Biomolecular Detection and Quantification 11 (2017) 4–20

Contents lists available at ScienceDirect

Biomolecular Detection and Quantification

journal homepage: www.elsevier.com/locate/bdq

Review article

Molecular techniques for the personalised management of patients with chronic myeloid leukaemia

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ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form
28 December 2016
Accepted 18 January 2017
Available online 14 February 2017
Handled by Jim Huggett

Keywords:
CML
Molecular monitoring
MRD
dPCR
NGS

ABSTRACT

Chronic myeloid leukemia (CML) is the paradigm for targeted cancer therapy. RT-qPCR is the gold standard for monitoring response to tyrosine kinase-inhibitor (TKI) therapy based on the reduction of blood or bone marrow *BCR-ABL1*. Some patients with CML and very low or undetectable levels of *BCR-ABL1* transcripts can stop TKI-therapy without CML recurrence. However, about 60 percent of patients discontinuing TKI-therapy have rapid leukaemia recurrence. This has increased the need for more sensitive and specific techniques to measure residual CML cells. The clinical challenge is to determine when it is safe to stop TKI-therapy. In this review we describe and critically evaluate the current state of CML clinical management, different technologies used to monitor measurable residual disease (MRD) focus on comparingRT-qPCR and new methods entering clinical practice. We discuss advantages and disadvantages of new methods.

Contents

Abbreviations: ABL1, Abelson murine leukaemia virus; ALL, acute lymphoblastic leukaemia; allo-SCT, Allogeneic Stem Cell Transplantation; AP, accelerated phase; ARQ, armored RNA Quant; ATP, adenosine triphosphate; BC, blast crisis; BCR, breakpoint cluster region; BM, bone marrow; BMT, bone marrow transplantation; Bp, base pair; CAP, College of American Pathology; cDNA, coding or complimentary DNA; CES, capillary electrophoresis sequencing; CML, chronic myeloid leukaemia; CMR, complete molecular response/remission; CP, chronic phase; DESTINY, De-Escalation and Stopping Treatment of Imatinib, Nilotinib or sprYcel in Chronic Myeloid Leukaemia; dMIQE, Minimum Information for Publication of Quantitative Digital PCR Experiments; DNA, deoxyribonucleic acid; dPCR, digital polymerase chain reaction; EAC, Europe Against Cancer; ELN, European Leukaemia Net; emPCR, emulsion PCR; EURO-SKI, European Stop Tyrosine Kinase Inhibitor Study; gDNA, genomic deoxyribonucleic acid; GUSB, glucuronidase beta gene; IC, inhibotory concentration; InDels, insertions and deletions; IRIS, interferon and cytarabine versus STI571; IS, International Scale; Kbp, Kilo Base Pairs; KDa, Kilo Dalton; LoD, limit of detection; LoQ, limit of quantification; LPC, leukemic progenitor cells; LSC, leukemic stem cell; Mbp, mega base pair; M-bcr, major-breakpoint cluster region; m-bcr, minor-breakpoint cluster region; μ-bcr, micro-breakpoint cluster region; MMR, major molecular response/remission; MR, deep molecular response/remission; MRD, minimal residual disease; mRNA, messenger RNA; μg, microgram; μl, microliter; NCCN, National Comprehensive Cancer Network; NEQAS, National External Quality Assessement Service; nM, manomolar; NGS, next generation sequencing; NTC, No Template Control; PB, Peripheral Blood; PCR, Polymerase Chain Reaction; PFS, Progression Free Survival; Ph, Philadelpia; QC, Quality Control; Q-PCR, quantitative polymerase chain reaction; RT, reverse transcription; RT-dPCR, reverse transcription-digital polymerase chain reaction; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SCT, stem cell transplant; SMRT, single-molecule real-time sequencing; STIM, stop imatinib; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitor; WHO, World Health Organisation; ZMW, zero-mode wave-guided.

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http://dx.doi.org/10.1016/j.bdq.2017.01.001

1. Introduction

Chronic myeloid leukaemia (CML) was recognized as a clinical entity in the early 19th century on the grounds extensive splenomegaly and leukocytosis [1–3]. In 1960, almost 100 years later, a consistent chromosome termed the Philadelphia (Ph) chromosome was described in the cells of patients with CML by Nowell and Hungerford [4]. In 1973, Janet Rowley [5] reported the Ph chromosome resulted from a reciprocal translocation between chromosomes 9 and 22. In 1980s the fusion of two genes, *BCR* and *ABL1*, was identified as causing CML [6–9], *BCR-ABL1* results in constitutive activation of the ABL1 tyrosine kinase domain which accounts for the disease phenotype [10,11]. In the late 1990s the BCR-ABL1 protein was recognised as a potential drug-able target and led to the development of several ABL1 tyrosine kinase inhibitors (TKI). Their introduction into clinical use has changed the course of CML: a one-time fatal disease is now a condition associated with a life expectancy similar to the normal age-matched population [12].

CML is a tri-phasic disease. It usually presents in a chronic phase (CP) marked by over-production of mature granulocytes (with <10% blasts in the blood and bone marrow. Untreated chronic phase CML invariably transforms into acute phase resembling acute lymphoid or myeloid leukaemia with >20% blasts in the blood or bone marrow. Many patients have in intermediate phase termed accelerated phase which is often poorly-defined with 10–20% blasts [13].

CML has a world-wide annual incidence of 1–2/100,000 population with a slight male predominance and accounts for 15% of adult leukaemia [14] in the Western hemisphere. Median age at onset is 60 years with a wide range [15].

2. The molecular features of CML

Most cases of CML have t(9;22). Other chromosome rearrangements such as complex translocations or insertions occur in some cases [16,17]. t(9;22) is also detected in 25–30% of adults and 5–10% of children with acute lymphoblastic leukaemia (ALL) [18–20]. Some of these patients may have had a clinically undetected chronic phase.

The molecular hallmark of CML is the exchange of genetic material between the long arms of chromosomes 9 and 22 [t(9;22)(9q34.1;22q11.2)]. This translocation joins the 5′ part of the *BCR* (the gene covers ~138.5Kbp region; 23 exons) on chromosome 22 and the 3′ part of *ABL1* (the gene covers ~174Mbp; 11 exons) on chromosome 9 forming the *BCR-ABL1* fusion oncogene [21].

The breakpoint in *ABL1* is typically in the 150 kb intronic region between exons 1a and 1b. Rarely the breakpoint is upstream exon 1b or downstream of exon 1a [22–24] but almost invariably upstream of exon 2.

Breakpoints in *BCR* are more variable but tend to occur within three main breakpoint cluster regions: the major (M-*bcr*) [8], minor (*m-bcr*) [25,26] and micro (μ-*BCR*) [27] regions. Breakpoints in the M-*BCR* region are associated with two major transcripts designated e13a2 (b2a2) and e14a2 (b3a2). The exons within the M-*BCR* region previously numbered b1-5 were later renamed e12-e16 after the successful mapping of the entire *BCR* gene [28]. Both transcripts