Quantitative Real-time Polymerase Chain Reaction (Coupled with High-resolution Melting) for Simultaneous Detection and Quantification of Four *BCR::ABL1* Isoforms

T. Zhou[1], A. Mohamed[2], B. Legendre[2], H. Newell[2], K.S.J. Elenitoba-Johnson[1]
1. Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
2. Precipio Inc, New Haven, Connecticut, USA.

INTRODUCTION

The diagnosis and management of chronic myeloid leukemia (CML) critically rely on detecting and serially quantifying *BCR::ABL1*.

Currently, no clinical assay simultaneously detects multiple *BCR::ABL1* isoforms; each isoform requires a separate analysis, which is labor-intensive and cost-inefficient.

This technical constraint results in several important limitations in clinical practice:

- Baseline limits: testing is typically limited to p210/ p190, which may miss p230 or atypical isoforms as primary drivers when co-expressed with low-level p210/p190.
- Monitoring gap: standard monitoring tracks only baseline transcripts, potentially overlooking emerging novel isoforms during evolution, falsely suggesting disease stability and delaying therapeutic adjustments.
- Coverage gap: the prevalence and significance of e13a3/e14a3 (p203)—anecdotally linked to asciminib resistance—remain unclear.

AIM

- To develop a single multiplex assay that simultaneously detects and quantifies four clinically relevant *BCR::ABL1* transcripts—e1a2/e1a3 (p190), e13a2/e14a2 (p210), e19a2/e19a3 (p230), and e13a3/e14a3 (p203).
- To evaluate its analytical performance and clinical utility.

METHOD

We developed the **BloodHound** assay, employing multiplex RT-qPCR. This assay achieves a limit of detection of 0.001%, 99.9% sensitivity, and > 99% specificity, when validated against droplet digital PCR and Sanger sequencing.

Using **BloodHound**, we characterized the distribution and co-expression of the four transcripts in 895 samples (**Table 1**), from patients with suspected (n = 566), established (n = 296), relapsed (n = 32) CML, or unknown status (n = 1).

Sanger sequencing and Qiagen ipsogen IS assay were used for orthogonal confirmation.

RESULTS

Overall *BCR::ABL1* detection: 187/895 samples (20.9%)

- Suspected CML: 3.5% (20/566)
- Established CML under monitoring: 33.1% (98/296)
- Relapsed disease: 100% (32/32)

Isoform distribution

- p210 alone: 161/187 (86.1%)
- p190 alone: 2/187 (1.1%)
- p230 alone: 1/187 (0.5%)
- P203: not detected.
- p190 and p210 co-expression: 23/187, 12.3%) was more frequent than historically appreciated, particularly in baseline diagnostic specimens (25.0%).
- When co-expressed, p210 levels were markedly higher than p190 (median p210:p190 ratio = 2,152).

Orthogonal confirmation:

- Qualitative results showed 100% concordance with Sanger sequencing.
- Quantitative values correlated tightly with the standardized Qiagen ipsogen IS assay (r = 0.998; median absolute difference = 0.02%, **Fig. 1**).

Figure 1. Correlation of p210 transcript levels between the BloodHound™ assay and the parallel Qiagen assay.

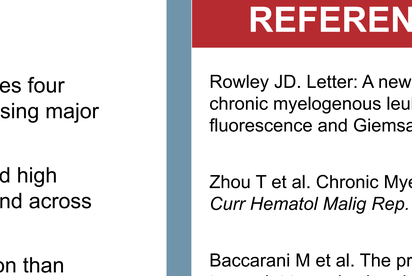

Table 1. Specimen types and disease status

Characteristics	Values
Total specimens tested; N	895
Specimen types; N (%)	
BM	49 (5.5%)
PB	846 (94.5%)
Overall disease status; N (%)	
Suspected/baseline CML	566 (63.2%)
Post-therapy	296 (33.1%)
Relapse	32 (3.6%)
Unknown	1 (0.1%)

Table 2. Distribution of BCR::ABL1 transcripts

BCR::ABL1 positivity	n/N (%)
Total positive specimens	187/895 (20.9%)
p190 alone	2/187 (1.1%)
p210 alone	161/187 (86.1%)
p230 alone	1/187 (0.5%)
p203 alone	0
p190 + p210 co-expression	23/187 (12.3%)
All other co-expression	0
Suspected/baseline CML	20/566 (3.5%)
p190 alone	0
p210 alone	14/20 (0.0%)
p230 alone	1/20 (5.0%)
p190 + p210 co-expression	5/20 (25.0%)
Post-therapy	135/296 (45.6%)
p190 alone	2/135 (1.5%)
p210 alone	118/135 (87.4%)
p230 alone	0
p190 + p210 co-expression	15/135 (11.1%)
Relapse	32/32 (100.0%)
p190 alone	0
p210 alone	29/32 (90.6%)
p230 alone	0
p190 + p210 co-expression	3/32 (9.4%)

Technical and Clinical Advantages of Bloodhound

- Single-reaction multiplexing
- Pre-plated panel with embedded primers and controls, simplifying implementation and reducing handling errors
- High analytical performance
- Compatible with standard laboratory equipment
- Robust intra-laboratory, inter-laboratory, and inter-primer set standardization
- Potentially interlaboratory standardization of non-p210 quantification

CONCLUSIONS

- BloodHound simultaneously detects and quantifies four *BCR::ABL1* isoforms in a single reaction, addressing major gaps in CML testing.
- The assay shows robust clinical performance and high analytical concordance with standard methods and across diagnostic, monitoring, and relapse settings.
- Co-expression of p190 and p210 is more common than previously reported
- BloodHound has the potential to significantly streamline clinical workflows, enhance diagnostic precision, and provide deeper insights into CML clonal dynamics.

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CONTACT INFORMATION

Precipio, Inc
WWW.Precipiodx.com
Contact@precipiodx.com
203-787-78888