

**Preliminary evaluation  
of the GeneXpert Dx System  
for CML patients monitoring  
through the Xpert BCR-ABL Monitor™ assay:  
comparison with traditional RT-qPCR  
methods**

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**3<sup>rd</sup> International qPCR Symposium**

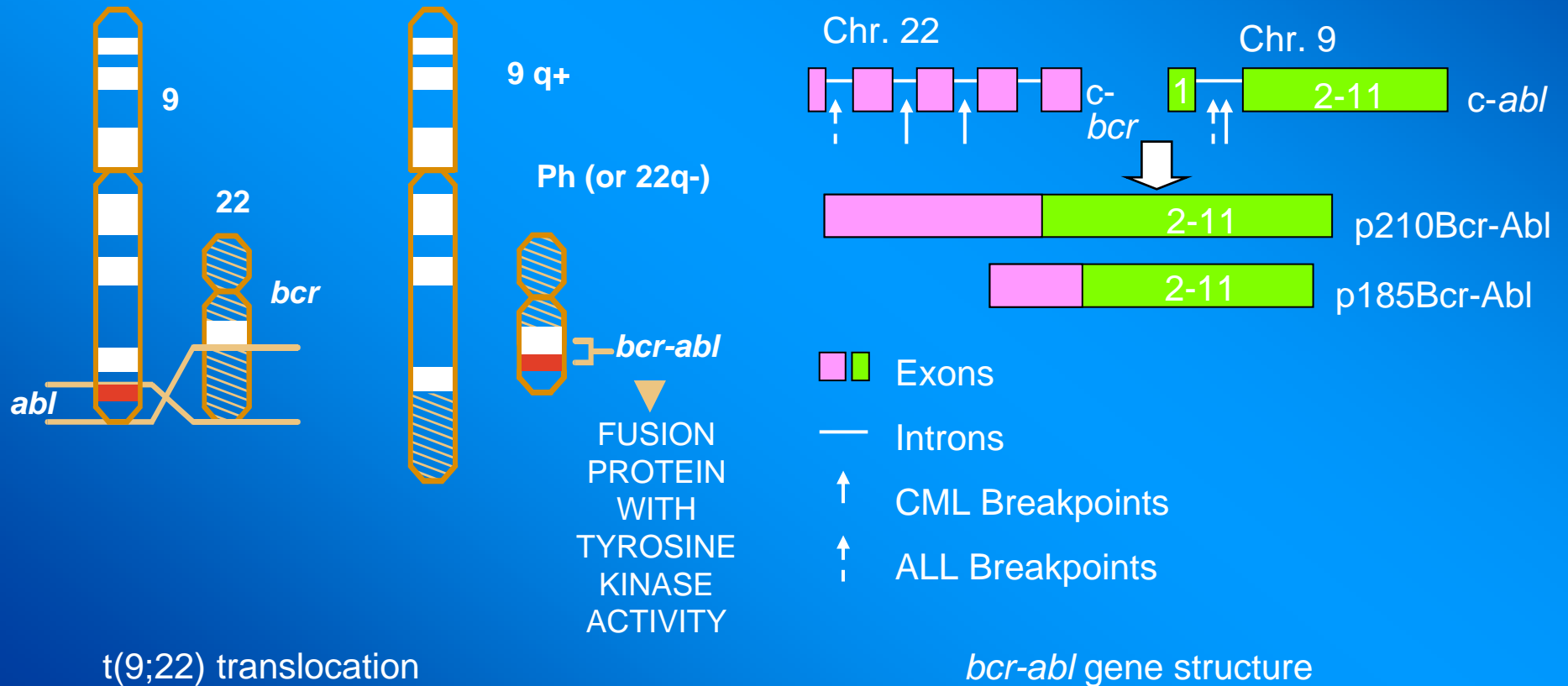
**Freising-Weihenstephan - 2007**

# CML (Chronic Myeloid Leukemia)

Rare clonal myeloproliferative disease (1-1,5 cases/10<sup>5</sup> inhabitants per year; 15-20% of all leukemias) characterised by a chronic phase (4-6 years), followed by a blastic phase (6 months)

Median age at diagnosis: 48 years

Male : female = 1.4 : 1



# Molecular monitoring in CML

- 1999 **Standardization of qualitative PCR**

van Dongen JJM et al. *Leukemia* 13:1901-1928 BIOMED-1 Concerted Action for the investigation of minimal residual disease in acute leukemia.

- 2003 **Standardization of quantitative PCR** (RT reaction, primer and probe sequences, PCR conditions) and evaluation of the **best reference genes** (Europe Against Cancer Program)

Gabert J et al. *Leukemia* 17:2318-2357

Beillard E et al. a Europe against cancer program. *Leukemia* 17:2474-2486

- 2006 Recommendations for **harmonizing** methodologies and **results expression** through an **International Scale of Measurement for BCR-ABL**

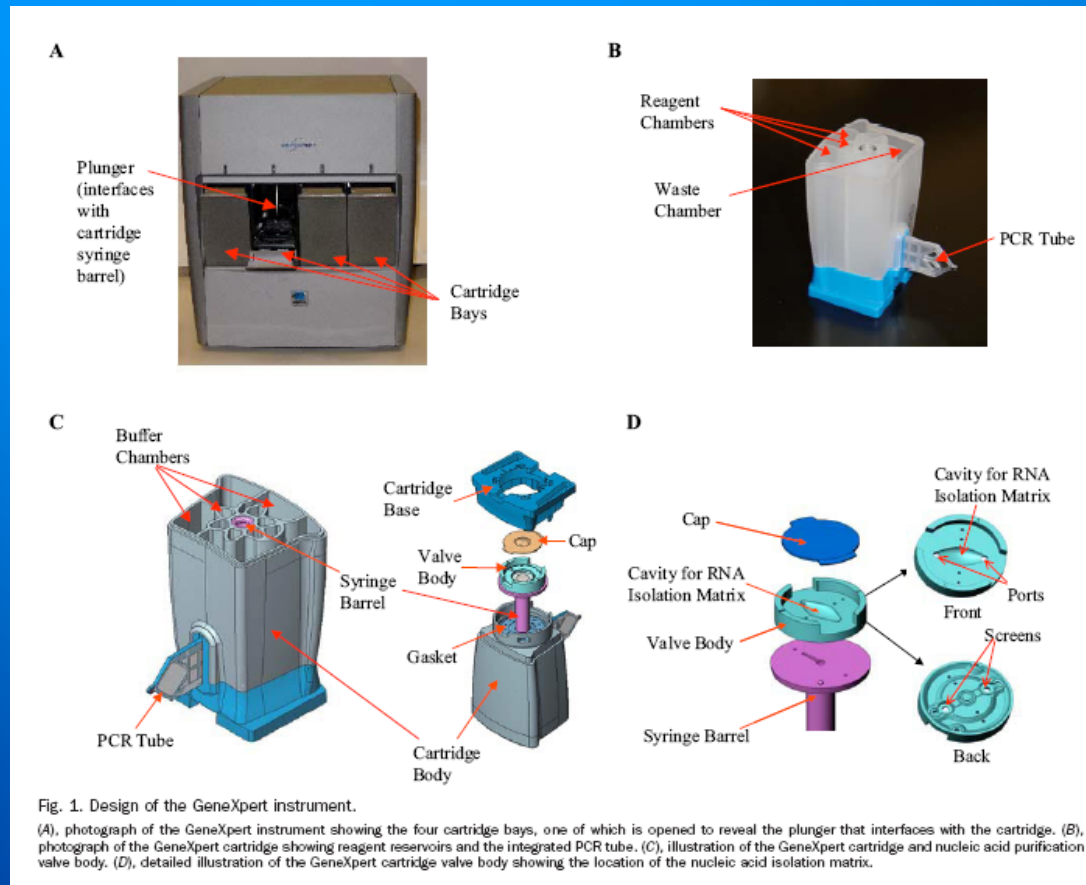
Hughes T. et al. *Blood* 108(1):28-37; Branford S. et al. *Leukemia* 20:1925-1930

- 2006 Italian CML Network: common reference **material and standardization process**

## Aim of the study

1. Analysis of **20 samples** with the **Xpert BCR-ABL Monitor™ assay** in combination with the **GeneXpert Dx System (Cepheid)**
2. Comparison of the results with 2 conventional real-time PCR methods used in CML monitoring:
  - a **home-made method** (Sybr Green I)
  - an **IVD CE commercial method** (TaqMan), the *M-Bcr FusionQuant kit* (Ipsogen)

# The GeneXpert Dx system



Fully automated platform (1-4 position):

- nucleic acid isolation
- reverse transcription
- first round PCR
- nested qPCR

multi-chamber single-use disposable cartridge

quantitative real-time thermal-cycler (Smartcycler technology)

integrated cartridge processor

analysis software

# Patients and Samples

19 Chronic Myeloid Leukemia (CML) **patients:**

- 18 submitted to Glivec therapy, constantly monitored at our Division and by our Laboratory with real-time PCR
- 1 at first analysis, with CML diagnosis from another Centre

12 males and 7 females  
median age: 52 (range:18-83)

**Patients choice:** random, according to the Laboratory reception order

**Samples:**

- 19 fresh peripheral blood samples (10 ml) in K<sub>2</sub>EDTA
- 1 fresh bone marrow sample (2 ml) in K<sub>2</sub>EDTA, from a patient also submitted to peripheral blood analysis

Samples stored max 24 h at 4°C before analysis

# Common pre-RTqPCR procedures

Mononuclear cells (MNCs) isolation by density gradient centrifugation with Lympholite (2 aliquots)

RNA isolation with RNeasy Total RNA kit and QiaVac system (QIAGEN)

Qualitative and quantitative RNA evaluation by spectrophotometric analysis (Biophotometer, Eppendorf)

# Conventional RTqPCR methods

## HOME-MADE ASSAY

Reverse transcription of 1 µg of total RNA with SuperScriptII (Invitrogen) as described in: van Dongen JJM et al. (1999) *Leukemia* 13:1901-1928

1/10 of cDNA product submitted to qPCR in 25 µl reaction volume with SybrGreen QPCR MasterMix (Eurogentec)

BCR-ABL and ABL primer sequences as described in: Gabert J et al. EAC Program (2003) *Leukemia* 17:2318-2357

GeneAmp5700 (Applied Biosystems)

40 cycles (30" 95°C; 30" 60°C; 30" 72°C)

Dissociation curves analysis

## IVD CE ASSAY

Reverse transcription of 1 µg of total RNA with SuperScriptII (Invitrogen) as described in: Gabert et al. (2003) *Leukemia* 17:2318-2357

1/10 of cDNA product submitted to qPCR in 25 µl reaction volume with Ipsogen M-bcr FusionQuant kit according manufacturer conditions

BCR-ABL and ABL primer and probe sequences as described in: Gabert J et al. EAC Program (2003) *Leukemia* 17:2318-2357

GeneAmp5700 (Applied Biosystems)

50 cycles

# Xpert BCR-ABL Monitor™ assay

In Vitro Diagnostic assay, whose intended use is the molecular monitoring of p210 BCR-ABL transcript in peripheral blood samples of CML patients, through the GeneXpert Dx System

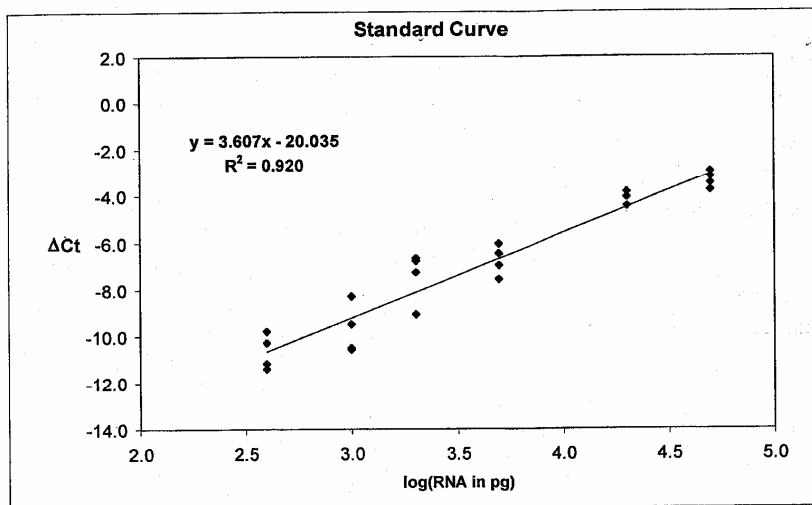


Figure 1. The lot-specific standard curve.

valid ABL Ct range = 12-18

Valid BCR-ABL Ct range = 12-32

2 packages of 10 cartridges  
(IVD version) with same lot  
ID (01401)

standard curve: K562 RNA  
diluted in PC3 RNA

efficiency = 1.89

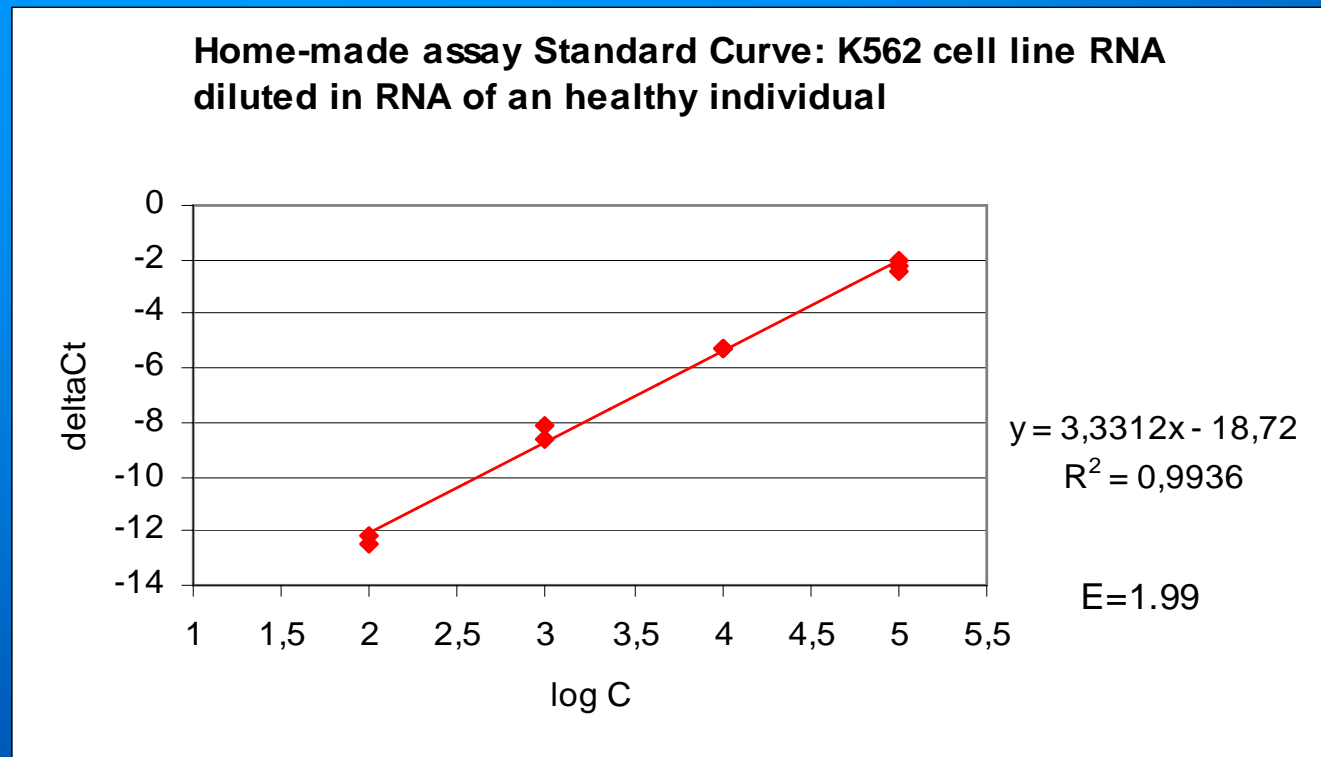
analysis volume proposed:

200 μl (20 if ABL Ct < 12)

analysis volume used: 20,50  
or 200 μl

# Home-made assay

Standard curve design similar to that of the Xpert BCR-ABL Monitor



# Xpert BCR-ABL Monitor™ assay report

GeneXpert PC

**Test Report**

Sample ID: Xpert B 221206130745  
Test Type: Specimen

**Assay Information**

Assay	Assay Version	Assay Type
Xpert BCR-ABL Monitor	2	In-Vitro Diagnostics

Test Result: POSITIVE

**Test and Analyte Result**

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result	Target Delta Ct	Curve Fit
BCR-ABL	26.6	515	POS	PASS	-14.3	PASS
ABL	12.3	518	PASS	PASS		PASS

User: <None>  
Status: Done  
Reagent Lot ID\*: 01401  
Expiration Date\*: 14/1/2007  
Cartridge S/N\*: 1136998  
S/W Version: 1.2  
Notes: 62212SLMC

Start Time: 22/12/2006 13:08:31  
End Time: 22/12/2006 14:59:06  
Module Name: A1  
Module S/N: 502676  
Instrument S/N: 701557

For In Vitro Diagnostics Use Only.

**BCR-ABL/ABL  
ratio =  $E^{\Delta Ct}$**

# ABL Ct value with Xpert

ABL Ct values of valid samples only:

56,3% < 13

87,5% < 14

(the unique sample with a Ct > 17 has been evaluated with a volume of 20 µl)

Low Ct=large detection range, but near the "dangerous zone"

	ABL Ct						
	N	Mean	Sd	Ct <13	13-13,99	14-16,99	>17
All	19	13,88	3,55	11	5	1	2
Valid samples only	16	13,24	1,46	9	5	1	1

(valid ABL Ct range = 12-18)

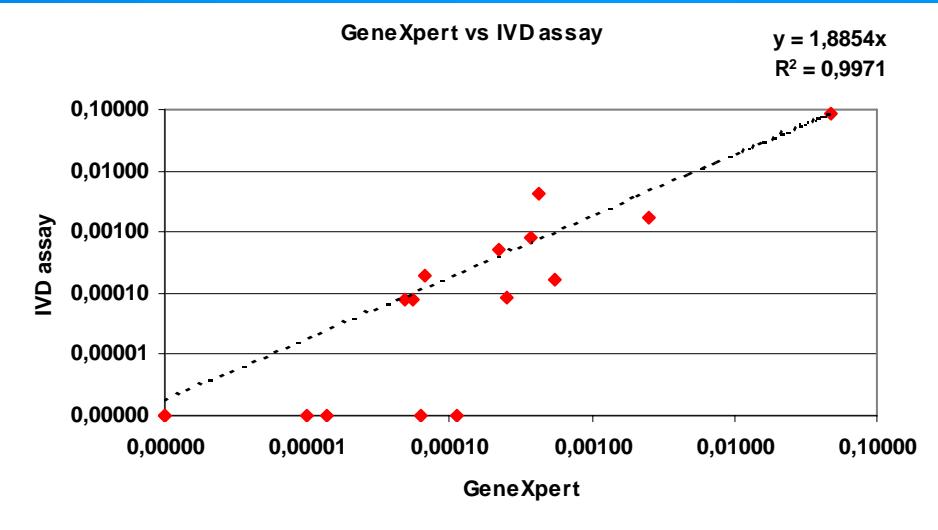
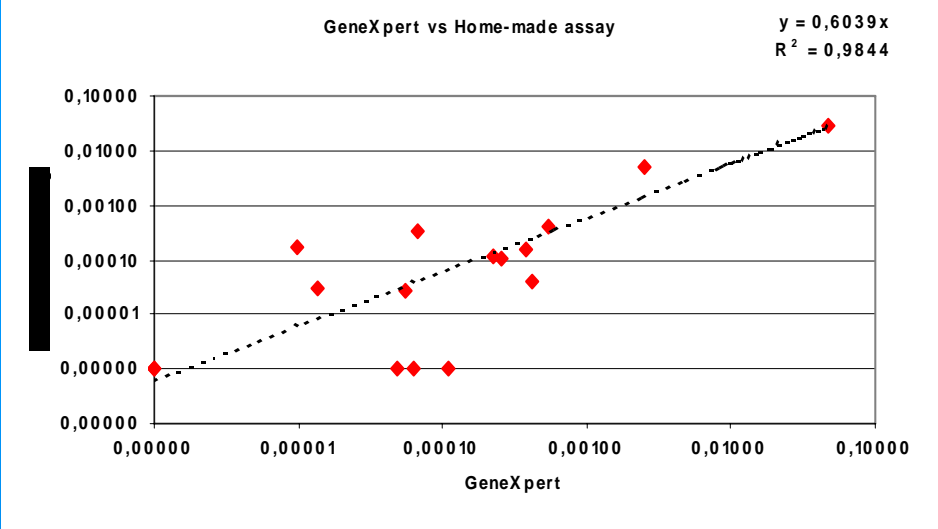
# GeneXpert results

QUALITATIVE RESULTS	
VALID SAMPLES	16
NEGATIVE WITH ALL	2
NEGATIVE WITH BOTH RTqPCR METHODS/ POSITIVE WITH GENEXPERT	2
NEGATIVE WITH ONE RTqPCR METHODS/ POSITIVE WITH GENEXPERT	2
POSITIVE WITH ALL	10

20 samples analyzed:  
16 produced a valid result  
4 samples (25%) were  
not evaluable (analysis  
not repeated)

FAILED SAMPLES: analysis results and error messages							
N.	ABL			BCR-ABL			TEST RESULT
	Ct	curve fit	result	Ct	curve fit	result	
1	12,5	NA	FAIL	12,3	PASS	INVALID	INVALID
4	NA	NA	NO RESULT	NA	NA	NO RESULT	ERROR (aborted)
8	27,4	NA	FAIL	NA	NA	INVALID	INVALID
12	12	PASS	PASS	31,2	FAIL	INVALID	INVALID

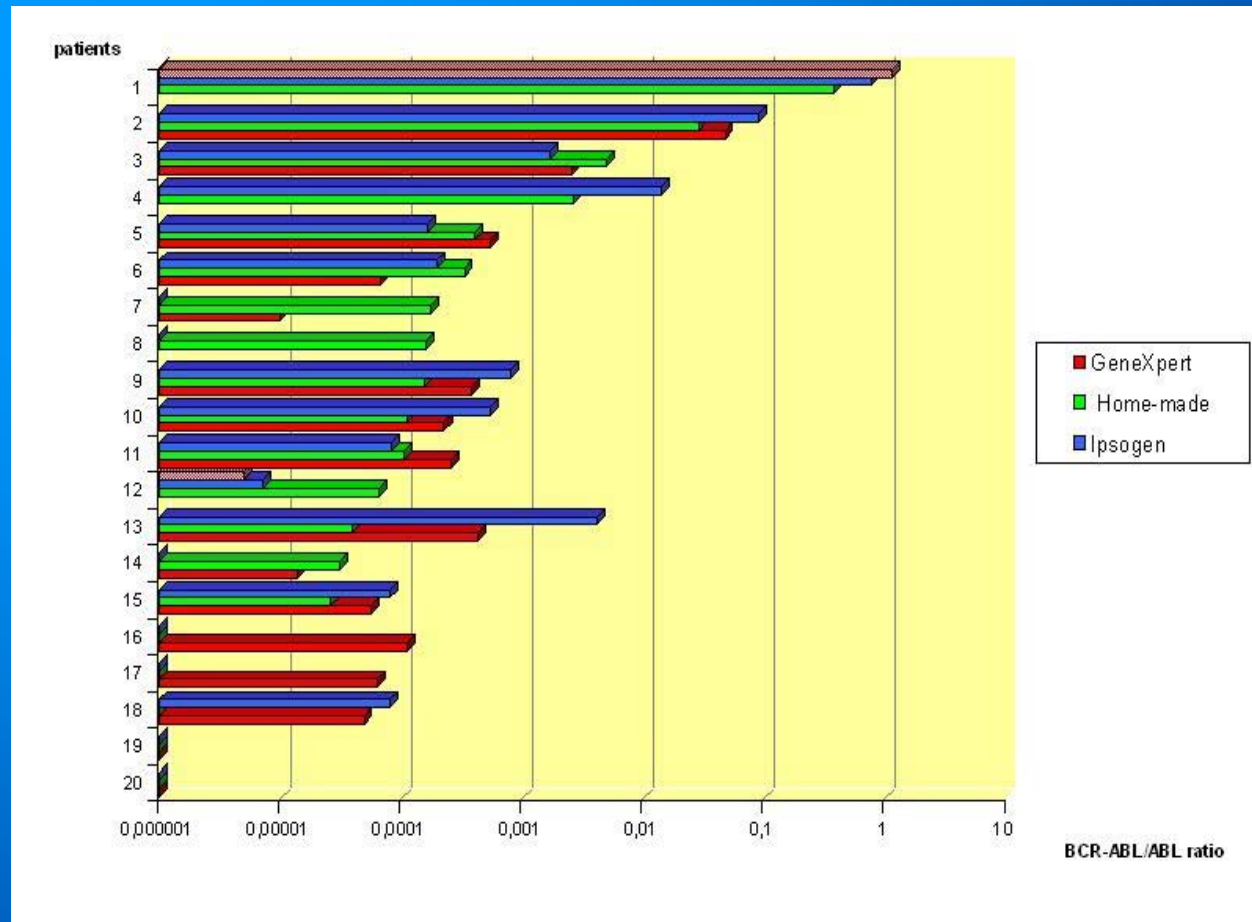
NA= NOT AVAILABLE



# Samples comparison

The 20 samples analyzed: results are expressed as BCR-ABL/ABL ratio for both GeneXpert and Home-made assay; Ipsogen results are expressed as NCN (Normalized Copy Number)

Sample	Copy n.	
	ABL	BCR/ABL
1	6145,47	4673,67
2	3751,25	333,64
3	4125,05	6,99
4	3970,27	56,02
5	4480,15	0,73
6	1429,64	0,28
7	836,11	0,00
8	3609,79	0,00
9	3965,76	3,18
10	5856,67	3,12
11	10117,73	0,84
12	8385,75	0,06
13	9125,33	37,38
14	5549,18	0,00
15	4793,95	0,38
16	462,09	0,00
17	2939,11	0,00
18	4031,87	0,32
19	9205,26	0,00
20	3725,89	0,00



# Bone marrow evaluation

	BCR-ABL/ABL ratio		
	Genexpert	Home-made	Ipsogen
Peripheral blood	0,00252	0,00491	0,00169
Bone marrow	0,00055	0,00040	0,00016

Bone marrow evaluation was successful and analysis of peripheral blood/bone marrow in the same patients provided comparable results with the 3 methods

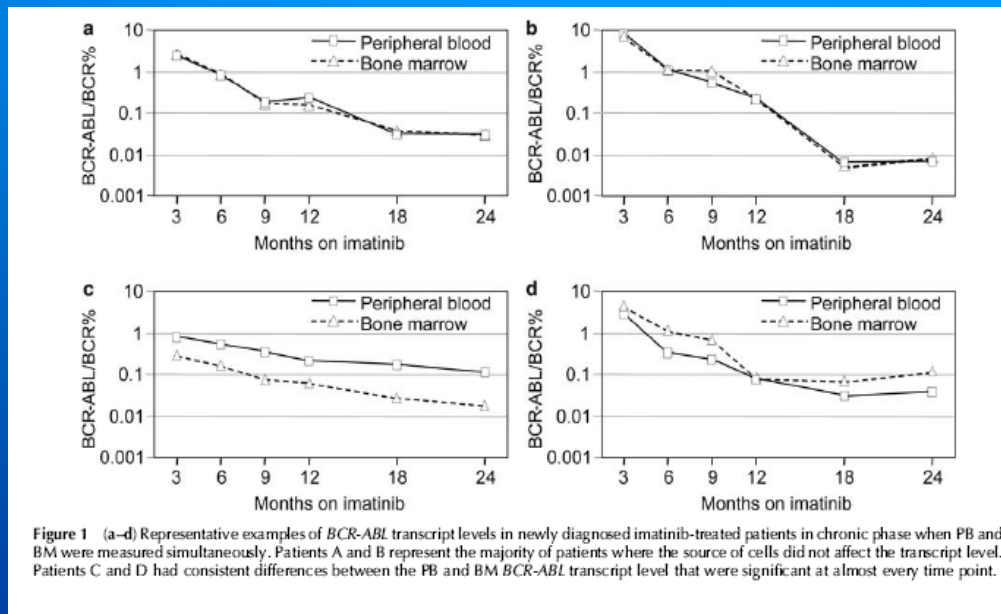


Figure 1 (a-d) Representative examples of BCR-ABL transcript levels in newly diagnosed imatinib-treated patients in chronic phase when PB and BM were measured simultaneously. Patients A and B represent the majority of patients where the source of cells did not affect the transcript level. Patients C and D had consistent differences between the PB and BM BCR-ABL transcript level that were significant at almost every time point.

The examined patient represents a situation that may be observed during molecular monitoring (fig.C), that is peripheral blood showing higher transcripts levels than bone marrow

Branford S. et al.  
Leukemia 20: 1925-30  
(2006)

# Cell number comparison

sample n.	WBC x10 <sup>3</sup> /μl	neutrophil %	GeneXpert		RT-qPCR		
			volume μl	analyzed cell n. x10 <sup>6</sup>	isolated MNC n. x10 <sup>6</sup>	tot RNA μg	estimated cell n. x10 <sup>6</sup> in RT
1	50,64	89,7	50	2,53	15,00	8,06	1,86
2	3,71	34,8	200	0,74	10,89	10,26	1,06
3	4,41	56,7	200	0,88	8,59	11,75	0,73
4	7,34	59,8	200	1,47	13,28	18,23	0,73
5*	na	na	50	na	15	33,69	0,45
6	3,63	38,2	200	0,73	10,10	12,85	0,79
7	5,38	62,3	200	1,08	9,13	12,28	0,74
8	na	na	200	na	10	19,06	0,52
9	5,52	70	200	1,10	7,45	11,21	0,66
10	5,48	46	200	1,10	13,32	14,34	0,93
11	5,74	52,1	200	1,15	12,37	19,09	0,65
12	5,47	39,4	200	1,09	14,92	16,60	0,90
13	5,6	46,8	200	1,12	13,41	9,96	1,35
14	5,55	60,2	200	1,11	9,94	15,40	0,65
15	4,82	47,3	200	0,96	11,43	6,81	1,68
16	6,03	53	200	1,21	12,75	13,15	0,97
17	7,93	69,3	200	1,59	10,96	6,48	1,69
18	7,12	63,1	200	1,42	11,82	11,29	1,05
19	5,12	58,1	200	1,02	9,65	13,66	0,71
20	5,72	44,2	20	0,11	14,36	19,31	0,74
mean	8,07	55,06		<b>1,13</b>	11,72	14,17	<b>0,94</b>
sd	10,68	13,47		0,47	2,26	6,04	0,40
min	3,63	34,80		0,11	7,45	6,48	0,45
max	50,64	89,70		2,53	15,00	33,69	1,86

\* bone marrow sample

# Conclusions

## Technical issues:

- User friendly
- Better standardization
- CML with high WBC count and transcript levels?
- Bone marrow or other samples (RNA, isolated cells)?
- WBC count before analysis (advisable)
- absolute copy number?

## Economical issues:

- cost of repeated samples

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