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Chronic myeloid leukemia: Relevance of cytogenetic and molecular assays

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Contents

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ABSTRACT

Chronic myeloid leukemia (CML) is the prototype cytogenetic malignancy. Even before the development of basic G- and R-banding techniques, CML was found to be associated with a persistent chromosomal abnormality, the Philadelphia (Ph) chromosome. Banding technology later showed the marker chromosome to be a translocation between the breakpoint cluster region (*BCR*) on chromosome 22q11.2 and the Abelson proto-oncogene (*ABL*) on chromosome 9q34. Further advances in cytogenetic and molecular biology have also contributed to the understanding, diagnosis, and treatment of CML. Fluorescent in situ hybridization (FISH) has revealed cryptic translocations in most cases of Ph-negative CML. Additional rare chromosomal variant translocations have been discovered as well.

The understanding of cytogenetic and molecular physiopathology of CML has led to the use of tyrosine kinase inhibitors as treatment for this disease with spectacular success. Over the 40 years since being identified as the first cytogenetic disease, CML has become the greatest success in translating the basic science of oncology into the treatment of patients with cancer.

In this review we will not only summarize the biology of CML, recent progress in the delineation of mechanisms and treatment strategies, but also we will discuss the laboratory tools used for diagnosing CML, for monitoring during treatment and for revealing point mutations and additional chromosomal abnormalities. In doing so, we will describe in detail our individual research on CML, identifying why and how these tests were performed to help to explain CML subgroups and clinical significance of additional chromosomal abnormalities.

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1. Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of a pluripotent stem cell; it was first described in 1845, when several cases of splenomegaly, anemia and massive granulocytosis were illustrated (Bennett, 1845). Neumann deduced that the disease originated in the bone marrow and called "myeloid leukemia" (Neumann, 1878). In 1960, the discovery of the Philadelphia (Ph) chromosome led to a better understanding of the pathogenesis of the disease (Nowell and Hungerford, 1960).

Thirteen years later, the Ph chromosome was shown to be generated by a specific translocation involving chromosomes 9 and 22 (Rowley, 1973).

The natural history of CML is characterized by a biphasic evolutive course. Patients are usually diagnosed in the chronic phase (CP) of the disease but they eventually progress to a terminal, acute leukaemia-like phase, the so-called blast crisis (BC) of CML, sometimes preceded by an accelerated phase (AP) (Melo and Barnes, 2007).

In 1980, the *BCR-ABL* fusion oncogene was described and found to be transcribed into a functional protein. P210 *BCR-ABL* differed both in terms of its subcellular localization and its tyrosine kinase activity, from the endogenous c-ABL protein (Faderl et al., 1999a; Wang et al., 1984).

The progress in the understanding of the molecular physiopathology of CML has led to the development of several novel therapeutic approaches targeting various steps of the malignant transformation (Faderl et al., 1999a; Wang et al., 1984; Deininger et al., 2000).

In this review we will summarize the biology of CML with the recent progress in the delineation of mechanisms and treatment strategies and we will discuss the laboratory tools used for diagnosing CML, for monitoring during treatment and for revealing point mutations and additional chromosomal abnormalities. In doing so, we will describe in detail our individual research in CML, identifying why and how these tests were performed to help to explain CML subgroups and clinical significance of additional chromosomal abnormalities.

1.1. Treatment of CML

For many years, the treatment strategy for CML patients was based on chemotherapeutic agents such as busulfan and hydroxyurea. But these agents failed to eliminate the malignant clones (Hehlmann et al., 1994). In the mid-1970s, allogeneic stem cell transplantation leaded to the disappearance of the Ph-positive clone in CML patients (Kantarjian et al., 1995).

In 1980s, the introduction of interferon-alpha to the clinical treatment leads to complete cytogenetic response and long-term survival, but not in all patients (Kantarjian et al., 1995). In subsequent years allogeneic SCT6 and INFα7 therapy became the treatment of choice offered to CML patients.

Recognition that the *BCR-ABL* chimeric protein is a pivotal contributor to the disease pathogenesis and progression, led, in the late 1990s, to the synthesis of a tyrosine kinase inhibitor (TKI) that inhibited ABL and other tyrosine kinases and revolutionized CML therapy (Kantarjian et al., 1995; Druker, 2008). Imatinib was found to specifically inhibit the tyrosine kinase enzyme and prevent its activity (Druker et al., 1996). A new generation of TK inhibitors, nilotinib (Tasigna^{R}), dasatinib (Sprycel^{R}), Bosutinib (Bosulif^{TM}), Ponatinib (Iclusig^{TM}) and Omacetaxine (Synribo^{TM}) are now available and are used if imatinib therapy fails (Kantarjian et al., 1995; Buchdunger et al., 1995).

1.2. Response definition

The association between cytogenetic response (CyR) and improved survival made the cytogenetic response the gold standard of CML therapy.

Many studies precisely defined the conditions for the optimal, suboptimal, or failure to TKI treatment (Baccarani et al., 2009; Baccarani et al., 2013). In the previous versions of the ELN recommendations (Baccarani et al., 2009), complete cytogenetic response (CCyR) was defined as 0% Ph+ metaphases, partial cytogenetic response (PCyR) as 1–35% Ph+ metaphases, minor cytogenetic response (mCyR) as 36–65% Ph+ metaphases, minimal cytogenetic response (min CyR) as 66–95% Ph+ metaphases and no response (NR) as >95 of Ph+ metaphases (Baccarani et al., 2009).

Cytogenetics must be performed by chromosome banding analysis (CBA) of marrow cell metaphases, counting at least 20 metaphases, at 3, 6, and 12 months until a CCyR is achieved (Baccarani et al., 2009).

Response definitions were based on the total peripheral blood cell count normalization and the achievement of CCyR by CBA, relative to the time from diagnosis (Baccarani et al., 2009; Baccarani et al., 2013, 2008; Hughes et al., 2006). CBA can be substituted by FISH only when a CCyR has been achieved.

However, more sensitive monitoring assays, such as the polymerase chain reaction, show that even in the state of CCyR, more than 2.5×107 leukemic cells might still be present in the circulation (Baccarani et al., 2008).

This finding led to the definition of a third response level, the molecular response, based on molecular assays that detect residual leukemic cells (Baccarani et al., 2009; , 2013, 2008; Hughes et al., 2006, 2008 Hughes et al., 2006).

Currently, the response to first line treatment was not limited only to imatinib, but it is irrespective to the TKI that is used (Baccarani et al., 2013). The responses are defined as "optimal", "warning or "failure". Optimal response is associated with the best long-term outcome—that is, with a duration of life comparable with that of the general population, indicating that there is no indication for a change in that treatment. Failure means that the patient should receive a different treatment to limit the risk of progression and death. (Baccarani et al., 2013)

For the follow-up of CML patients, the European LeukemiaNet (Baccarani et al., 2013) requires molecular response monitoring every 3 months until a major molecular response (MMR) (at least) is achieved, then every 3 to 6 months.

BCR-ABL transcript levels $\leq$10% at 3 months, <1% at 6 months, and $\leq$0.1% from 12 months onward define optimal response, whereas >10% at 6 months and >1% from 12 months onward define failure. Similarly, PCyR at 3 months and CCyR from 6 months onward define optimal response, whereas no CyR at 3 months, less than PCyR at 6 months, and less than CCyR from 12 months onward define failure. Between optimal and failure, there is an intermediate warning zone requiring more frequent monitoring. Similar definitions are provided for response to second-line therapy. Specific recommendations are made for patients in the accelerated and blastic phases, and for allogeneic stem cell transplantation. Optimal responders should continue therapy indefinitely, with careful surveillance, or they can be enrolled in controlled studies of treatment discontinuation once a deeper molecular response is achieved. (Baccarani et al., 2013).

1.3. Molecular basis of CML

The BCR-ABL fusion protein acts as an oncoprotein by activating several signaling pathways that lead to transformation. *Myc, Ras, c-Rafn MAP/ERK, SAPK/JNK, STAT, NFKB, PI-3*kinase and *c-Jun* are included as signal cascade molecules (Cortez et al., 1997;