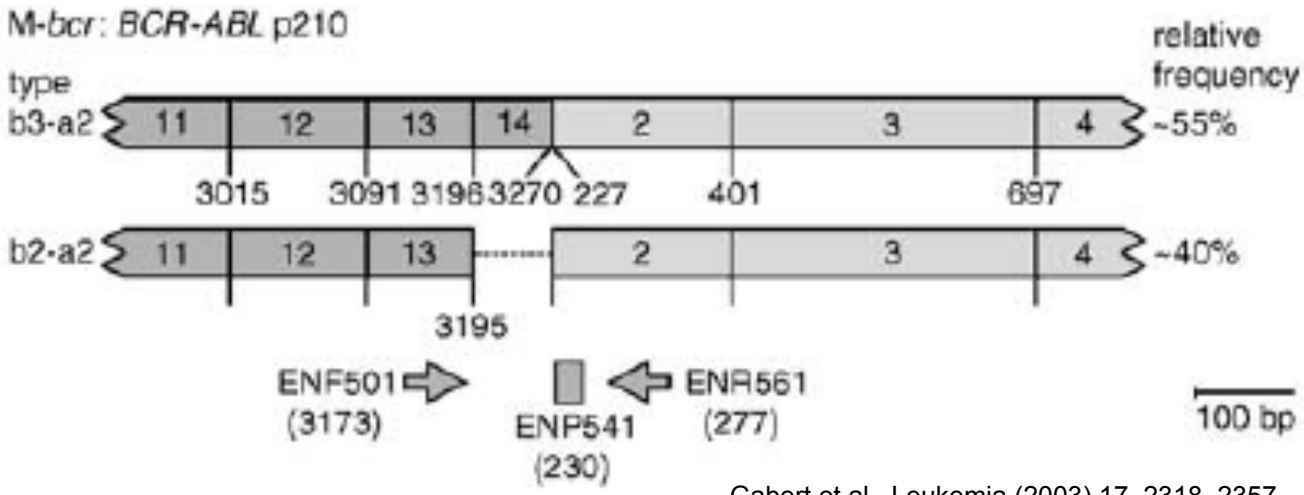
Génération signal fluorescent



Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

PCR quantitative *BCR-ABL1*:

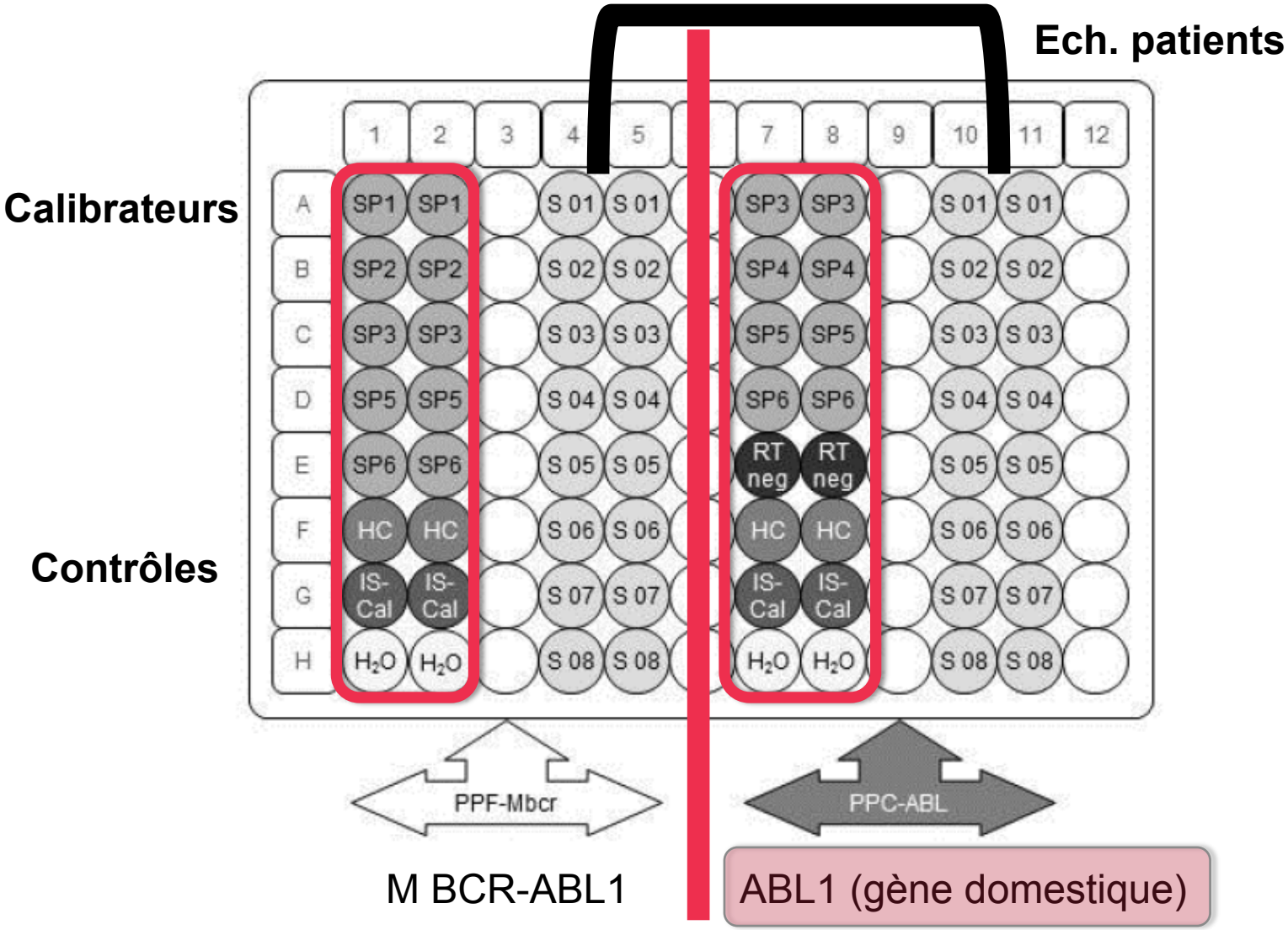
les sondes utilisées confèrent la spécificité, ne permettent de quantifier que e13/e14-a2/a3



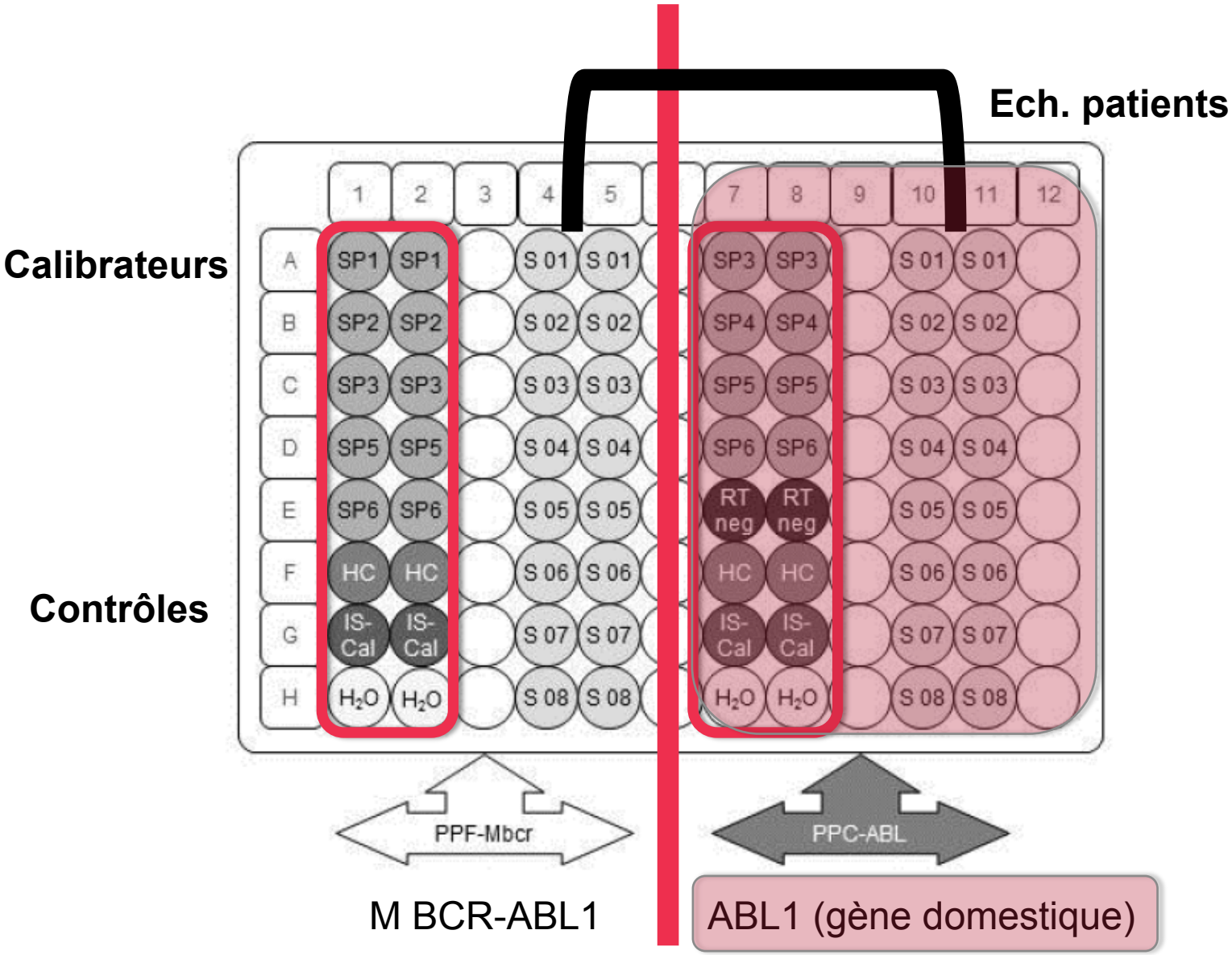
Gabert et al., Leukemia (2003) 17, 2318–2357

- **Spécificité: faible risque de faux positif**
- **Sensibilité: détection limitée aux formes e14/e13-a2a3**

COMPOSITION D'UNE PLAQUE(SÉRIE) DE RT q-PCR

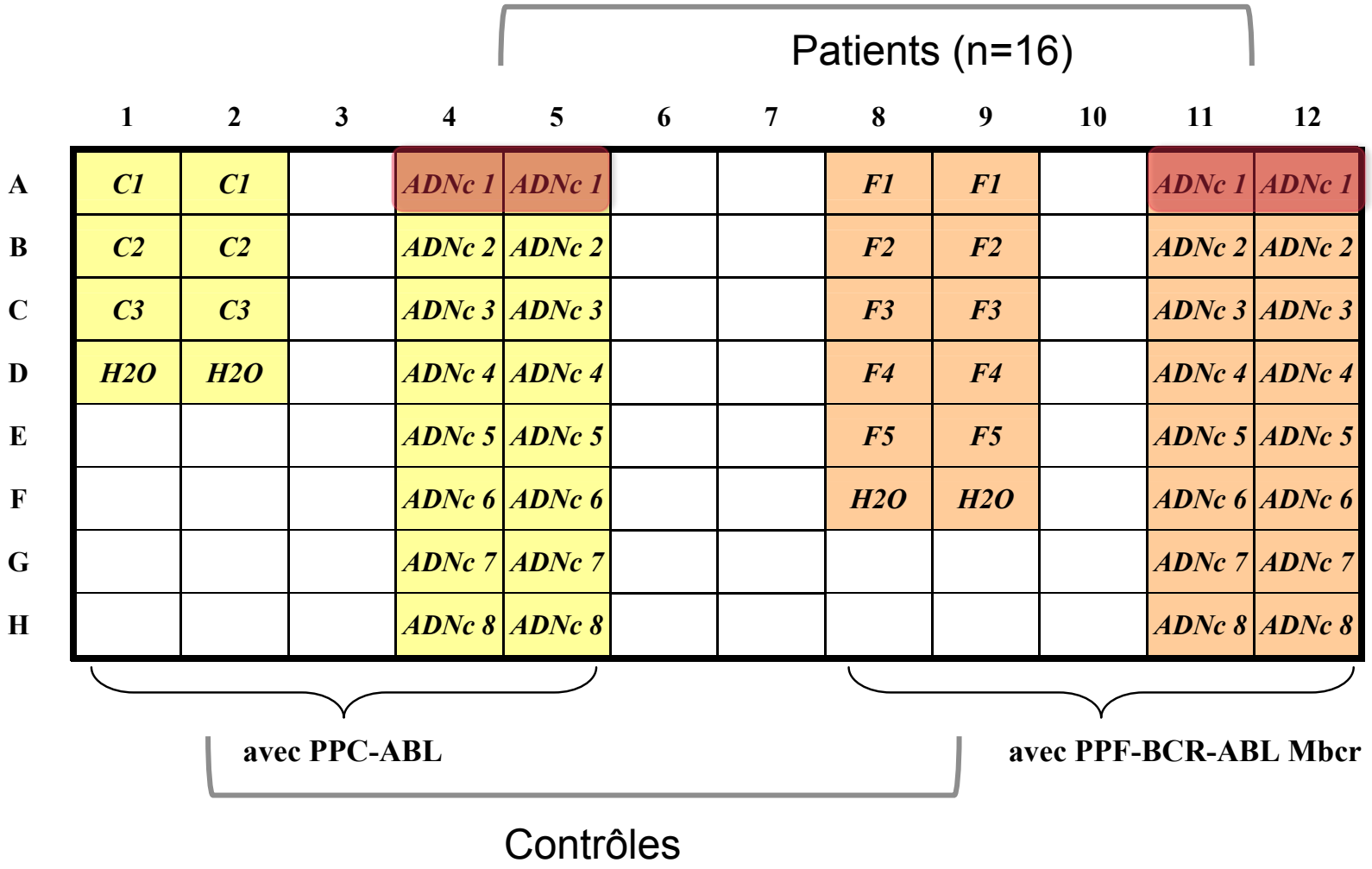


Constitution d'une plaque(série) de RT q-PCR



Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

REMPLISSAGE D'UNE PLAQUE(SÉRIE) DE qPCR



Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Composition d'une plaque 96 puits (série) de q-PCR *BCR-ABL1*

Plate Design

ABL1

	1	2	3	4	5	6	7	8	9	10	11	12
A	H2O ABL	H2O ABL	SP6 ABL	SP6 ABL	SP5 ABL	SP5 ABL	SP4 ABL	SP4 ABL	SP3 ABL	SP3 ABL	HC ABL	HC ABL
B	ISCAL ABL	ISCAL ABL	RTNEG ABL	RTNEG ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL	130916-0... ABL
C	130916-0... ABL	130916-0... ABL	130917-0... ABL	130917-0... ABL	130919-0... ABL	130919-0... ABL	130920-0... ABL	130920-0... ABL	130923-0... ABL	130923-0... ABL	130923-0... ABL	130923-0... ABL
D	130924-0... ABL	130924-0... ABL	130924-0... ABL	130924-0... ABL	130924-0... ABL	130924-0... ABL	130924-0... ABL	130924-0... ABL	130925-0... ABL	130925-0... ABL	130926-0... ABL	130926-0... ABL

BCR-ABL1

	1	2	3	4	5	6	7	8	9	10	11	12
E	H2O Mbcr	H2O Mbcr	SP6 Mbcr	SP6 Mbcr	SP5 Mbcr	SP5 Mbcr	SP3 Mbcr	SP3 Mbcr	SP2 Mbcr	SP2 Mbcr	SP1 Mbcr	SP1 Mbcr
F	HC Mbcr	HC Mbcr	ISCAL Mbcr	ISCAL Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr	130916-0... Mbcr
G	130916-0... Mbcr	130916-0... Mbcr	130917-0... Mbcr	130917-0... Mbcr	130919-0... Mbcr	130919-0... Mbcr	130920-0... Mbcr	130920-0... Mbcr	130923-0... Mbcr	130923-0... Mbcr	130923-0... Mbcr	130923-0... Mbcr
H	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130924-0... Mbcr	130925-0... Mbcr	130925-0... Mbcr	130926-0... Mbcr	130926-0... Mbcr

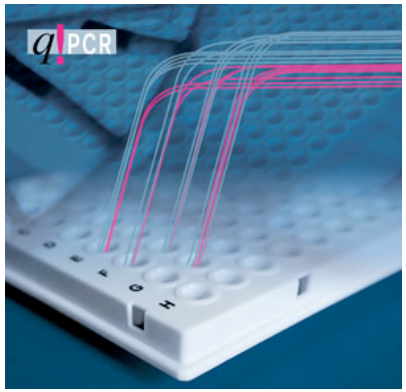
- SP : Single Plasmid
- IS-Cal : International Scale Calibrator
- HC : High Control
- RT neg : Reverse Transcription Non Template Control
- H2O : Water Control

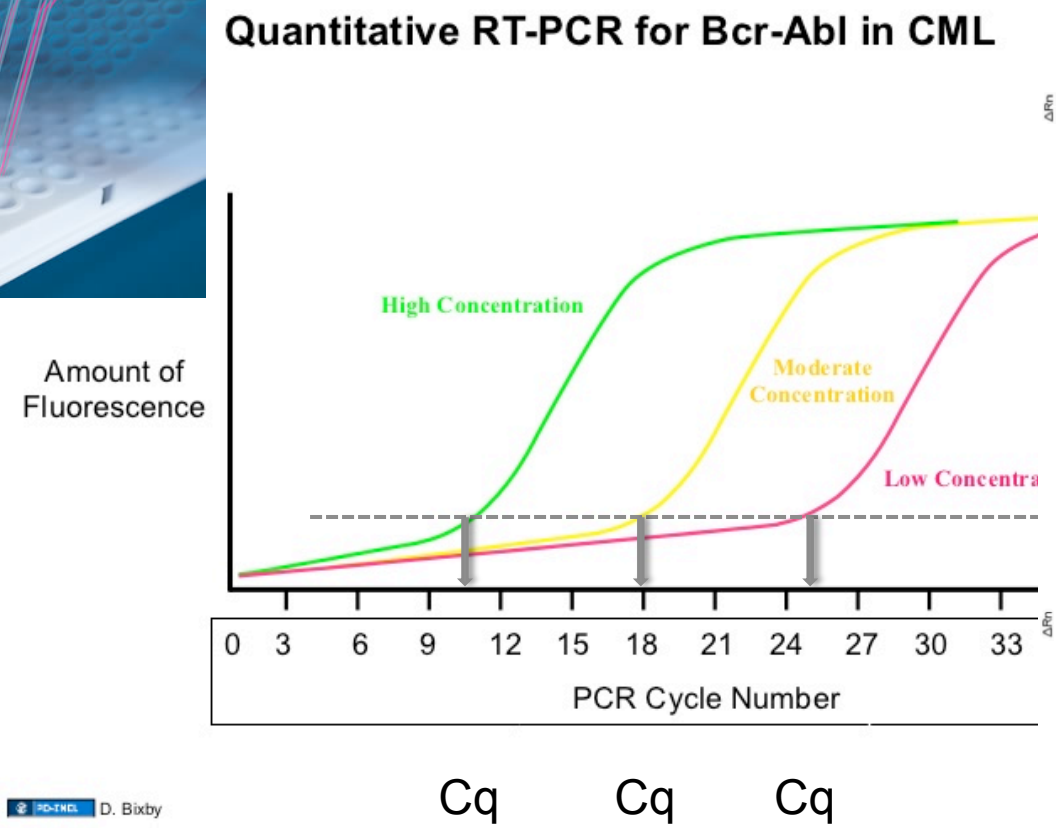
-> travail par série de 16 patients

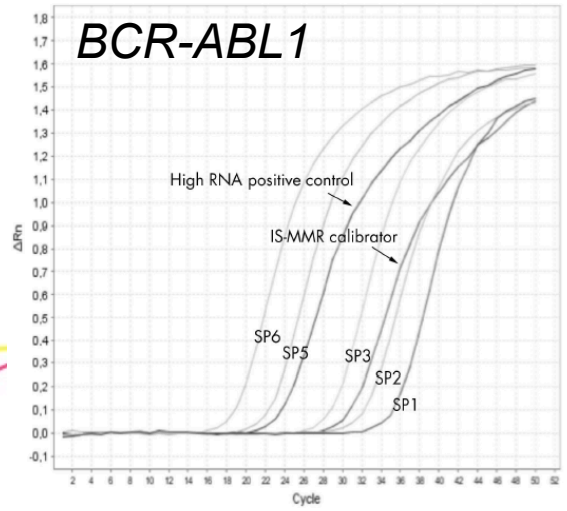
Temps pour remplir 1 plaque = +:- 2 semaines

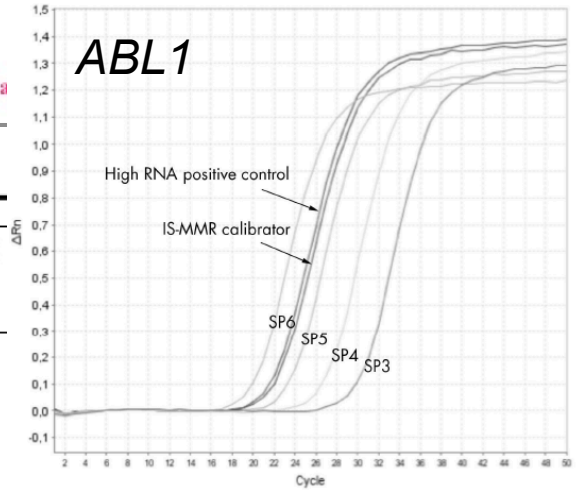
Principe d'analyse des données :
mesure du signal fluorescent pour les standards calibrés (*BCR-ABL1* et *ABL1*)

Detection of BCR-ABL Mbcr with standards SP1, SP2, SP3, SP5, and SP6. 101, 102, 103, 105, 106 copies/5 µl.





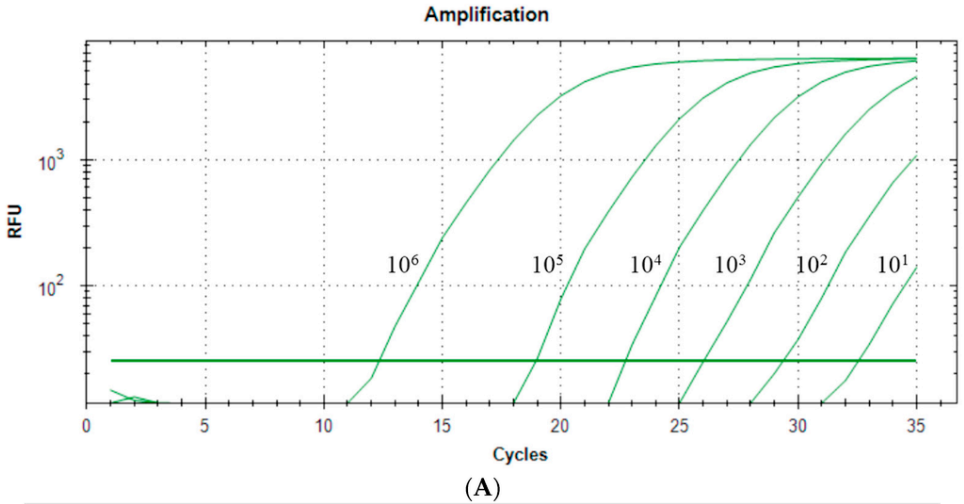




Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Principe d'analyse des données :
construction d'une droite de calibration pour *BCR-ABL1* et *ABL1*

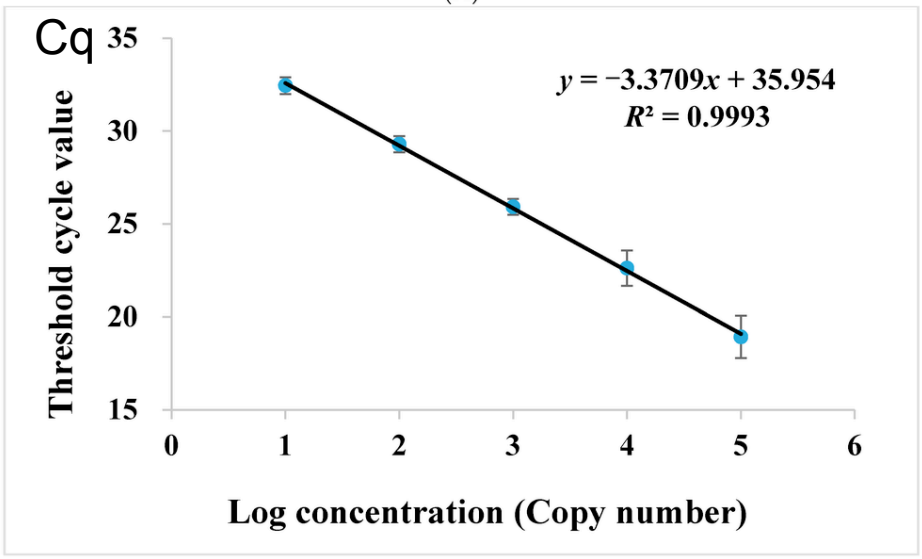
Mesuré



(A)

Cq

Extrapolé

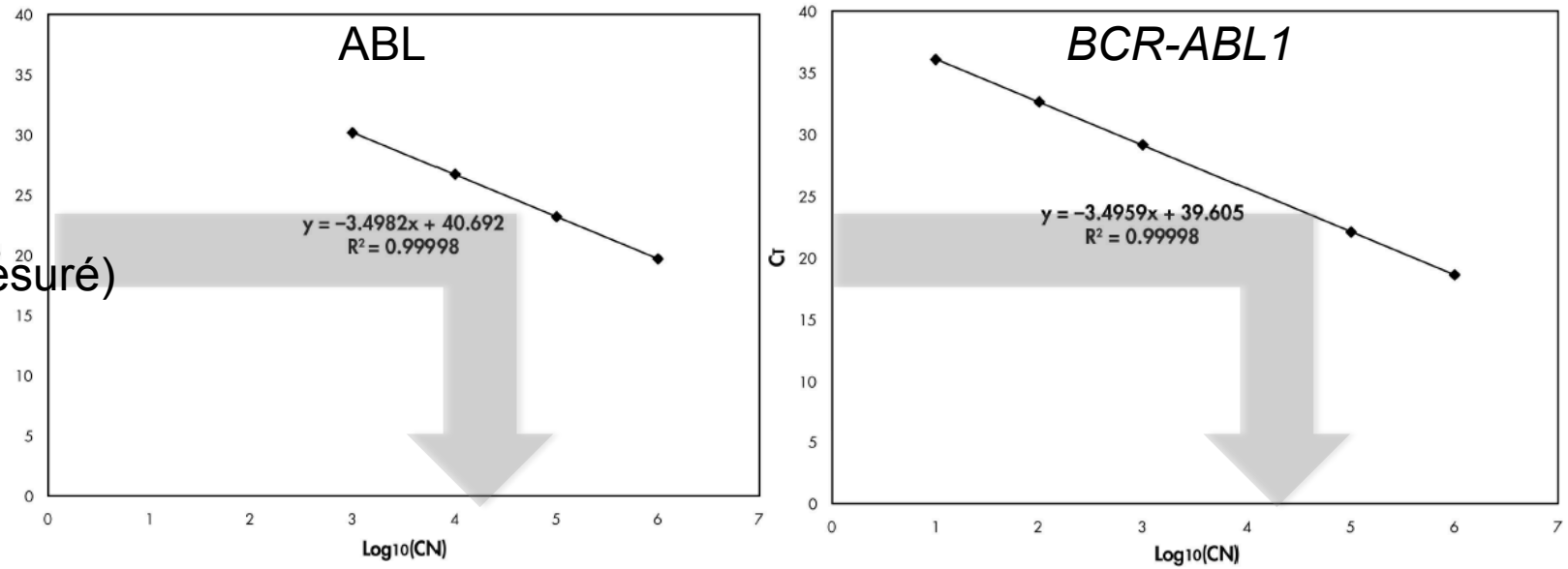


(B)

Principe du calcul du nombre de copies :
extrapolation construction de courbes de standards

Theoretical standard curve for ABL calculated from 4 standard dilutions

Theoretical standard curve for BCR-ABL calculated from 5 standard dilutions



Ct (Mesuré)

Nombre de copies (extrapolé)

Détermination nombre de copies d'*ABL1* et de *BCR-ABL1* pour chaque échantillon

Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

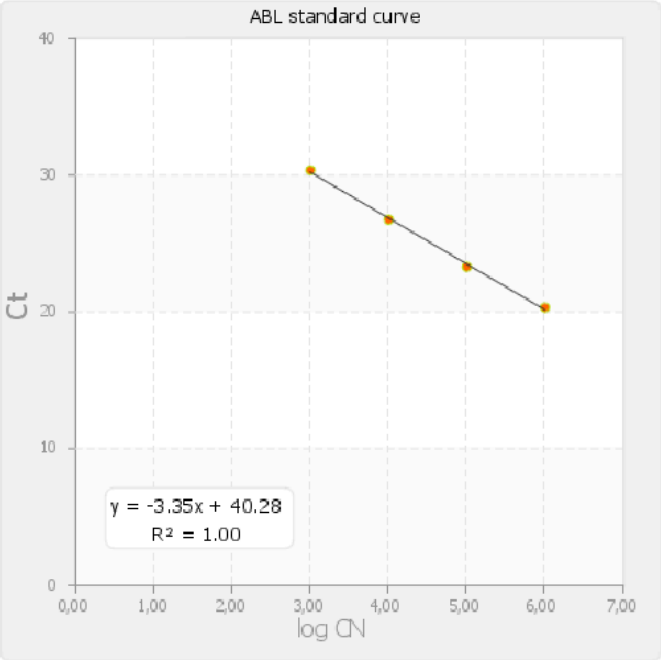
Principe d'analyse des données : la mesure du Ct permet le calcul du nombre de copies d'*ABL1* et *BCR-ABL1* pour chaque échantillon testé

	ABL Results				Mbcr Results								
Sample Name	Ct	Mean Ct	Log CN	CN	Ct	Mean Ct	Log CN	CN	NCN	IS-NCN	MMR** status	Warnings	Lab Conclusion on MMR status
130916-0135	24.78	24.80	4.61	4.105e+4	36.23	36.09	0.69	4.908	0.01196	0.01099	MMR		
	24.83				35.95								
130916-0168	23.44	23.48	5.01	1.019e+5	31.53	31.73	1.97	92.94	0.0912	0.08385	Inconclusive		
	23.52				31.94								
130916-0175	23.04	23.06	5.13	1.36e+5	Unde.			0	0	0	MMR	WRQ09: Warning: The NCN calculated for this sample is under the reference limit of detection. BCR-ABL Mbcr is detected but not quantified.	
	23.08				Unde.								
130917-0063	24.29	24.26	4.78	5.966e+4	29.20	29.07	2.75	560.2	0.9389	0.8632	No MMR		
	24.23				28.95								

ABL

Name	Ct	Mean Ct	Log CN	Warnings
SP3	30.47	30.37	3.00	
	30.26			
SP4	26.72	26.74	4.00	
	26.77			
SP5	23.28	23.30	5.00	
	23.32			
SP6	20.37	20.33	6.00	
	20.29			
H2O	41.19			WRO13: Warning: A Ct value was detected for one of the replicates of H2O (water) controls for the ABL detector. Some reagents or samples may have been contaminated. The results interpretation can be wrong.
	Undet.			
RT neg	Undet.			
	Undet.			

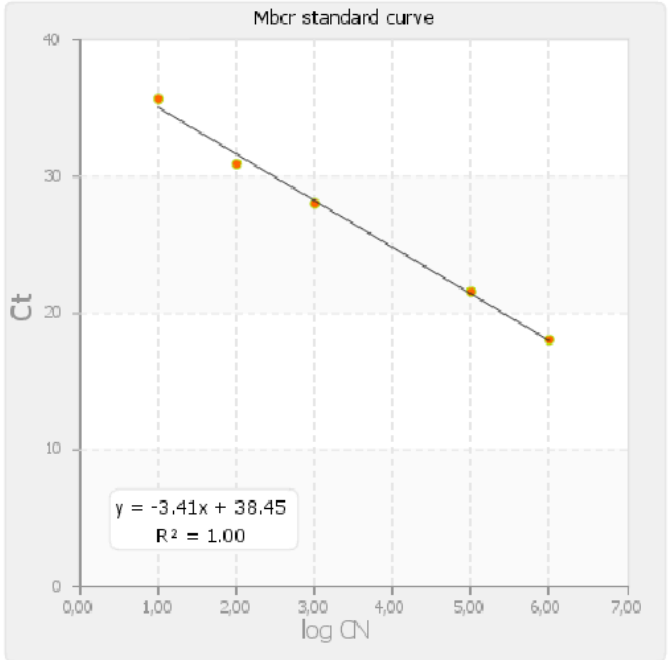
		Warnings
Slope	-3.355	
Intercept	40.28	
R^2	0.998	



BCR-ABL

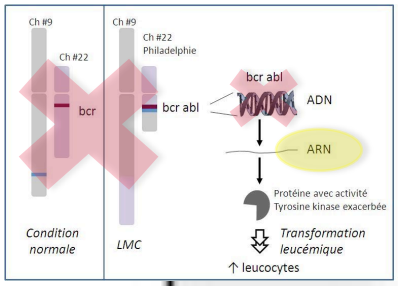
Name	Ct	Mean Ct	Log CN	Warnings
SP1	36.42	35.69	1.00	
	34.95			
SP2	30.65	30.92	2.00	
	31.19			
SP3	28.17	28.02	3.00	
	27.87			
SP5	21.52	21.57	5.00	
	21.62			
SP6	18.17	18.07	6.00	
	17.97			
H2O	33.26			WRO16: Warning: A Ct value was detected for one of the replicate of H2O (water) controls for the Mbcr detector. Some reagents or samples may have been contaminated. The results interpretation can be wrong.
	Undet.			

		Warnings
Slope	-3.41	
Intercept	38.45	
R^2	0.995	

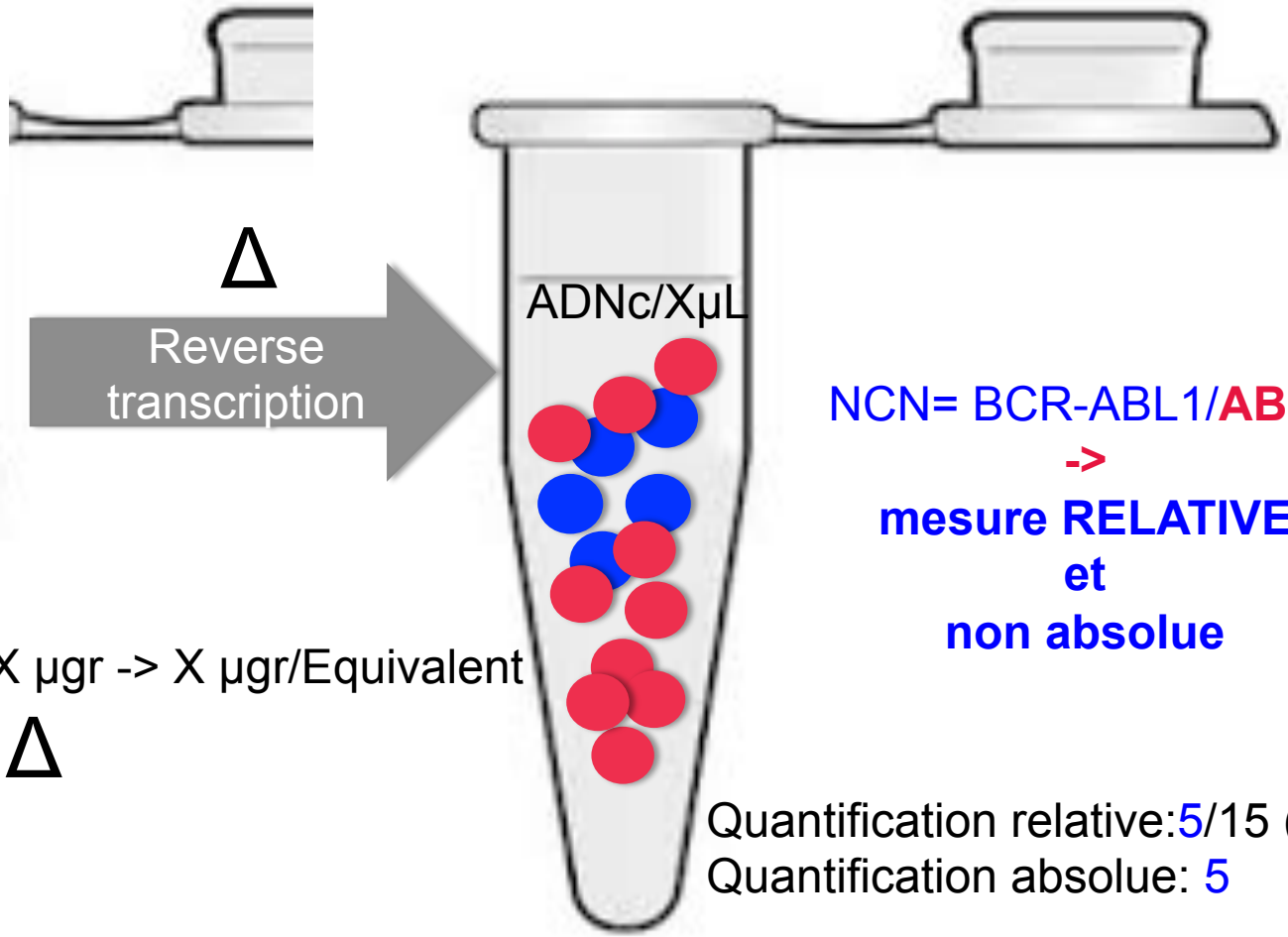


Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Gène domestique (housekeeping):
normalisation (dégradation/erreur de pipetage)



Ratio copies BCR-ABL1/ copies gène de référence (**ABL1**)



X µgr -> X µgr/Equivalent

Δ

NCN= BCR-ABL1/**ABL1**

->

mesure RELATIVE

et

non absolue

Quantification relative:5/15 (33%)

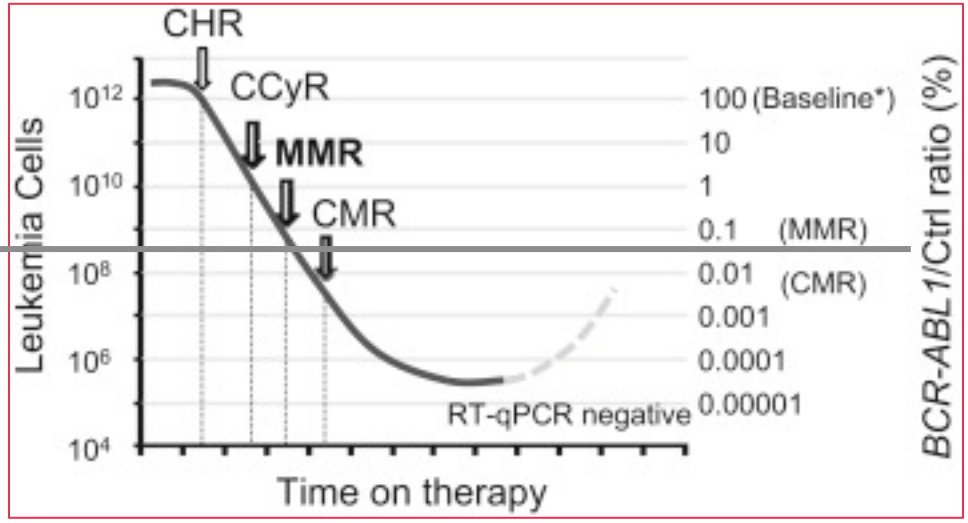
Quantification absolue: 5

- **Différents gènes domestiques peuvent être utilisés, dont Abelson (*ABL1*)**

LE NOMBRE DE COPIES D'*ABL1* CONDITIONNE LA LIMITE DE DÉTECTION DU TEST (SENSIBILITÉ ANALYTIQUE OU LOD), UN PARAMÈTRE ESSENTIEL POUR LA MESURE DES RÉPONSES MR3/4/4.5/5….

$$NCN = \frac{\text{BCR-ABL Mbcr}_{CN}}{\text{ABL}_{CN}} \times 100$$

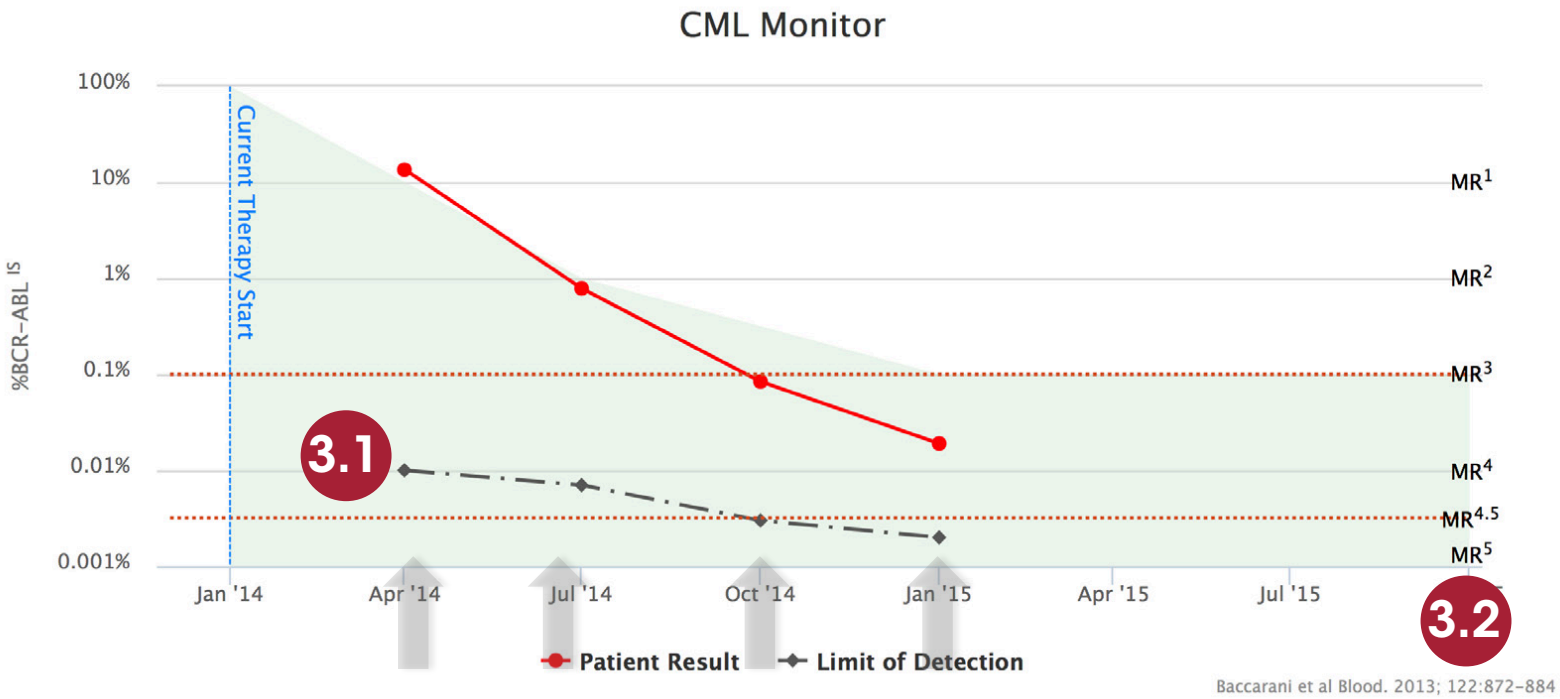
> 10.000 MMR



ChaoJie Zhen et al., Volume 15, Issue 5, September 2013, Pages 556–564

Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

LA LIMITE DE DÉTECTION DU TEST, varie au cours du temps !!!



Cross *et al* 2015[3] recommend the following control gene copy numbers are necessary to score molecular response:
MR4.0 = 10,000-31,999 copies of ABL1
MR4.5 = 32,000-99,999 copies of ABL1

MR5.0 ≥100,000 copies of ABL1

La limite de détection du test, est conditionnée par le nombre de copies du gène de référence

ETAPES DE VALIDATION ANALYTIQUE:
critères de validation de la série testée

Criteria	Acceptable values/results
Variations in C_T values between replicates	≤2 C_T if mean C_T value >36 ≤1.5 C_T if mean C_T value ≤36
Slope for standard curves	Between –3.0 and –3.9
R^2 for standard curves	At least >0.95 better if >0.98
SP1 standard dilution (BCR-ABL 10 copies plasmid)	Must be detected and included in the standard curve
Quality control on ABL_{CN} value for biological samples, high positive RNA control, and the IS-MMR-Calibrator	ABL_{CN} >10,000 copies of ABL to reach the optimal sensitivity
PCR (water) and reverse transcription (RT negative) controls	For each ABL_{CN} = 0 and $Mbcr_{CN}$ = 0
NCN obtained for IS-MMR Calibrator (NCN_{cal})	Must be within the interval 0.05–0.3
High positive RNA control	Must be detected
NCN obtained for the high positive RNA control converted to the international scale ($IS\text{-}NCN_{HC}$)	Status: No major molecular response

Sources de variabilité: analytique

Table 1 Details of the methods used in 57 participating laboratories

Protocol variable	*Number (%)*
RQ-PCR platform	
LightCycler (Roche Diagnostics)	16 (28)
TaqMan (Applied Biosystems, Foster City, CA, USA)	33 (58)
Rotor-Gene (Qiagen, Hilden, Germany)	4 (7)
Stratagene Q-PCR system (Stratagene, La Jolla, CA, USA)	4 (7)
Control genes	
Total *ABL* (Gabert *et al.*[30] (EAC protocol), $n=36$; Emig *et al.*[25], $n=5$; in-house methods, $n=5$)	46 (80)
GUSB (β-glucuronidase) (EAC)	4 (7)
B2M (β-2 microglobuline) (EAC)	2 (4)
G6PD (Glucose-6-phosphate dehydrogenase)	4 (7)
PBGD (Porphobilinogen deaminase)	1 (2)
Reference material	
Ipsogen plasmids[20]	28 (49)
pME-2 plasmids[23]	7 (12)
Local in-house plasmids	11 (19)
RNA calibrator (Roche t(9;22) kit, Roche Diagnostics)	4 (7)
Others[a]	7 (12)
cDNA synthesis	
Random hexamer priming	55 (96)
Random nonamer priming	2 (4)
Reverse transcriptase	
MMLV (Invitrogen, Karlsruhe, Germany)	38 (66)
SuperScript (Invitrogen)	11 (19)
AMV (Roche Diagnostics)	5 (9)
Transcriptor (Roche Diagnostics)	2 (4)
Multiscribe (Applied Biosystems)	1 (2)

Abbreviation: RQ-PCR, real-time quantitative PCR.
[a]pGD210, K562 calibrator, RNA dilutions, pGEM-Teasy-b3a2-plasmid.

MC Müller, Leukemia (2009) 23, 1957–1963

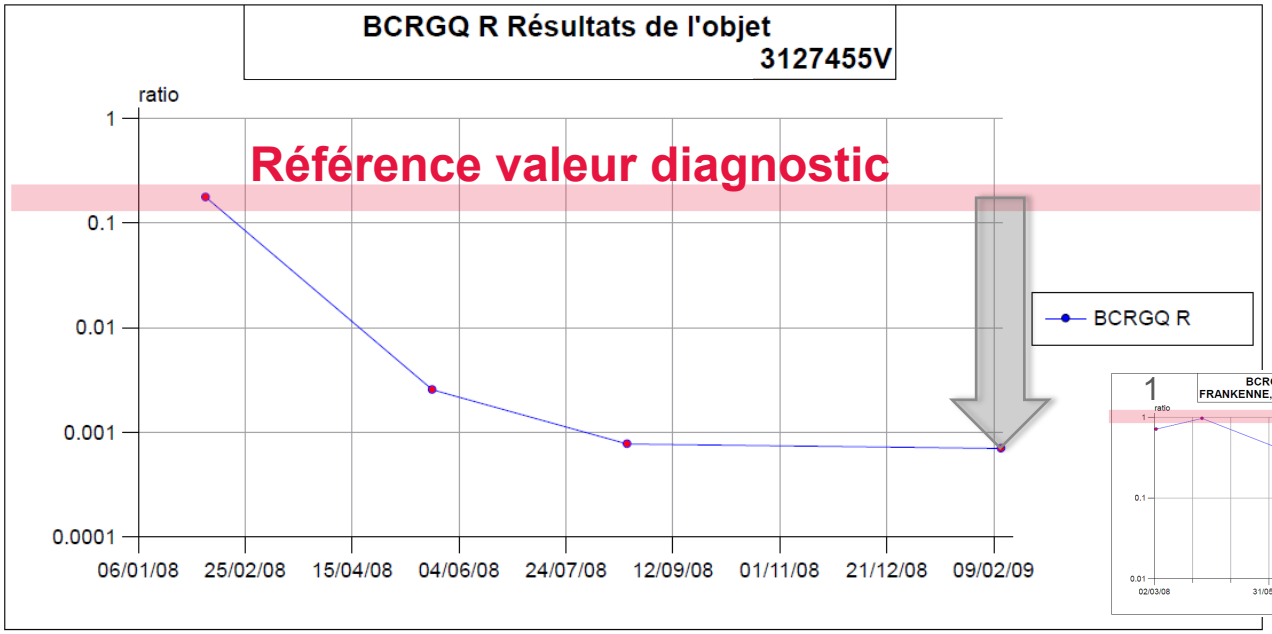
Sources de variabilité: rapportage

Table 1. Ways That Individual Laboratories May Report *BCR-ABL* Transcript Levels[a,22-24]

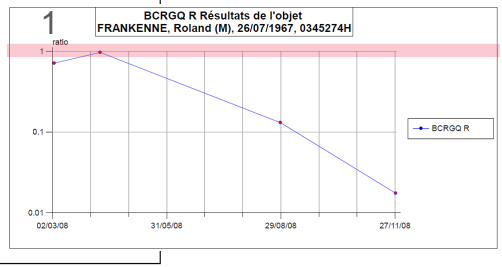
Value Reported	Method for Determination of Value	How Expressed
BCR-ABL copy number	Absolute copy number	Absolute copy number
BCR-ABL ratio	Ratio of *BCR-ABL* to a control gene	Percentage or fraction
Relative to single diagnostic sample	Highest *BCR-ABL* transcript level from a patient's single diagnostic sample converted to $\log_{10}$ scale and used as the baseline	Log reduction (ie, patient's current *BCR-ABL* transcript levels converted to $\log_{10}$ scale) from specified baseline
Relative to laboratory median of diagnostic samples	Median *BCR-ABL* transcript levels derived from RNA, cDNA, plasmid DNA, or cell line samples converted to $\log_{10}$ scale and used as baseline	Log reduction (ie, patient's current *BCR-ABL* transcript levels converted to $\log_{10}$ scale) from specified baseline
Relative to lab mean of diagnostic samples	Mean *BCR-ABL* transcript levels derived from RNA, cDNA, plasmid DNA, or cell line samples converted to $\log_{10}$ scale and used as baseline	Log-reduction (ie, patient's current *BCR-ABL* transcript levels converted to $\log_{10}$ scale) from specified baseline
Relative to previous patient sample	*BCR-ABL* transcript levels from patient's last test result or baseline test result converted to $\log_{10}$ scale and used as the baseline	Log-reduction (ie, patient's current *BCR-ABL* transcript levels converted to $\log_{10}$ scale) from specified baseline
Relative to diluted and undiluted K562 cells	*BCR-ABL* transcript levels derived from standard curve based on serial samples of diluted and undiluted K562 cells converted to $\log_{10}$ scale and used as the baseline	Log-reduction (ie, patient's current *BCR-ABL* transcript levels converted to $\log_{10}$ scale) from specified baseline Percentage, per IS
Per IS	Anchored to a lab-specific pretreatment standardized value baseline *BCR-ABL* ratio (100% IS) and a 3-log reduction from the same (0.1% IS); mathematical conversion to IS by multiplication of the *BCR-ABL*/control ratio by a laboratory-specific conversion factor	

Conséquences de la variabilité:
résultats non convertis sur l'échelle internationale (« International scale », I.S) non comparables

Avant standardisation

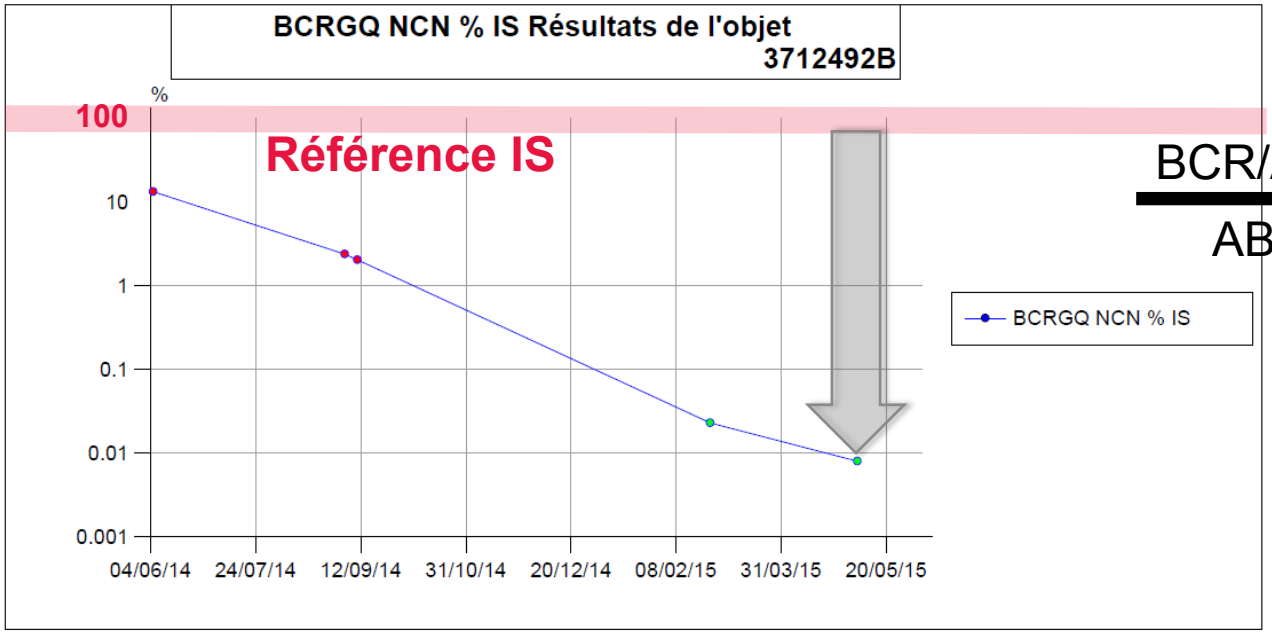


$$\frac{\text{BCR/ABL1}}{\text{ABL1}}$$



Après standardisation

(Novembre 2013)



$$\frac{\text{BCR/ABL1}}{\text{ABL1}} \times 100 \times \text{FC}$$

LE RECOURS A DES KITS COMMERCIAUX CERTIFIÉS INCLUANT CES CALIBRATEURS PERMET CETTE STANDARDISATION

Table 2. Selected List of Commercially Available RT-PCR Kits and Reagents for Processing Samples to Assay *BCR-ABL* Levels[a]

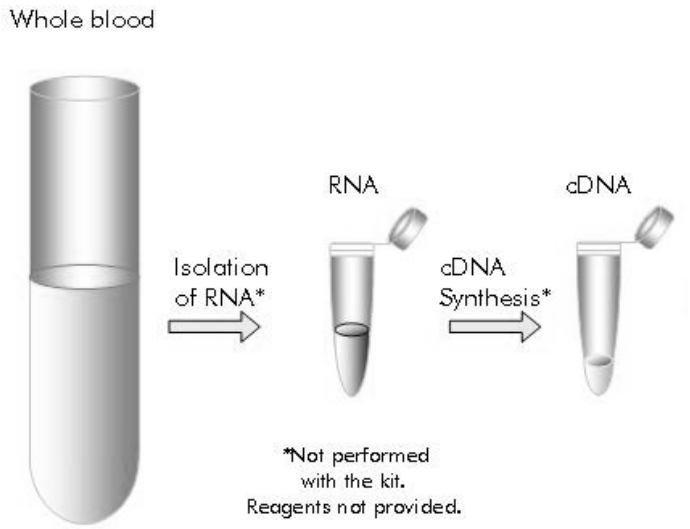
Manufacturer and Location	Product Name	Selected Product Claims/Components	Principal Reagents/Components
Ipsogen SA, Marseilles, France	*BCR-ABL* Mbcr IS-MMR Kit	• qRT-PCR to detect and quantify specific *BCR-ABL* fusion gene transcripts relative to *ABL* control gene expression in sample RNA • *BCR-ABL* IS: NCN results converted to the IS • Good sensitivity	• IS-MMR Calibrator to convert NCN results to the IS and report MMR • A high-positive control is provided to check for quality process of the experiment • Single plasmid for *BCR-ABL* and *ABL*, limiting variability
MolecularMD, Portland, OR	One-Step qRT-PCR *BCR-ABL* Kit	• One-step protocol: reverse transcription and quantitative PCR reactions are performed in the same well, saving time and money • Exceptional sensitivity down to 3 copies of *BCR-ABL* • Integrated conversion factor, enabling results on the IS	• Convenient RNA controls emulating high-level (10%) and low-level (0.1%) residual disease levels
Life Technologies Corporation, Carlsbad, CA	Asuragen *BCR/ABL*1 Quant Test	• An LOD with >50% positivity was obtained at a 0.001% ratio • Sensitive: precise quantification at low *BCR/ABL*1:*ABL*1 ratios aids in measuring MMR, minimal residual disease, and estimating risk of relapse • IS harmonization: has performance characteristics required for reporting quantitative *BCR/ABL*1 results on the IS	• Standardization through ARQtechnology: armored RNA calibrators and extractable control help ensure consistent and reliable results; consists of blend of precisely quantified *BCR/ABL*1, *ABL*1, and *BCR/ABL*1 Quant Norm ARQs
Cepheid, Sunnyvale, CA	GeneXpert System *BCR/ABL* Assay (for research use only)	• Fully automated RT-PCR system that combines integrated sample preparation with amplification and detection • *BCR-ABL* assay: closed system, nested RT-PCR assay for rapid, standardized research test reporting in approximately 2 hours	• Microfluidic single-use cartridge includes processing chamber with reagents, filters, and capture technologies necessary to extract, purify, amplify, and detect target nucleic acids

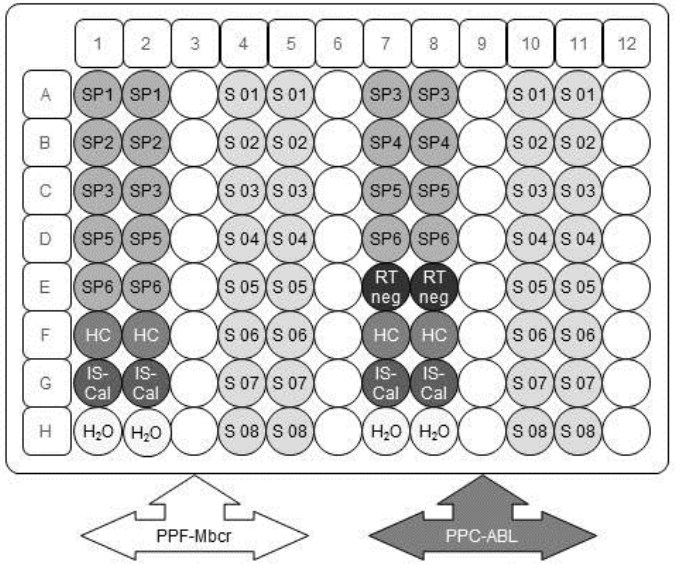
PCR quantitative *BCR-ABL1*:

Solution commerciale utilisée au CHU Lg

IPSOGEN® BCR-ABL1 M BCR IS-MMR

QIAGEN® Sample and Assay Technologies





RNA isolation, cDNA synthesis, and qPCR

Trousse commerciale certifiée CEE IVD

https://www.qiagen.com/us/resources/resourcedetail?id=11ca6274-2ed2-4751-a4d6-a1eb98c8204a&lang=en

Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

D'AUTRES SOLUTIONS COMMERCIALES SONT DISPONIBLES…

Rapports de PCR quantitative *BCR-ABL1 I.S* : buts ?

ELN European LeukemiaNet

UPDATE 2013

European LeukemiaNet Recommendations for the Management of Chronic Myeloid Leukemia (CML)

Response definitions for any TKI first line, and 2nd line in case of intolerance, all patients (CP, AP, and BC)

Time	Optimal response	Warning	Failure
Baseline		High risk Major route CCA/Ph+	
3 mos.	BCR-ABL^{IS} ≤10%* Ph+ ≤35% (PCyR)	BCR-ABL^{IS} >10%* Ph+ 36-95%	No CHR* Ph+ >95%
6 mos.	BCR-ABL^{IS} <1%* Ph+ 0% (CCyR)	BCR-ABL^{IS} 1-10%* Ph+ 1-35%	BCR-ABL^{IS} >10%* Ph+ >35%
12 mos.	BCR-ABL^{IS} ≤0.1%* (MMR)	BCR-ABL^{IS} 0.1-1%*	BCR-ABL^{IS} >1%* Ph+ >0%
Then, and at any time	MMR or better	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Loss of MMR, confirmed** Mutations CCA/Ph+

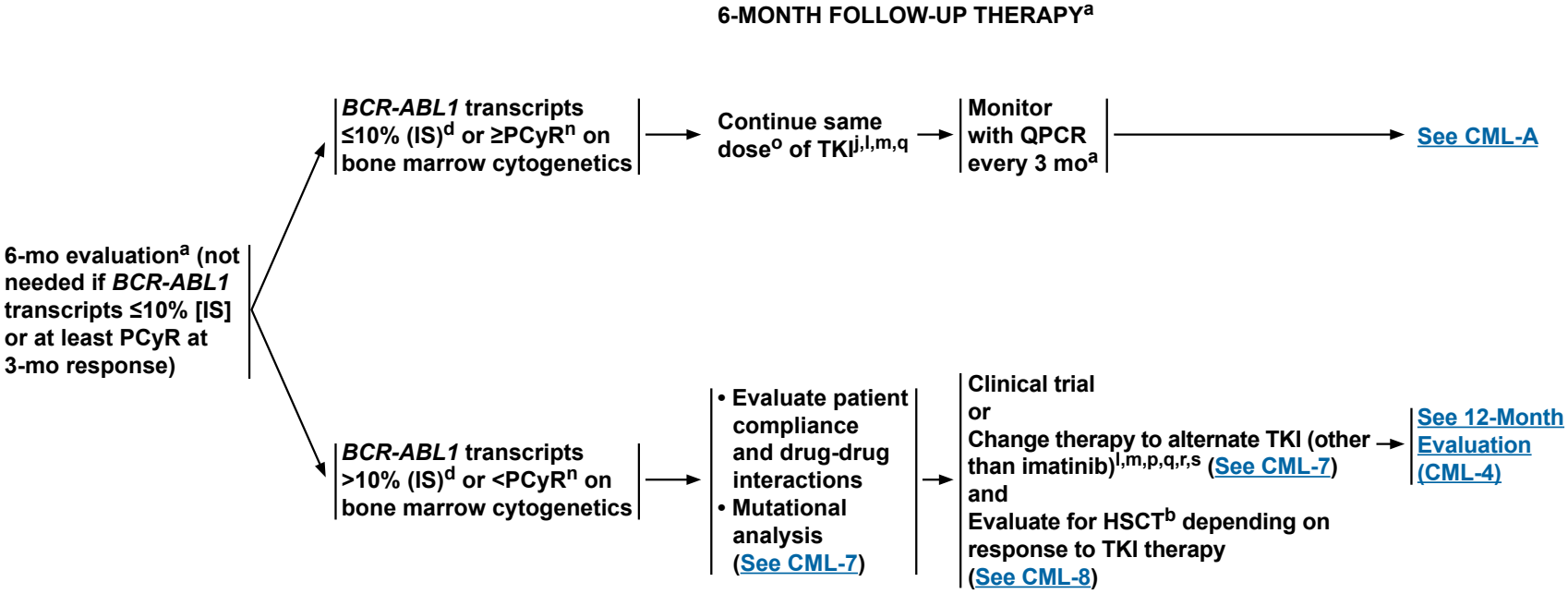
*and/or **in 2 consecutive tests, of which one ≥1% IS: BCR-ABL on International Scale

NCCN GUIDELINES 2014

National Comprehensive Cancer Network®

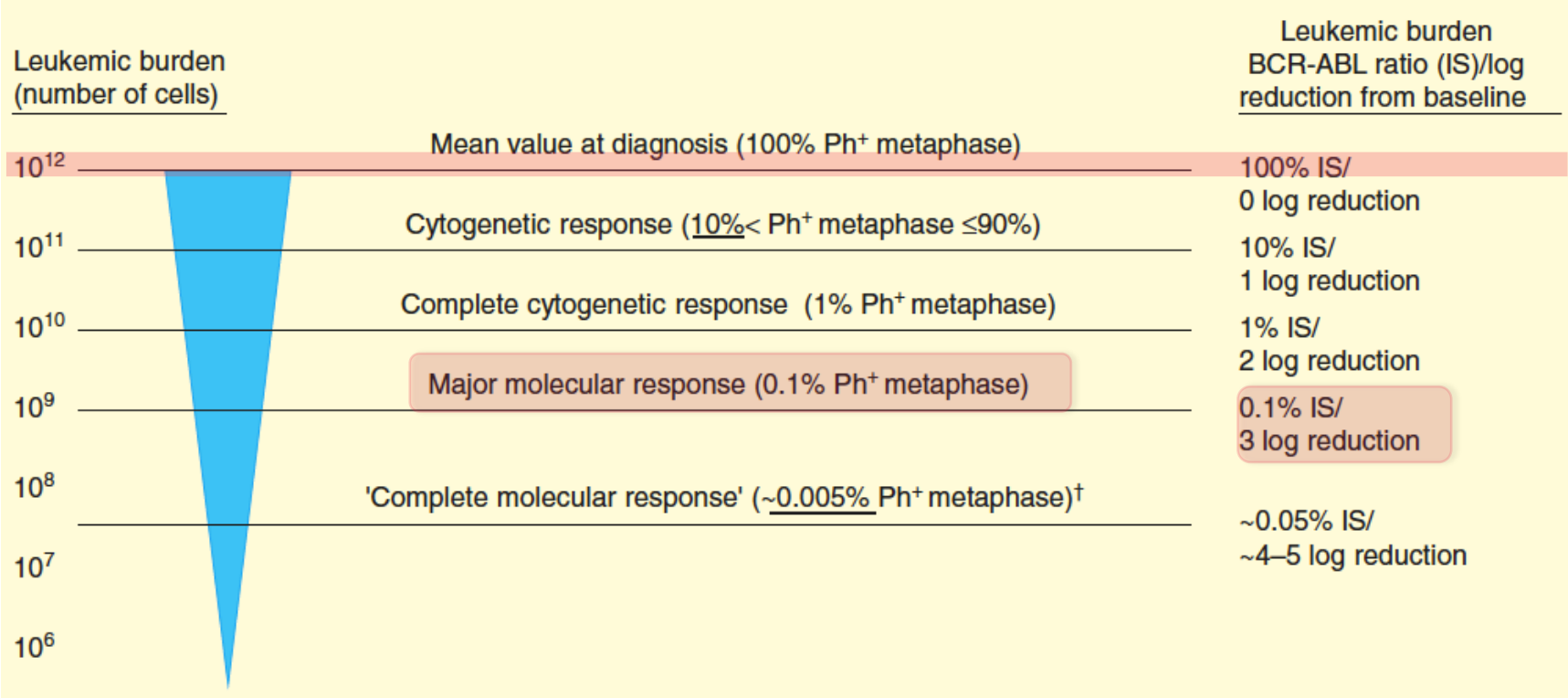
NCCN Guidelines Version 3.2014
Chronic Myelogenous Leukemia

NCCN Guidelines Index
CML Table of Contents
Discussion



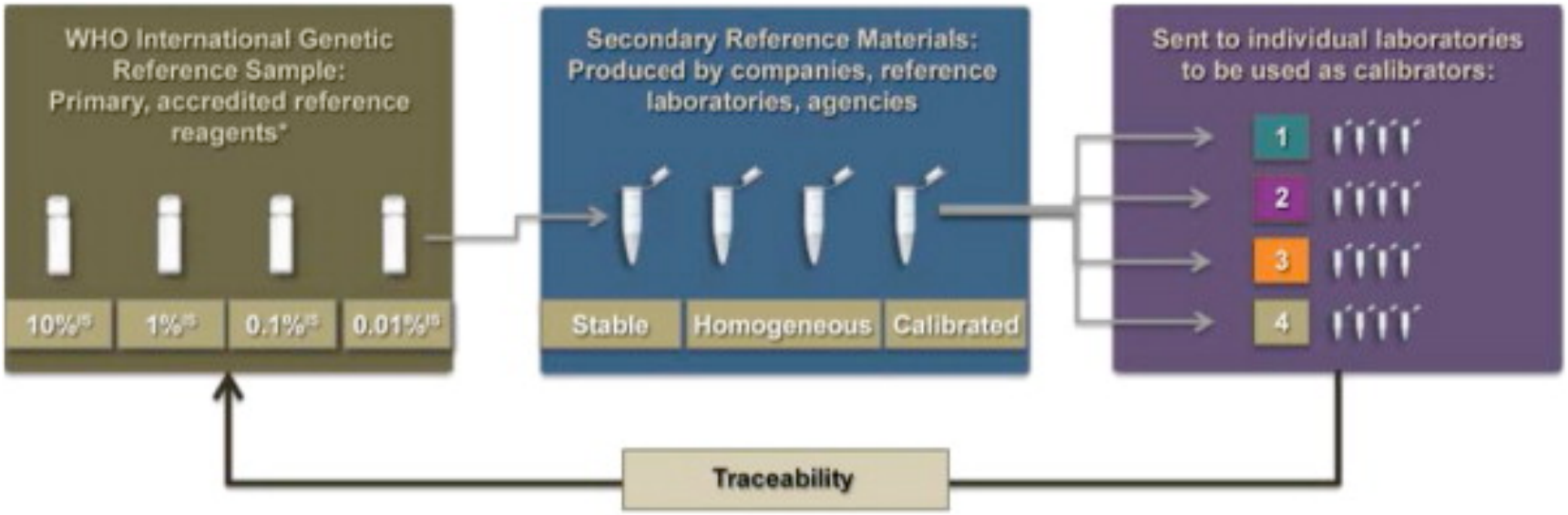
http://www.nccn.org/professionals/physician_gls/f_guidelines.asp

LEUKEMIC BURDEN IN CML PATIENTS AT DIAGNOSIS AND TKI TREATMENT RESPONSE LANDMARKS DURING MONITORING USING CYTOGENETICS AND BCR–ABL RNA RQ-PCR STANDARDIZED TO THE INTERNATIONAL SCALE

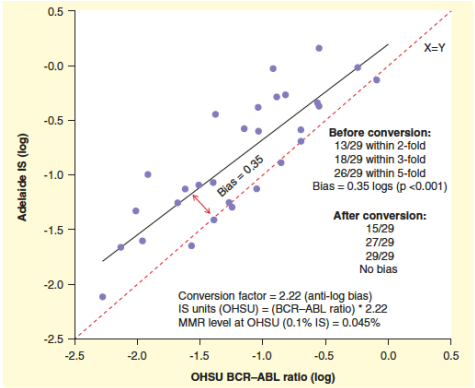


Martin Luu and Richard D, Expert Rev. Mol. Diagn. 13(7), 749–762 (2013)

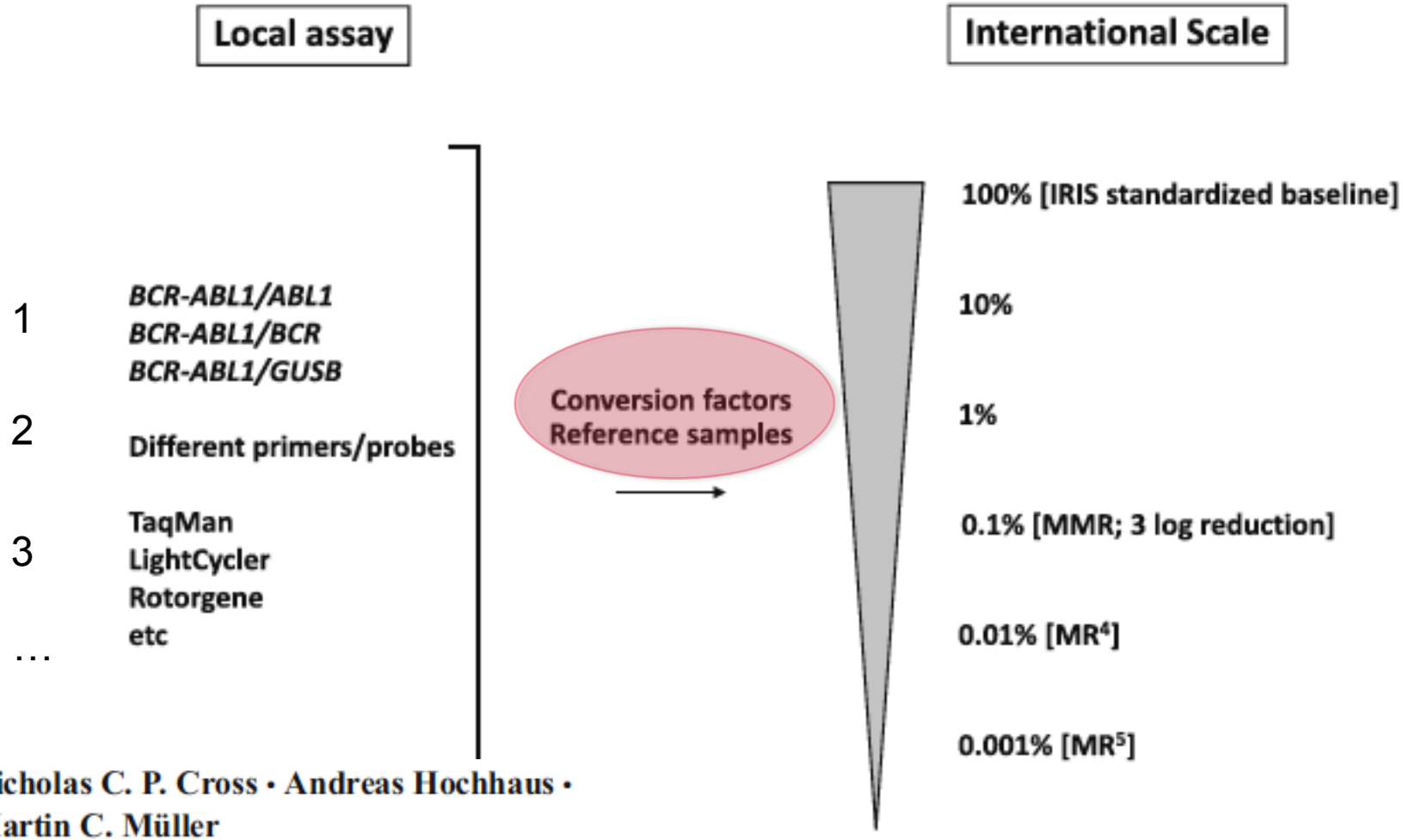
Comment réduire la variabilité: la standardisation *via* l'utilisation de matériaux de références calibrés et certifiés (par l'OMS)



*Approximately 3500 vials of each primary reference reagent were produced.



Sources multiples de variabilité requièrent une conversion: les matériaux de références calibrés permettent de générer un facteur de conversion et de convertir les résultats locaux sur une échelle internationale standardisée



Nicholas C. P. Cross · Andreas Hochhaus · Martin C. Müller

Ann Hematol (2015) 94(Suppl 2):S219–S225
DOI 10.1007/s00277-015-2315-1

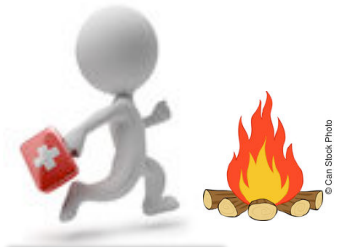
Une échelle internationale standardisée: Pour quoi faire ?

Descendre de 3 étages…vers la sortie !

© Can Stock Photo - csp13258469

© Can Stock Photo - csp2132313

Une échelle internationale standardisée: Pour quoi faire ?



Descendre de 3 étages…vers la sortie !

3

2

1

0

-1

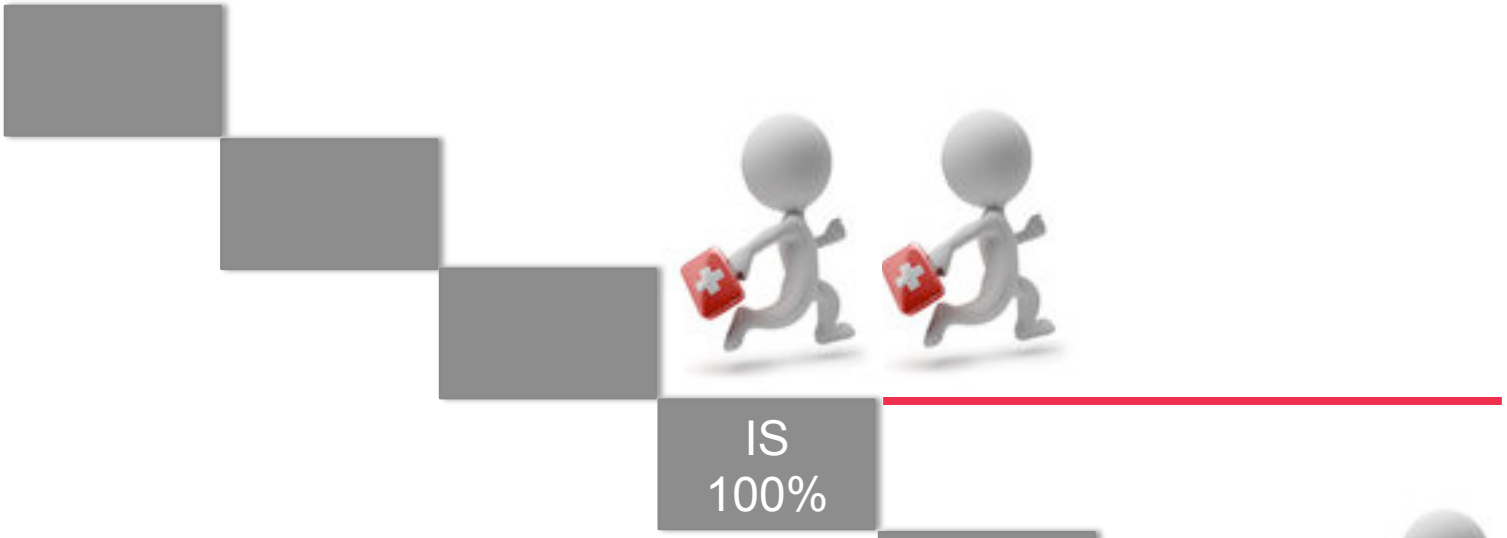
-2

-3

3 Etages

3 Etages

Une échelle internationale standardisée: Pour quoi faire ?



Comment réduire la variabilité: la standardisation *via* l'utilisation de matériaux de références calibrés et certifiés (par l'OMS)

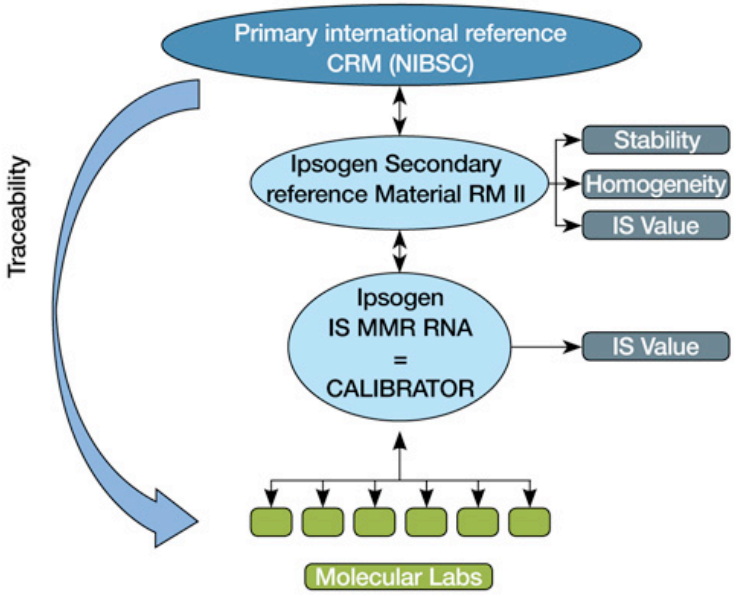
OPEN Leukemia (2015) 29, 369–376 ... www.nature.com/leu

ORIGINAL ARTICLE

A certified plasmid reference material for the standardisation of *BCR–ABL1* mRNA quantification by real-time quantitative PCR

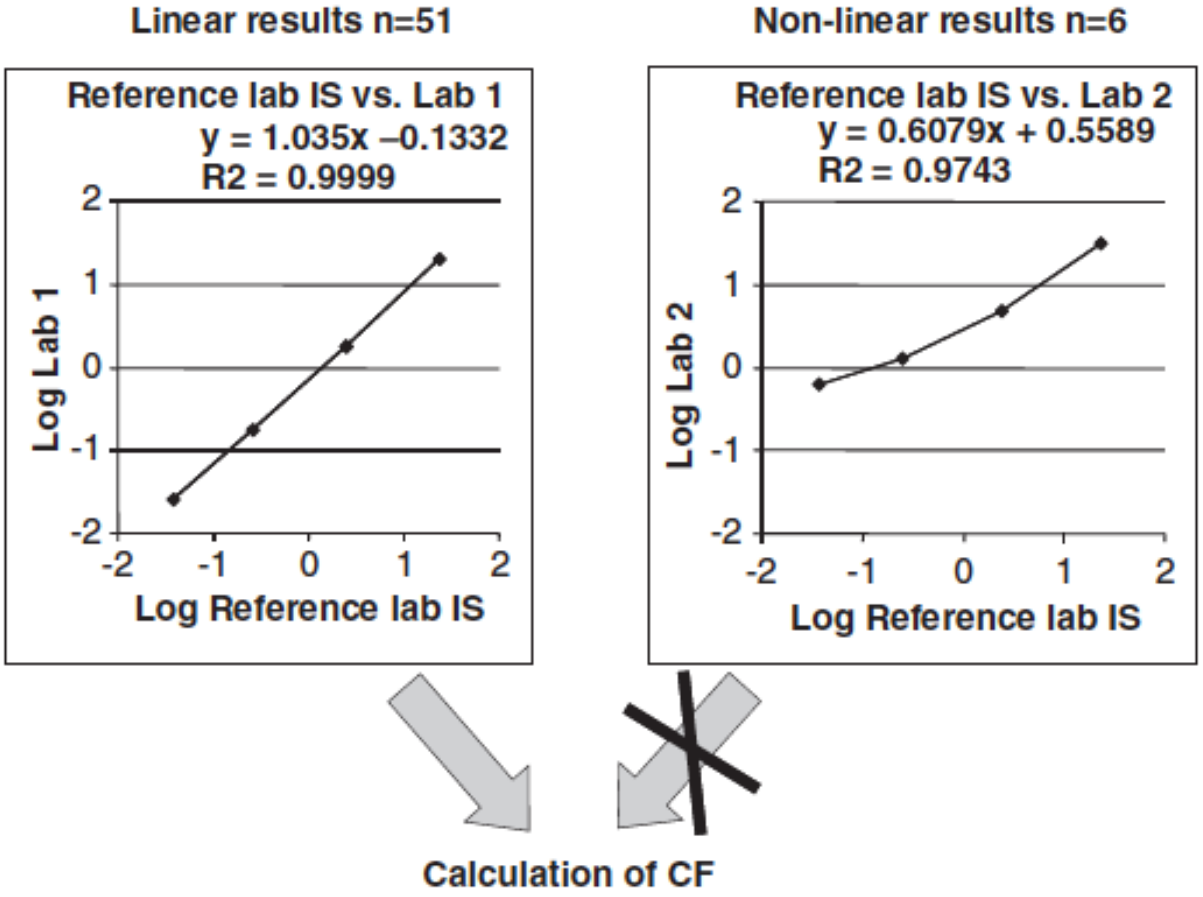
H White[1,2], L Deprez[3], P Corbisier[3], V Hall[1], F Lin[1,2], S Mazoua[3], S Trapmann[3], A Aggerholm[4], H Andrikovics[5], S Akiki[6], G Barbany[7], N Boeckx[8,9], A Bench[10], M Catherwood[11], J-M Cayuela[12], S Chudleigh[13], T Clench[14], D Colomer[15], F Daraio[16], S Dulucq[17], J Farrugia[18], L Fletcher[19], L Foroni[20], R Ganderton[21], G Gerrard[20], E Gineikienė[22], S Hayette[23], H El Housni[24], B Izzo[25], M Jansson[26], P Johnels[27], T Jurcek[28], V Kairisto[29], A Kizilors[30], D-W Kim[31], T Lange[32], T Lion[33], KM Polakova[34], G Martinelli[35], S McCarron[36], PA Merle[37], B Milner[38], G Mitterbauer-Hohendanner[39], M Nagar[40], G Nickless[41], J Nomdedéu[42], DA Nymoen[43], EO Leibundgut[44], U Ozbek[45], T Pajič[46], H Pfeifer[47], C Preudhomme[48], K Raudsepp[49], G Romeo[50], T Sacha[51], R Talmaci[52], T Touloumenidou[53], VHJ Van der Velden[54], P Waits[55], L Wang[56], E Wilkinson[57], G Wilson[58], D Wren[59], R Zadro[60], J Ziermann[61], K Zoi[62], MC Müller[63], A Hochhaus[61], H Schimmel[3], NCP Cross[1,2] and H Emons[3]

Fig. 1 IS-MMR calibrator traceability against WHO reference materials.



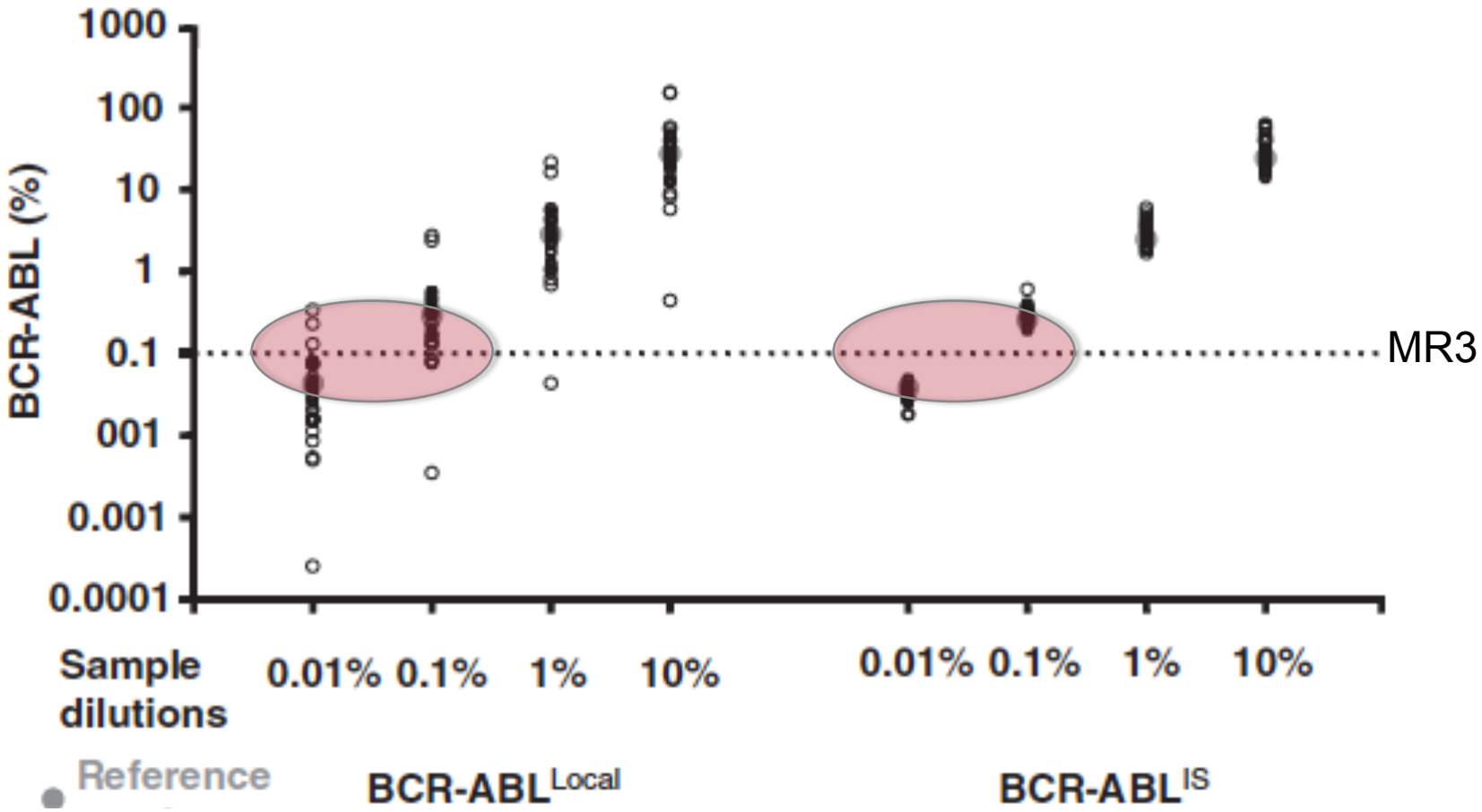
Impact de la conversion sur l'échelle internationale du NCN:

pondération valeur locale par facteur de conversion



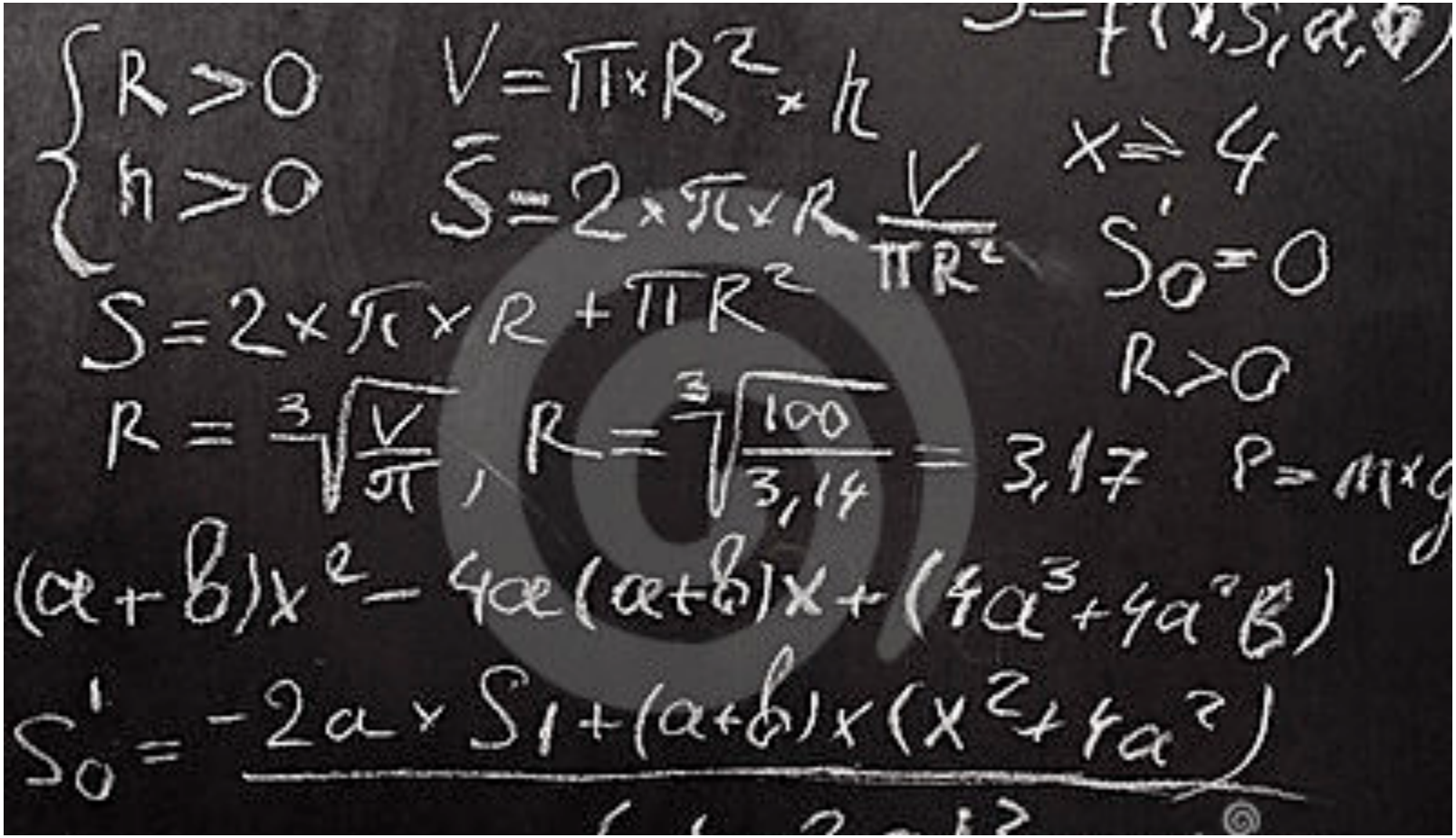
MC Müller, Leukemia (2009) 23, 1957–1963

Impact de la conversion sur l'échelle internationale du NCN:

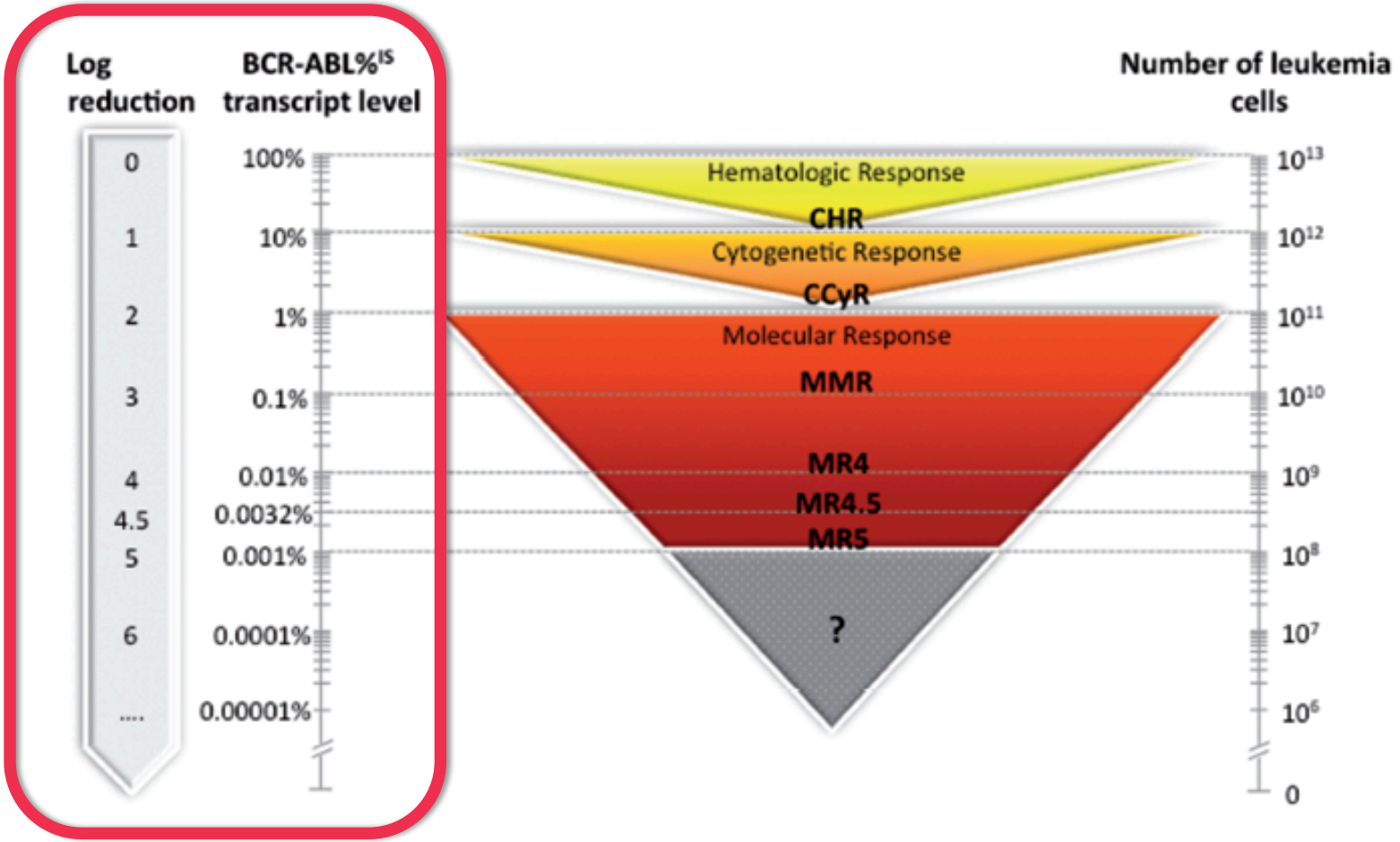


MC Müller, Leukemia (2009) 23, 1957–1963

2. COMMENT LE RATIO *BCR-ABL* I.S % EST-IL CALCULÉ ET POURQUOI LE NOMBRE DE COPIES DU GÈNE DE MÉNAGE EST-IL SI IMPORTANT ?



2. COMMENT LE RATIO *BCR-ABL* I.S % EST-IL CALCULÉ ET POURQUOI LE NOMBRE DE COPIES DU GÈNE DE MÉNAGE EST-IL SI IMPORTANT ?



NORMALISATION DU NOMBRE DE COPIES (NCN), pour chaque échantillon

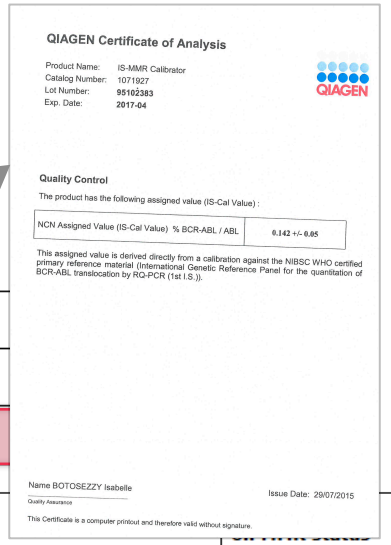
$$NCN = \frac{\text{BCR-ABL Mbcr}_{CN}}{{}^{*}\text{ABL}_{CN}} \times 100$$

* ou GUS, B2M…

Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Détermination du facteur de conversion (CF):

écart entre valeur du calibrateur certifié fournie par producteur et valeur calculée localement


QIAGEN Certificate of Analysis

Product Name: IS-MMR Calibrator
Catalog Number: 1071927
Lot Number: 95102383
Exp. Date: 2017-04

QIAGEN

Quality Control

The product has the following assigned value (IS-Cal Value) :

NCN Assigned Value (IS-Cal Value)	% BCR-ABL / ABL	0.142 +/- 0.05

This assigned value is derived directly from a calibration against the NIBSC WHO certified primary reference material (International Genetic Reference Panel for the quantitation of BCR-ABL translocation by RQ-PCR (1st I.S.)).

Name BOTOSEZZY Isabelle
Quality Assurance

Issue Date: 29/07/2015

This Certificate is a computer printout and therefore valid without signature.

	ABL Results				Mbcr Results							
Sample Name	Ct	Mean Ct	Log CN	CN	Ct	Mean Ct	Log CN	CN	NCN	IS-NCN	MMR** status	Warnings
HC	21.67 21.63	21.65	5.55	3.578e+5	23.37 22.81	23.09	4.50	3.189e+4	8.912	8.194	No MMR	
IS-Cal	21.61 21.35	21.48	5.60	4.021e+5	29.51 29.62	29.57	2.60	402.4	0.1001	IS given value* 0.092		

Sample Name	Ct	Mean Ct	Log CN	CN	Ct	Mean Ct	Log CN	CN	NCN	IS-NCN	MMR** status	Warnings	
130916-0048	24.44 23.92	24.18	4.80	6.303e+4	35.59 36.30	35.95	0.73	5.413	0.008588	0.007896	MMR		
130916-0133	25.45 25.39	25.42	4.43	2.691e+4	38.59 38.42	38.51	-0.02	0.9608	0.00357	0.003282	MMR	WRQ09: Warning: The NCN calculated for this sample is under the reference limit of detection. BCR-ABL Mbcr is detected but not quantified.	

Conversion sur l'échelle internationale du NCN:

pondération valeur locale du nombre de copies de chaque échantillon par facteur de conversion

Facteur de conversion

$$\text{IS-NCN}_{\text{sample}} = \frac{\text{NCN}_{\text{sample}} \times \text{IS-Cal value}}{\text{NCN}_{\text{cal}}}$$

Détermination d'un facteur de conversion (CF)

CF=valeur calibrateur IS / valeur mesurée du calibrateur

Adapted from Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Illustration calcul NCN I.S local :

CHU Lg

BMH.BCRGQ_NCN_IS.ANA.A07 | | Version : 3

MBCRABL Run : 160127 BCRG IS-MMR

Valeur IS-Cal: 0,142

Facteur conv: 0,953

A B A/B A/B*100 NCN*CF

Nom Ech.	Ct ABL	Ct moy	SD Ct	Qty	Qty moy	Somme Qtt	Ct BCRG	Ct moy	SD Ct	Qty	Qty moy	Ratio	NCN	LoB	LoD	IS-NCN	Rép molec	Statut
IS-MMR-Calib	21,15 20,84	21,04	0,1500	384350,01 445644,25	414997	829994	29,24 29,11	29,17	0,0940	589,74 647,27	618,50	0,00149	0,14904	D	Q	0,142		
CTRL pos. haut	20,30 20,94	20,62	0,4517	696140,72 445169,71	570155	1140310	23,16 22,97	23,06	0,1312	41737,37 47528,77	44633,07	0,07828	7,82823	D	Q	7,459		No MMR
160112-0011 160112-0011	23,09 23,26	23,15	0,1618	103436,15 88173,59	95805	191610	24,62 24,70	24,66	0,0557	14962,05 14159,74	14560,90	0,15198	15,19848	D	Q	14,481		No MMR
160112-0047 160112-0047	23,65 23,82	23,74	0,1218	67121,91 59518,83	63320	126641	33,86 33,97	33,92	0,0793	23,19 21,45	22,32	0,00035	0,03525	D	Q	0,034		MMR
160112-0056 160112-0056	23,55 23,98	23,77	0,2979	71743,77 53475,00	62609	125219	35,92	17,96		5,47	2,74	0,00004	0,00437	D	NQ	0,004		MMR
160112-0057 160112-0057	23,86 24,07	23,97	0,1435	57605,41 50268,64	54092	108184	35,14 35,50	35,37	0,3125	9,43 6,92	8,18	0,00015	0,01512	D	Q	0,014		MMR
160113-0085 160113-0085	23,50 23,51	23,51	0,0090	74051,52 73992,28	74322	148644	37,98			1,30	0,65	0,00001	0,00087	ND	NQ	0,001		MMR
160114-0098 160114-0098	23,90 23,83	23,86	0,0494	56619,74 55449,82	56035	116070	38,63			0,82	0,41	0,00001	0,00071	ND	NQ	0,001		MMR
160114-0098 160114-0098	24,00 23,79	23,89	0,1569	52745,00 61577,97	57162	114323	33,20 33,10	33,15	0,0719	36,93 39,50	38,22	0,00067	0,06886	D	Q	0,064		Ind
160114-0105 160114-0105	23,02 23,17	23,10	0,1049	104007,71 83779,80	93894	197788					0,00					0,000	MR5	MMR
160114-0106 160114-0106	23,38 23,31	23,35	0,0478	81065,13 84961,63	83013	166027	38,58			0,85	0,43	0,00001	0,00061	ND	NQ	0,000		MMR
160115-0078 160115-0078	23,98 24,24	24,10	0,1967	54311,55 44641,24	49476	99953	31,68 31,55	31,61	0,0918	105,83 117,00	111,92	0,00225	0,22620	D	Q	0,216		No MMR
160115-0099 160115-0099	23,99 23,99	23,99	0,0031	53031,20 53195,53	53113	106227	33,87 33,87	33,87	0,0005	23,08 23,09	23,09	0,00043	0,04347	D	Q	0,041		MMR
160115-0035 160115-0035	23,94 23,83	23,89	0,0795	54727,83 59194,22	56961	113922	37,88 38,88	38,38	0,7041	1,39 0,69	1,04	0,00002	0,00182	ND	NQ	0,002		MMR
160115-0076 160115-0076	24,79 24,62	24,70	0,1220	30367,49 34241,99	32300	64599	29,55 29,82	29,68	0,1327	475,25 392,70	433,98	0,01344	1,34350	D	Q	1,280		No MMR
160115-0081 160115-0081	22,77 22,91	22,84	0,0993	124600,62 112971,51	118789	237578	32,15 32,41	32,28	0,1855	78,80 33,91	70,35	0,00059	0,05923	D	Q	0,056		Ind
160119-0035 160119-0035	23,91 24,19	24,05	0,1929	55928,96 46237,27	51083	102166	26,31 26,48	26,39	0,1187	4587,53 4088,73	4337,13	0,09490	9,49034	D	Q	9,039		No MMR
160119-0087 160119-0087	22,62 22,55	22,58	0,0508	138090,36 145193,42	141642	283284	22,49 22,58	22,54	0,0592	66396,76 62615,97	64506,36	0,45542	45,54196	D	Q	43,391		No MMR

IS-NCN: Non MMR Non concluant MMR

3. DISCUSSION INTERACTIVE SUR LE FORMAT DU RAPPORT DE LABORATOIRE IDÉAL

Rapports de PCR quantitative *BCR-ABL1* au CHU de Liège : situation actuelle

Rapports de PCR quantitative *BCR-ABL1*: buts ?

- **Objectiver la qualité/profondeur de la réponse à un traitement donné;**
- **Sur une échelle internationale standardisée;**
- **Décider d'analyses complémentaires éventuelles;**
- **Adapter (ou non) le traitement et évaluer les répercussions**

Illustration 1

CHU de Liège
Chilab Lg.

Centre Hospitalier Universitaire de Liège
Domaine Universitaire du Sart Tilman - B35 - 4000 LIEGE 1
www.chuliege.be

Agréation :
N° 8.62700.18.998

Prescrit par DR DE PASQUAL AURELIE

CENTRE DE GENETIQUE
Agréation : 8.62990.19.996 art.33 & bis
Génétique clinique - 04/366.71.24
Biochimie génétique - 04/366.76.95 - fax 04/366.84.74
Cytogénétique - 04/366.25.61 - fax 04/366.29.74
Génétique moléculaire - 04/366.24.78
Biologie moléculaire hématologique - 04/366.25.61

16841574598 LHCV

Impression du: **01/02/2016 à 18:56**

Réf du labo: 14-160119-0087
Votre Réf: 248518

Protocole DUPLICATA

Nom, prénom:
Né(e) le 29/03
Sexe: Masculin
Code Patient:
N° Traitement:

Date du prélèvement: **19/01/2016 10:40**

Date de réception: **19/01/2016 15:55**
Date de validation: **28/01/2016 10:27**

BIOLOGIE MOLECULAIRE HEMATOLOGIQUE

Echantillon

Moelle

La prescription reçue n'était pas cochée. Pourriez vous prendre contact rapidement avec notre secrétariat afin de nous communiquer les analyses à réaliser. Merci

Renseignements cliniques

LMC
Progression suspectée.
Premier prélèvement reçu le 02/11/2012.

RT-PCR Quantitatives

M BCR-ABL1/10E4ABL1	0.00100	%	0.02300	03/12/14

(NCN% IS):
(Ratio normalisé sur échelle internationale "IS")

Contrôle(s) amplificabilité - sensibilité

ABL1 (Nbr copies): 66614 copies = moyenne !!!

Le nombre de copies d'ABL1 conditionne la limite de détection (LOD) du test.

Si le transcrit BCR-ABL1 n'est pas détecté, un nombre minimum de:

- 10.000
- 32.000
- 100.000

copies d'ABL1 doit être présent pour déterminer si le patient est en:

- MR 4
- MR 4.5
- MR 5.

Commentaires

Le ratio normalisé M BCR-ABL1/ABL1sur l'échelle internationale (NCN IS BCR-ABL1) est <= à 0.0032%, ce qui correspond à une réduction de >= 4.5 log10 par rapport à la valeur de référence basale selon l'IRIS et à une réponse moléculaire 4.5 ou MR4.5.

Pour rappel une réponse moléculaire majeure (MMR) correspond à un ratio normalisé sur l'IS <=0.1%.

Votre patient présente donc PLUS qu'une réponse moléculaire majeure (MMR) selon les critères définis par l'European LeukemiaNet (ELN).

Illustration 2

RT-PCR Quantitatives

M BCR-ABL1/10E4ABL1	0.01000	%	0.00900	22/01/15

(NCN% IS):
(Ratio normalisé sur échelle internationale "IS")

Contrôle(s) amplificabilité - sensibilité

ABL1 (Nbr copies): 77090 copies

Le nombre de copies d'ABL1 conditionne la limite de détection (LOD) du test.

Si le transcrit BCR-ABL1 n'est pas détecté, un nombre minimum de:

- 10.000
- 32.000
- 100.000

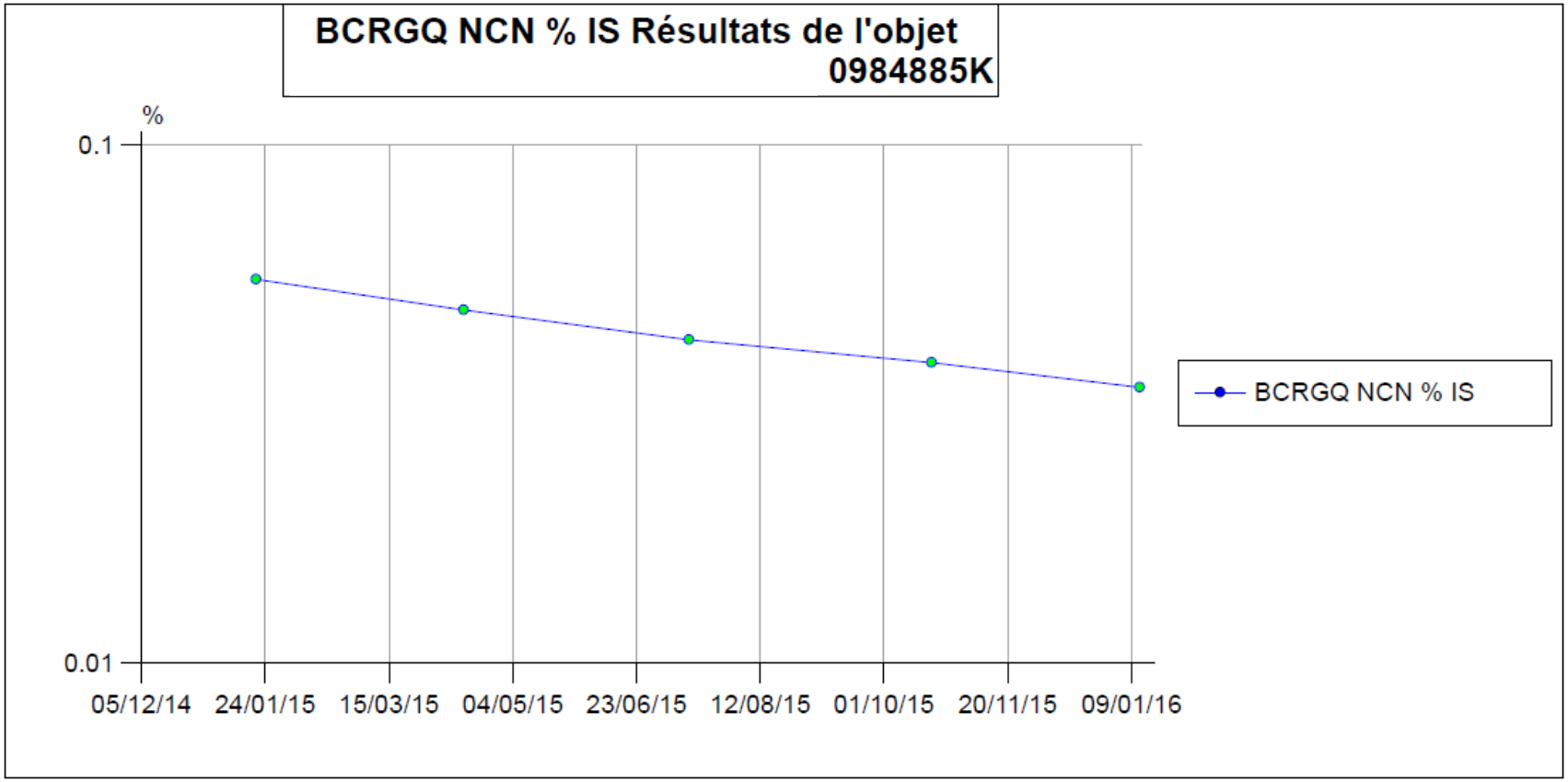
copies d'ABL1 doit être présent pour déterminer si le patient est en:

- MR 4
- MR 4.5
- MR 5.

Commentaires

Le ratio normalisé M BCR-ABL1/ABL1 rapporté sur l'échelle internationale (NCN-IS) est <= à 0.1%.

Ceci correspond à une réponse moléculaire majeure (MMR), ou MR3, selon les critères définis par l'European LeukemiaNet (ELN), soit une réduction >= 3 log10 par rapport à la valeur de référence basale selon l'IRIS.


BCRGQ NCN % IS Résultats de l'objet
0984885K
%
0.1
0.01
05/12/14
24/01/15
15/03/15
04/05/15
23/06/15
12/08/15
01/10/15
20/11/15
09/01/16
BCRGQ NCN % IS

enABL, BCR-ABL Report Optimization: le format idéal de rapport

First Name:
Last Name:
Sex:
Birth Date: 01/02/1944
Patient Number: 08642

Physician's Name:
Physician's Location:

Current Diagnosis: Chronic Myeloid Leukaemia – Chronic Phase
Date of Current Diagnosis: 01/01/2014
Clinical Details: 12 month follow-up

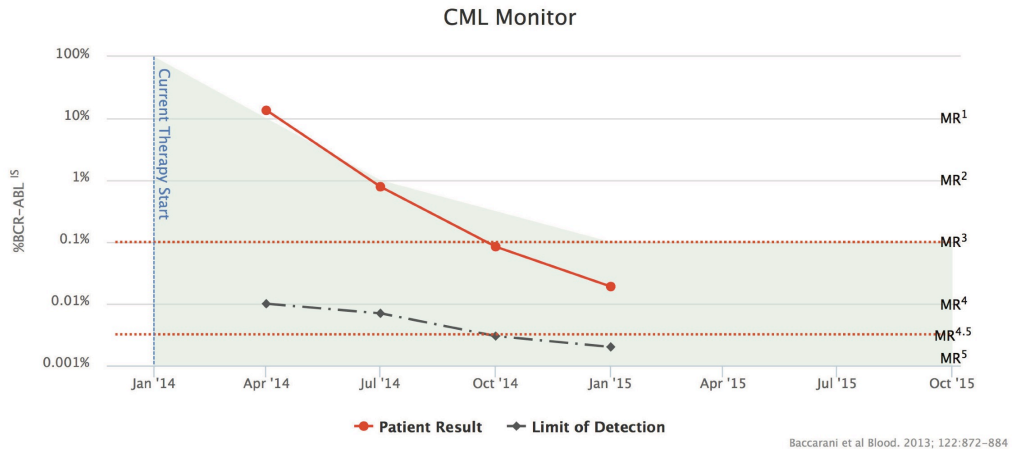
Current Therapy Start Date: 01/01/2014
Current Therapy: Nilotinib
Current Therapy Status: Patient on first-line therapy or second-line therapy due to first-line therapy intolerance

Results

Sample ID: 323421
%BCR-ABL IS: 0.019%
%BCR-ABL/CGx100: 0.022%

MMR Achieved: Yes
MR4.5 Achieved: No
Sample Quality: Pass

Interpretative Comments: The patient has achieved or maintained MMR, which is defined by ELN as an optimal response to therapy.



Baccarani et al Blood. 2013; 122:872–884

Date Taken	Sample ID	Sample Type	%BCR-ABL^{IS}
01/01/2015	323421	Peripheral Blood (PB)	0.019
10/10/2014	333444	Peripheral Blood (PB)	0.084
07/07/2014	222333	Peripheral Blood (PB)	0.783
04/04/2014	111222	Peripheral Blood (PB)	13.5

Technical details

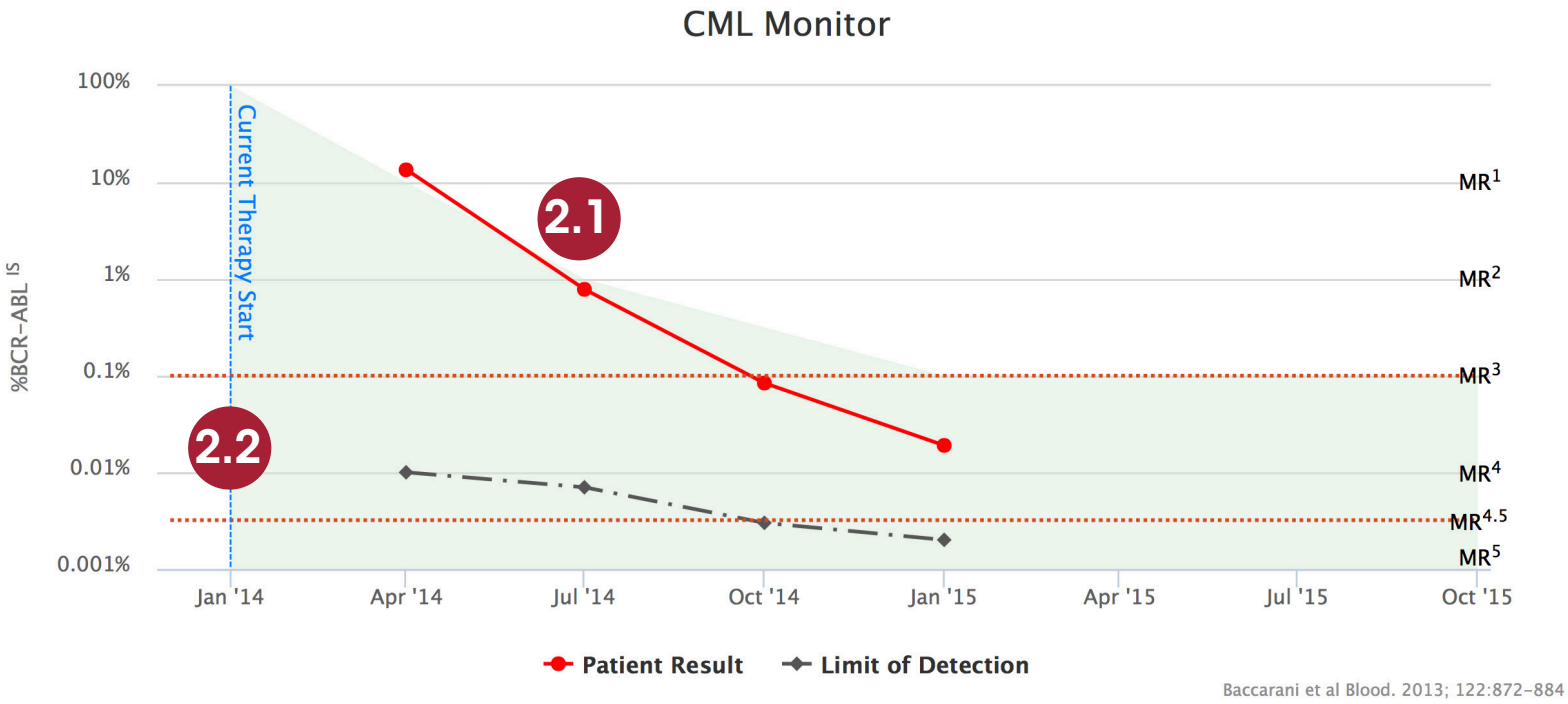
Control Gene (CG) used: ABL1
BCR-ABL Copy Number: 10
Control Gene Copy Number: 46000
Methodology used: Qiagen IS MMR
Transcript type: e13a2
Limit of Detection: 0.0023%

LES DONNÉES A PRÉSENTER DANS LE RAPPORT:

%BCR-ABL/CGx100	The raw BCR-ABL to control gene ratio expressed as percentage, which is then aligned to the International Scale with use of a conversion factor.
BCR-ABL Copy Number	The number of BCR-ABL1 molecules detected in the sample, the International Scale is only valid for major transcripts (e14a2 and 13a2).
Control Gene (CG) Used	This is the gene that is used to normalize the BCR-ABL result. This information is required in order to interpret the control gene copy number, particularly important when BCR-ABL is undetectable. Options include: • GUSB • ABL1 • BCR • G6PDH • B2M
Methodology used	This information provides the ordering physician context for the interpretation of the result. The description of the protocol used to perform the test should be informative but concise. Example: *Qiagen IS MMR Fusion Quant RQ-PCR performed on mRNA.* Excessive detail should be avoided.

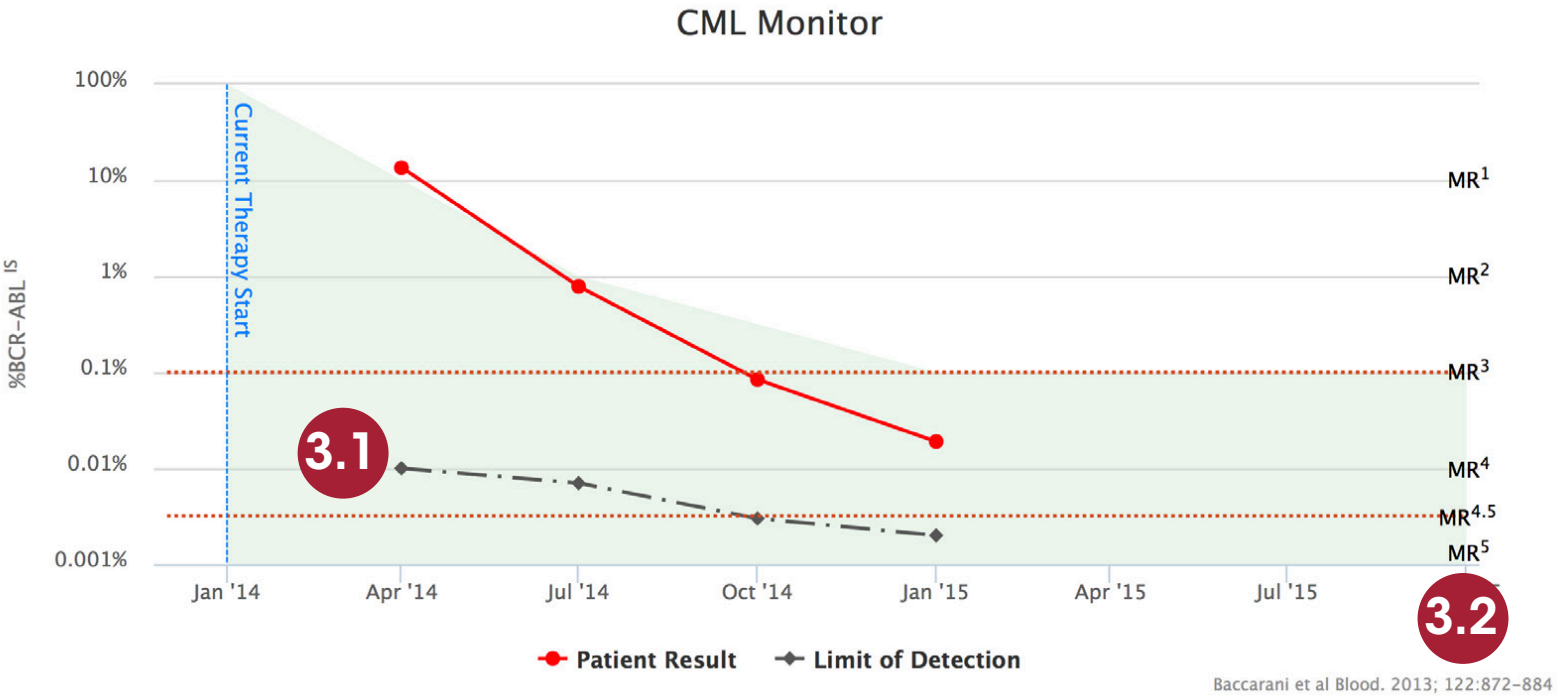
LES DONNÉES A PRÉSENTER DANS LE RAPPORT:

enABL, BCR-ABL Report Optimization:
implémentation d'une représentation visuelle des données historiques de suivi



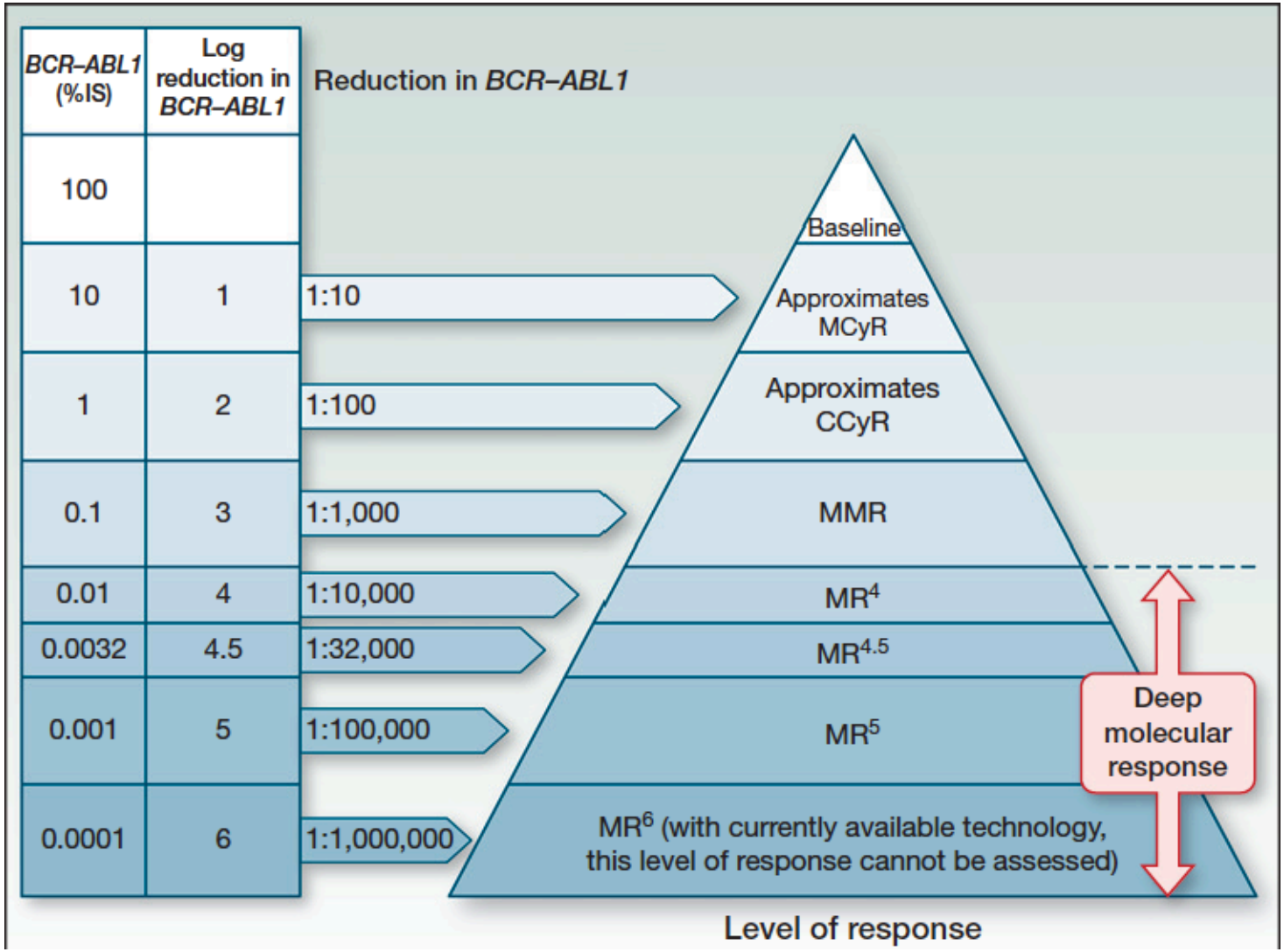
Baccarani et al Blood. 2013; 122:872–884

enABL, BCR-ABL Report Optimization:
implémenter la représentation de la limite de détection (LoD) et évaluation du niveau de la réponse moléculaire atteint



Cross *et al* 2015[3] recommend the following control gene copy numbers are necessary to score molecular response:
MR4.0 = 10,000-31,999 copies of ABL1 or 24,000-76,999 copies of GUSB
MR4.5 = 32,000-99,999 copies of ABL1 or 77,000-239,999 copies of GUSB
MR5.0 ≥100,000 copies of ABL1 or ≥240,000 copies of GUSB

Relation *BCR-ABL1* I.S et profondeur de la réponse moléculaire:



François-Xavier Mahon Clin Cancer Res; 20(2) January 15, 2014

OPEN

Leukemia (2015), 1–5

www.nature.com/leu

REVIEW

Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia

NCP Cross[1,2], HE White[1,2], D Colomer[3], H Ehrencrona[4], L Foroni[5], E Gottardi[6], T Lange[7], T Lion[8], K Machova Polakova[9], S Dulucq[10], G Martinelli[11], E Oppliger Leibundgut[12], N Pallisgaard[13], G Barbany[14], T Sacha[15], R Talmaci[16], B Izzo[17], G Saglio[6], F Pane[17,18], MC Müller[19] and A Hochhaus[20]

Leukemia advance online publication, 27 February 2015; doi:10.1038/leu.2015.29

Published definitions of MR

- MR4 (≥4-log reduction from IRIS baseline) =

either (i) detectable disease ⩽ 0.01% BCR-ABLIS or
(ii) undetectable disease in cDNA with 10 000–31 999 ABL1 transcripts

- MR4.5 (≥4.5-log reduction from IRIS
  detectable disease ⩽0.0032% BCR-ABLIS or

(ii) undetectable disease in cDNA with 32 000–99 999 ABL1 transcripts

- MR5 (≥5-log reduction from IRIS baseline) =

either (i) detectable disease ⩽ 0.001% BCR-ABLIS or
(ii) undetectable disease in cDNA with ⩾ 100 000 ABL1 transcripts

DEFINING DETECTABLE AND UNDETECTABLE DISEASE

- **The cutoff for positivity should correspond to a quantification cycle (Cq) of intercept +1 (which should generally lead to cutoffs of 41–42 Cq). In other words, samples with a Cq higher than intercept +1 should be considered as undetectable.**
- **The 'no-template control' wells and reagent blanks should ideally not cross the threshold at any point but should certainly be at least 2 Cq above the intercept Cq for that run. If this is not the case, then the run must be considered as failed.**
- **If replicate assays are performed for BCR-ABL1, any of the individual replicates are positive according to the criteria above, we recommend that the final result is considered as positive, that is, detectable disease**
- **all low level-positive replicates should be assigned a specific number of BCR-ABL1 transcripts by extrapolating below the lowest plasmid standard.**

Leukemia advance online publication, 27 February 2015; doi:10.1038/leu.2015.29

SCORING MR WHEN DISEASE IS DETECTABLE (1)

Table 1. Summary of reference gene numbers required for scoring deep molecular response

	MR^4	$MR^{4.5}$	MR^5
Minimum sum of reference gene transcripts irrespective of whether *BCR-ABL1* is detected or not[a]	10 000 *ABL1* 24 000 *GUSB*	32 000 *ABL1* 77 000 *GUSB*	100 000 *ABL1* 240 000 *GUSB*
$BCR\text{-}ABL^{IS}$ level for positive samples[b]	⩽0.01%	⩽0.0032%	⩽0.001%

[a]Numbers of reference gene transcripts in same volume of cDNA that is tested for *BCR-ABL1*. The minimum number in any individual replicate should be 10 000 *ABL1* or 24 000 *GUSB*. [b]Provided that the minimum reference gene copy numbers in the row above are fulfilled.

Example 3 (Lab CF = 0.5):

– BCR-ABL1 replicate 1: undetectable in 5 µl cDNA.
– BCR-ABL1 replicate 2: detectable in 5 µl cDNA, estimated 3 copies.
– ABL1 replicate 1: 9000 copies in 5 µl cDNA.
– ABL1 replicate 2: 8000 copies in 5 µl cDNA.

Result = inevaluable for MR.

Comment: Although the ((sum of BCR-ABL1)/(sum of reference gene)) × CF × 100 is o 0.01%, the sample should be considered as inevaluable for the assessment of MR as the ABL1 copy number in each replicate is <10000.

Example 1 (Lab CF = 0.8):

– BCR-ABL1 replicate 1: detectable in 2 µl cDNA, estimated 7 copies.
– BCR-ABL1 replicate 2: detectable in 2 µl cDNA, estimated 3 copies.
– ABL1 replicate 1: 24 000 copies in 2 µl cDNA.
– ABL1 replicate 2: 28 000 copies in 2 µl cDNA.

Result=(sum BCR-ABL1=10)/(sum ABL1=52000)×0.8×100= 0.015% = MMR but not MR4.

Leukemia advance online publication, 27 February 2015; doi:10.1038/leu.2015.29

SCORING MR WHEN DISEASE IS DETECTABLE (2)

Table 1. Summary of reference gene numbers required for scoring deep molecular response

	MR^4	$MR^{4.5}$	MR^5
Minimum sum of reference gene transcripts irrespective of whether *BCR-ABL1* is detected or not[a]	10 000 *ABL1* 24 000 *GUSB*	32 000 *ABL1* 77 000 *GUSB*	100 000 *ABL1* 240 000 *GUSB*
$BCR\text{-}ABL^{IS}$ level for positive samples[b]	⩽0.01%	⩽0.0032%	⩽0.001%

[a]Numbers of reference gene transcripts in same volume of cDNA that is tested for *BCR-ABL1*. The minimum number in any individual replicate should be 10 000 *ABL1* or 24 000 *GUSB*. [b]Provided that the minimum reference gene copy numbers in the row above are fulfilled.

Example 5 (Lab CF = 0.25):

– BCR-ABL1 replicate 1: undetectable in 2 µl cDNA.
– BCR-ABL1 replicate 2: detectable in 2 µl cDNA, estimated 3 copies.
– ABL1 replicate 1: 12 000 copies in 2 µl cDNA.
– ABL1 replicate 2: 14 000 copies in 2 µl cDNA.

Result = (sum BCR-ABL1 = 3)/(sum ABL1 = 26 000) × 0.25 × 100 = 0.0029%; sum of ABL1 < 32 000 = MR4.

Comment: Although the ((sum of BCR-ABL1)/(sum of reference gene)) × CF × 100 is < 0.0032%, the total ABL1 value is < 32 000 and should thus be considered as MR4.

Leukemia advance online publication, 27 February 2015; doi:10.1038/leu.2015.29

SCORING MR WHEN DISEASE IS UNDETECTABLE

Table 1. Summary of reference gene numbers required for scoring deep molecular response

	MR^4	$MR^{4.5}$	MR^5
Minimum sum of reference gene transcripts irrespective of whether *BCR-ABL1* is detected or not[a]	10 000 *ABL1* 24 000 *GUSB*	32 000 *ABL1* 77 000 *GUSB*	100 000 *ABL1* 240 000 *GUSB*
$BCR\text{-}ABL^{IS}$ level for positive samples[b]	⩽0.01%	⩽0.0032%	⩽0.001%

[a]Numbers of reference gene transcripts in same volume of cDNA that is tested for *BCR-ABL1*. The minimum number in any individual replicate should be 10 000 *ABL1* or 24 000 *GUSB*. [b]Provided that the minimum reference gene copy numbers in the row above are fulfilled.

Example 13:

– BCR-ABL1 replicate 1: undetectable in 5 µl cDNA.
– BCR-ABL1 replicate 2: undetectable in 5 µl cDNA.
– ABL1 replicate 1: 6000 copies in 5 µl cDNA.
– ABL1 replicate 2: 14 000 copies in 5 µl cDNA.

Result = inevaluable for MR.

Comment: One replicate is <10000 ABL1 and hence the sample should be considered as inevaluable for MR. As the two ABL1 replicates are discordant, the reference gene qPCR could be repeated.

Example 9:

– BCR-ABL1 replicate 1: undetectable in 5 µl cDNA.
– BCR-ABL1 replicate 2: undetectable in 5 µl cDNA.
– ABL1 replicate 1: 16 500 copies in 5 µl cDNA.
– ABL1 replicate 2: 18 000 copies in 5 µl cDNA.

Result = undetectable BCR-ABL1 in 34 500 ABL1 = MR4.5.

Leukemia advance online publication, 27 February 2015; doi:10.1038/leu.2015.29

enABL, BCR-ABL Report Optimization:
interprétation des recommandations ELN

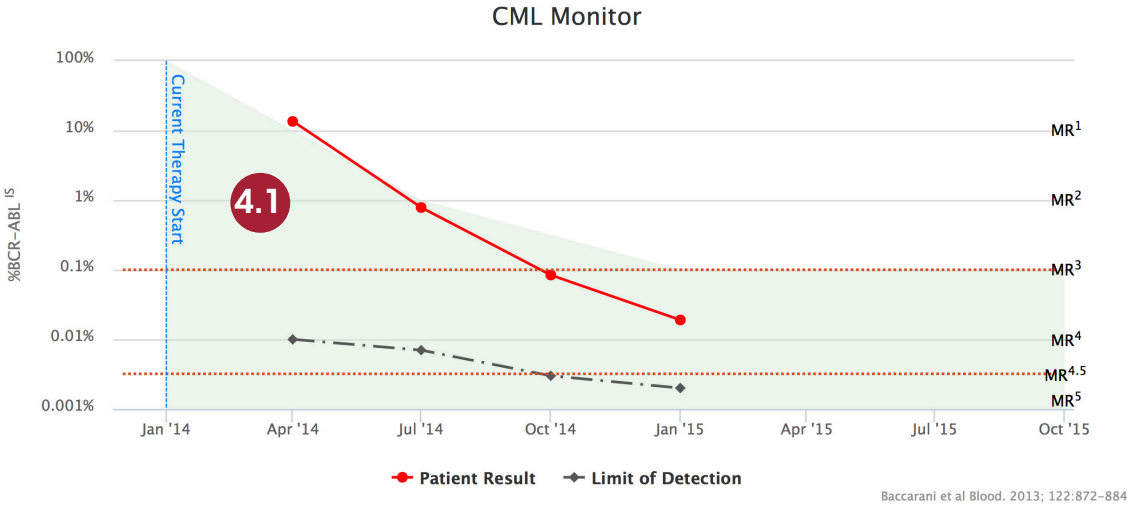
Key	Implementation Guidance	Common Mistakes to Avoid
4.1	The chart shading represents optimal response per the ELN recommendations[2] and differs dependent on line of therapy. The shading should be adjusted according to therapy start date and adhere to the following definitions. Note: There is no equivalent definition for patients on third-line therapy, therefore for these patients the graph should not be shaded.	Chart shading is not commonly incorporated into graphical representation of patients' results. Commonly only first-line response is included. Second-line criteria, and the lack of third-line response criteria, are essential for accurate ELN interpretation for all patients.

First-line Patients[2]

Time point	Green Area
3 Months	≤10% IS
6 Months	≤1% IS
>12 Months	≤0.1% IS

Second-line Patients[2]

Time point	Green Area
3 Months	≤10% IS
6 Months	≤10% IS
12 Months	≤1% IS
>12 Months	≤0.1% IS

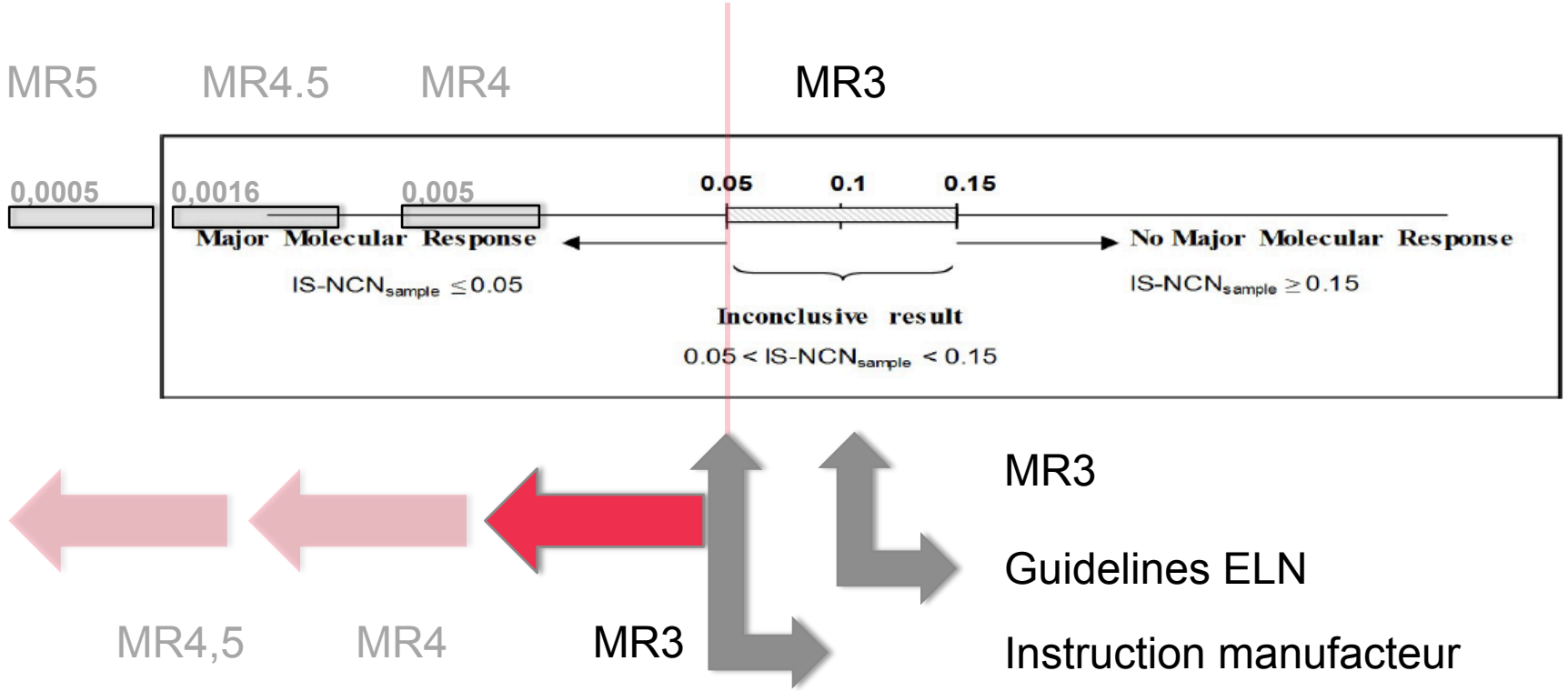


Baccarani et al Blood. 2013; 122:872–884

Zone grise :

la prise en compte de l'incertitude de mesure liée à la méthode peut paradoxalement générer une certaine « variabilité » dans la détermination du statut MR3/MR4….

Coefficient de variation sur l'IS-NCN: 100%



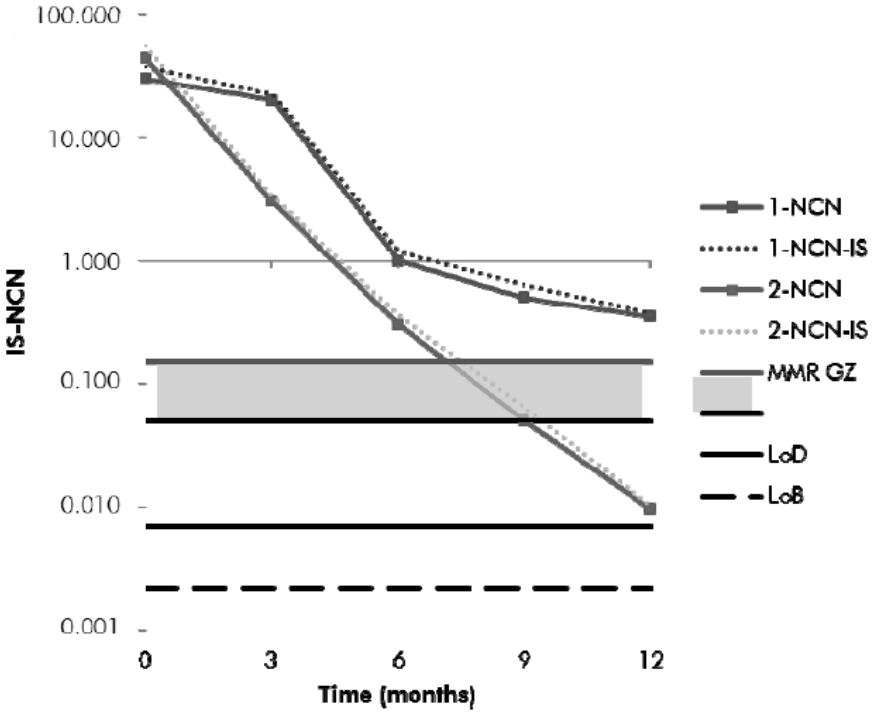
Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

Détermination du statut MMR (MR3) :

prise en compte de l'incertitude de mesure liée à la méthode

Critères d'interprétation kit QIAGEN

- **IS-NCN$_{sample}$ ≤0.05**: Major molecular response
- **0.05 <IS-NCN$_{sample}$ <0.15**: Gray zone around the MMR inconclusive result
- **IS-NCN$_{sample}$ ≥0.15**: No major molecular response



Manuel du kit ipsogen BCR-ABL1 Mbcr IS-MMR DX 01/2013 v1

4. DIFFÉRENCE ENTRE LABORATOIRE ACCRÉDITÉ ET LABORATOIRE STANDARDISÉ

ACCRÉDITATION

ISO 15189 est une norme internationale publiée par l'ISO en 2012 qui spécifie les exigences de qualité et de compétence propres aux laboratoires de biologie médicale (LBM). Son titre est "Laboratoire de biologie médicale. Exigences concernant la qualité et la compétence »

https://fr.wikipedia.org/wiki/ISO_15189

- **Laboratoire accrédité: certificat d'accréditation délivré en Belgique par BELAC, si respect norme ISO 15189**

Annexe au certificat d'accréditation
Bijlage bij accreditatie-certificaat
Annex to the accreditation certificate
Beilage zur Akkreditatierungszertifikat

128-MED

NBN EN ISO 15189:2012

Version/Versie/Version/Fassung	12
Date d'émission / Uitgiftedatum / Issue date / Ausgabedatum:	2015-10-26
Date limite de validité / Geldigheidsdatum / Validity date / Gültigkeitsdatum:	2016-02-23

Nicole Meurée-Vanlaethem
La Présidente du Bureau d'Accréditation
Voorzitster van het Accreditatiebureau
Chair of the Accreditation Board
Vorsitzende des Akkreditierungsbüro

L'accréditation est délivrée à/ De accreditatie werd uitgereikt aan/
The accreditation is granted to/ Die akkreditierung wurde erteilt für:

UNILAB Lg
Site du Sart-Tilman
CENTRE HOSPITALIER UNIVERSITAIRE DE LIEGE
Domaine Universitaire du Sart-Tilman, CHU B35
4000 LIEGE

Secrétariat:
Service public fédéral, Economie, P.M.E., Classes moyennes et Energie
Direction générale de la Qualité et de la Sécurité
Division Qualité et Innovation
Bd du Roi Albert II, 16 - 5ème étage - B-1000 Bruxelles
Website: http://economie.fgov.be
Numéro d'entreprise: 0314.595.348

Accréditation B E L A C Accreditatie

Tél: +32 2 277 54 34
Fax: +32 2 277 54 41

Internet: http://belac.fgov.be
E-Mail: Belac@economie.fgov.be

Secretariaat:
Federale Overheidsdienst, Economie, K.M.O., Middenstand en Energie
Algemene Directie Kwaliteit en Veiligheid
Afdeling Kwaliteit en Innovatie
Koning Albert II-laan 16 - 5de verd. - B-1000 Brussel
Website: http://economie.fgov.be
Ondernemingsnummer: 0314.595.348

.be

Laboratoire réalisant l'analyse	Domaine d'activité	CODE ESSAI	PROPRIETE MESUREE	ECHANTILLON	METHODE/APPAREIL
Biologie Moléculaire Hématologique (GNT.BMH)	Génétique - Biologie Moléculaire Hématologique	BCRG_BIOMED-1_T	Détection du transcrit M - BCR-ABL	Moëlle, sang	PCR nichée
Biologie Moléculaire Hématologique (GNT.BMH)	Génétique - Biologie Moléculaire Hématologique	BCRGQ_R	Analyse par PCR Quantitative en temps réel du Transcrit BCR-ABL Mbcr p210	Moëlle, sang	qPCR

http://www.chu.ulg.ac.be/jcms/c_3589182/accreditation

LA STANDARDISATION « EUTOS »

STANDARDISATION

Leukemia (2006) 20, 1925–1930

www.nature.com/leu

REVIEW

Rationale for the recommendations for harmonizing current methodology for detecting *BCR-ABL* transcripts in patients with chronic myeloid leukaemia

S Branford[1], NCP Cross[2], A Hochhaus[3], J Radich[4], G Saglio[5], J Kaeda[6], J Goldman[7] and T Hughes[8]

http://www.nature.com/leu/journal/v20/n11/full/2404388a.html

Leukemia (2009) 23, 1957–1963

www.nature.com/leu

SPOTLIGHT REVIEW

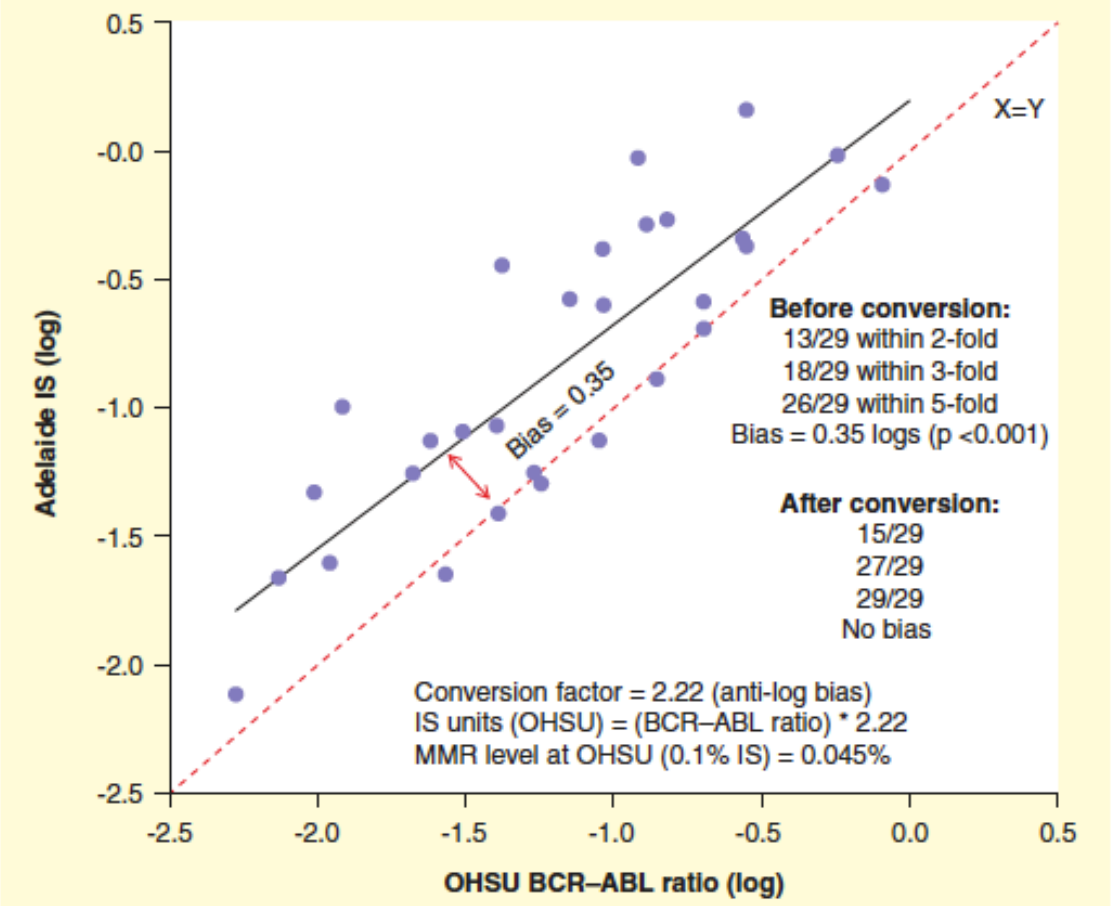
Harmonization of molecular monitoring of CML therapy in Europe

MC Müller[1], NCP Cross[2], P Erben[1], T Schenk[1], B Hanfstein[1], T Ernst[1,2], R Hehlmann[1], S Branford[3], G Saglio[4] and A Hochhaus[1,5]

[1]III. Medizinische Klinik, Universitätsmedizin Mannheim, Heidelberg University, Mannheim, Germany; [2]National Genetics Reference Laboratory, Salisbury District Hospital and University of Southampton School of Medicine, Salisbury, UK; [3]Institute of Medical and Veterinary Science, Adelaide, Australia; [4]Divisione di Medicina Interna e di Ematologia, Ospedale Universita di Torino, Turin, Italy and [5]Department Hematology/Oncology, Universitätsklinikum Jena, Jena, Germany

http://www.nature.com/leu/journal/v23/n11/abs/leu2009168a.html

STANDARDISATION EUTOS



« Participation in samples exchanges with the Adelaide Laboratory was initially the only mechanism to determine a laboratory-specific conversion factor (CF) to the IS. In order that an accurate IS CF is derived and validated, the protocol involves exchanging 20–30 samples between the field laboratory and the Adelaide Lab. »

Martin Luu and Richard D, Expert Rev. Mol. Diagn. 13(7), 749–762 (2013)

5. THE BELGIAN STANDARDIZATION PROJECT

Rationale:

"A novel approach for standardizing *BCR-ABL1* quantification on the International Scale on behalf of the Belgian working group on *BCR-ABL1* IS standardization' by Maes et al. ". The Journal of Molecular Diagnostics (submitted).

« Deals with the problem of standardization of *BCR-ABL1* quantification, offering an alternative approach for calibration to the IS scale that is achievable by almost all laboratories. »

PRINCIPLES - STRATEGY

- UK NEQAS LI *BCR-ABL1* quantification program;
- lyophilized cell line samples to over 100 laboratories every six months;
- Information on the 25th, 50th and 75th percentiles of *BCR-ABL1/ABL1* quantification converted using the International Scale (IS) in labs using *ABL1* as control gene for 16 samples taken between March 2011 and January 2014 (samples 110-125);
- Median IS *BCR-ABL1* values ranging between 0.01 % and 5.33 %;
- Combined with *BCR-ABL1/ABL1* and *ABL1* quantifications from 11 Belgian labs that use *ABL1* as control gene;
- Performance of the selected CF validated on newly collected samples taken between March 2014 and January 2015 (samples 126-131);
- For this validation, EQA results of 9 laboratories were available.
- Comparison of the selected CF with the CF obtained through commercial reference material (mostly Nanogen) available for five labs (labs 2, 3, 4, 8 and 12) using a Wilcoxon signed-rank test.

DESCRIPTION OF PROPOSED CONVERSION FACTORS

1. Optimization of the RT-qPCR BCR-ABL1 flow with improvement of the RT step efficacy and increase of the LOD;

2. The first two CFs were defined as:

   CF1 = mean($ratio_{IS}$)

   and

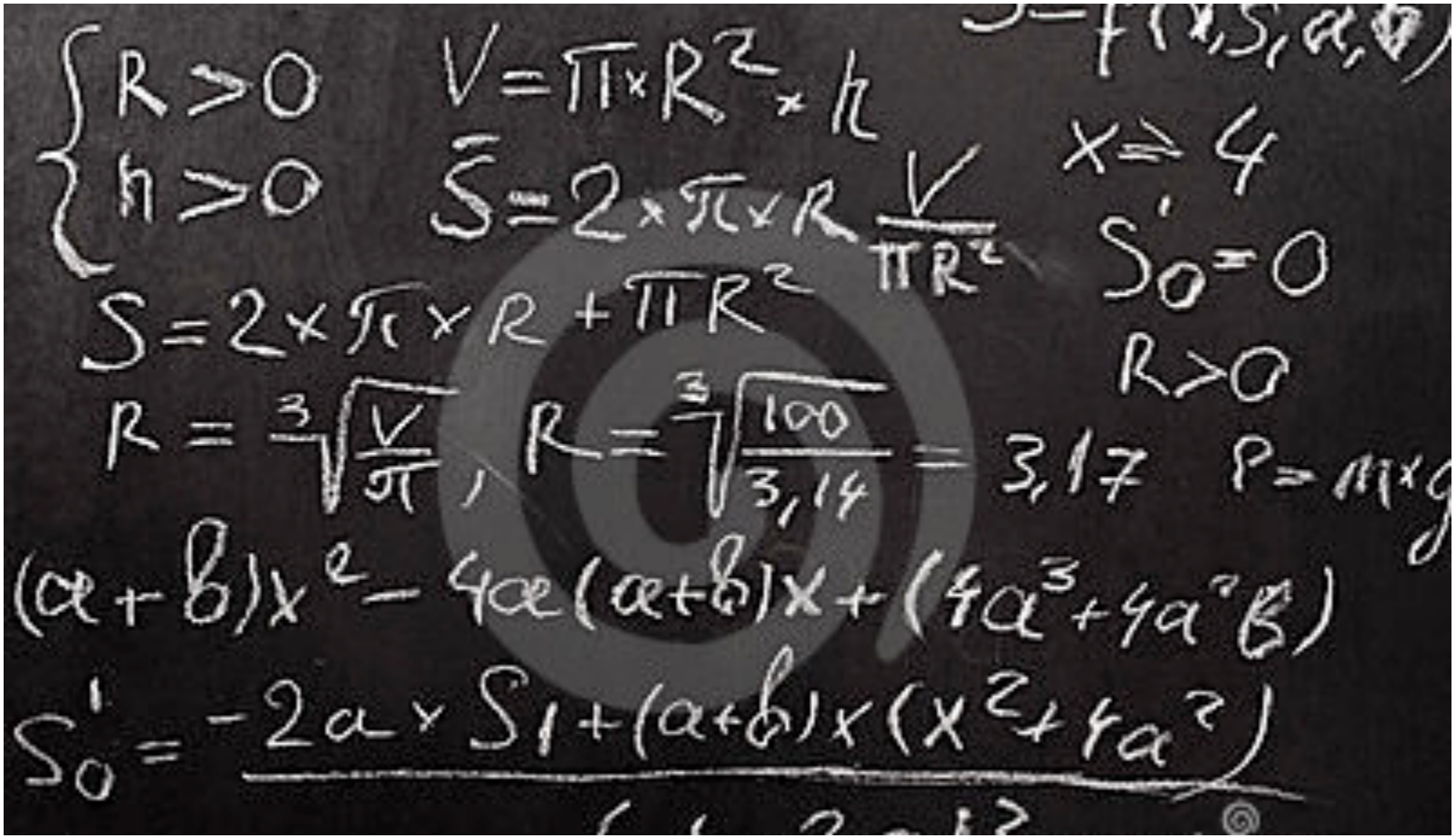
   CF2 = median ($ratio_{IS}$),

   with $ratio_{IS}$= ($median_{s}$/$measurement_{IS}$)

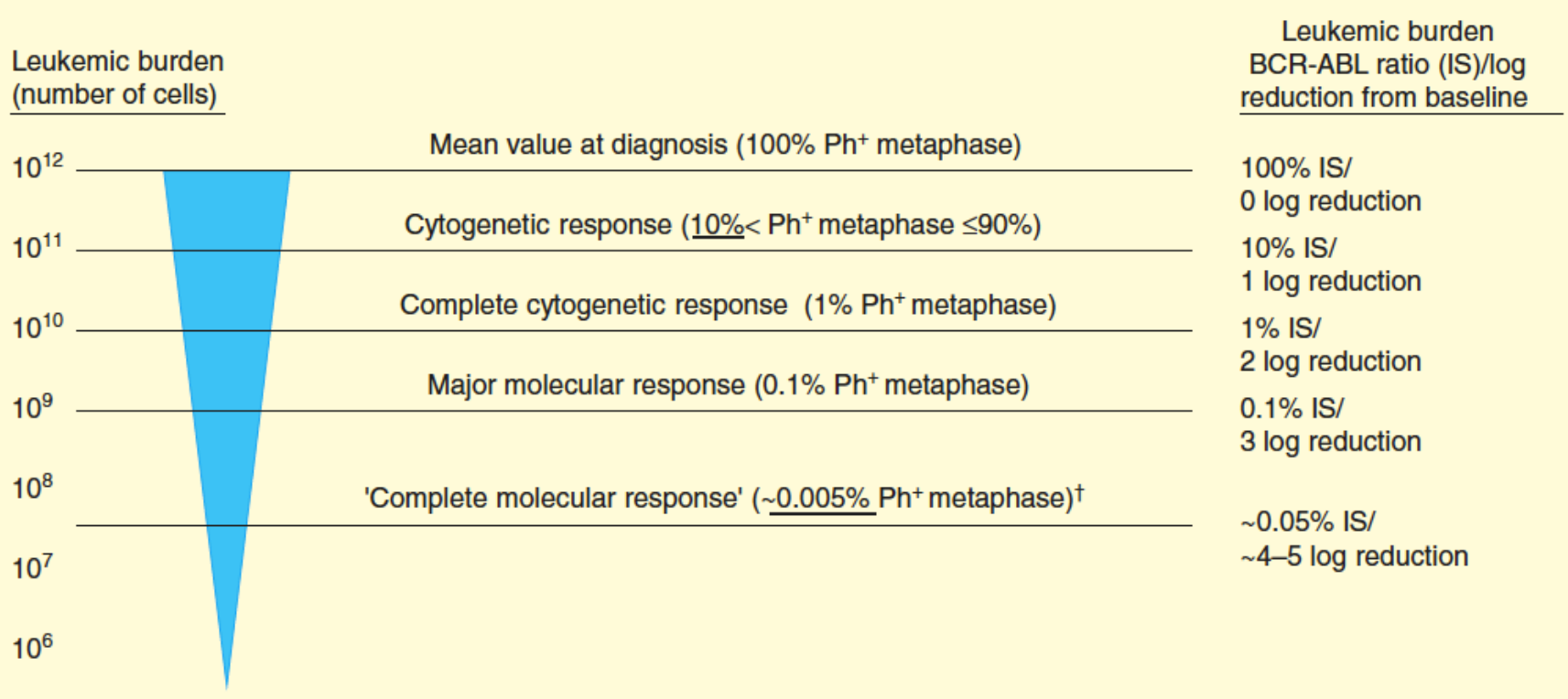
CONCLUSIONS: AIMS OF THE MEETING

- **How is *BCR-ABL1* mRNA Molecular monitoring performed at Unilab Lg *in practice ?***
- **What's should integrate a clear and concise molecular reporting to assist physicians in clinical decision making ?**
- **The Belgian *BCR-ABL1* Standardization Project**
- **Milestones in CML monitoring & implications for the future**

RATIO *BCR-ABL1/ABL1* IS



LEUKEMIC BURDEN IN CML PATIENTS AT DIAGNOSIS AND TKI TREATMENT RESPONSE LANDMARKS DURING MONITORING USING CYTOGENETICS AND BCR–ABL RNA RQ-PCR STANDARDIZED TO THE INTERNATIONAL SCALE



Martin Luu and Richard D, Expert Rev. Mol. Diagn. 13(7), 749–762 (2013)

Rapports de PCR quantitative *BCR-ABL1*: buts ?

ELN European LeukemiaNet

UPDATE 2013

European LeukemiaNet Recommendations for the Management of Chronic Myeloid Leukemia (CML)

Response definitions for any TKI first line, and 2nd line in case of intolerance, all patients (CP, AP, and BC)

Time	Optimal response	Warning	Failure
Baseline		High risk Major route CCA/Ph+	
3 mos.	BCR-ABL^{IS} ≤10%* Ph+ ≤35% (PCyR)	BCR-ABL^{IS} >10%* Ph+ 36-95%	No CHR* Ph+ >95%
6 mos.	BCR-ABL^{IS} <1%* Ph+ 0% (CCyR)	BCR-ABL^{IS} 1-10%* Ph+ 1-35%	BCR-ABL^{IS} >10%* Ph+ >35%
12 mos.	BCR-ABL^{IS} ≤0.1%* (MMR)	BCR-ABL^{IS} 0.1-1%*	BCR-ABL^{IS} >1%* Ph+ >0%
Then, and at any time	MMR or better	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Loss of MMR, confirmed** Mutations CCA/Ph+

*and/or **in 2 consecutive tests, of which one ≥1% IS: BCR-ABL on International Scale

MERCI POUR VOTRE ATTENTION

QUESTIONS ?

https://www.google.be/search?q=etages&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiQw_PYuuDKAhXJbBoKHXFQACUQ_AUIBygB&biw=1920&bih=899#tbm=isch&q=open+mind&imgrc=khYNFLB5AGcuHM%3A