Best Practice & Research Clinical Haematology 28 (2015) 32–42

Contents lists available at ScienceDirect

Best Practice & Research Clinical Haematology

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

4

Mutational profiling in patients with MDS: Ready for every-day use in the clinic?

Ulrike Bacher, MD [1], Alexander Kohlmann, PhD [1], Torsten Haferlach, MD [*]

MLL Munich Leukemia Laboratory, Munich, Germany

Keywords:
myelodysplastic syndrome (MDS)
mutations
amplicon deep-sequencing
next-generation sequencing
NGS
diagnostic algorithm

Multiple recurrent somatic mutations were identified in the majority of patients with myelodysplastic syndromes (MDS), but investigating the broad spectrum of molecular markers in MDS exceeds many laboratories' capacity when traditional molecular techniques are used. High-throughput second generation sequencing (=next-generation sequencing, NGS) has proven to be applicable for comprehensive biomarker mutation analyses allowing to increase diagnostic sensitivity and accuracy and to improve risk stratification and prognostication in addition to cytomorphology and cytogenetic analysis in patients with MDS. Amplicon deep-sequencing enables comprehensive biomarker analysis in a multitude of patients per investigation in an acceptable turn-around time and at affordable costs. Comprehensive myeloid marker panels were successfully introduced into diagnostic practice. Therefore, molecular mutation analysis is ready for use in all patients with suspected MDS, may contribute to risk stratification in possible candidates for allogeneic stem cell transplantation, and should become an integral part of clinical research studies in MDS patients.

* Corresponding author. MLL Munich Leukemia Laboratory, Max-Lebsche-Platz 31, 81377 Munich, Germany. Tel.: +49 89 990 17 100; Fax: +49 89 990 17 109.
E-mail address: torsten.haferlach@mll.com (T. Haferlach).

URL: http://www.mll.com

[1] Equal contribution.

http://dx.doi.org/10.1016/j.beha.2014.11.005
1521-6926/

Introduction

Thus far, cytomorphology and cytogenetics in combination were providing the basis for the diagnosis of myelodysplastic syndromes (MDS). Also, prognostic classification and risk assessment as suggested by the IPSS [1]/IPSS-R [2] and the WHO 2008 guidelines [3] centrally focus on these aspects. In cases with normal karyotypes and without increase of blasts or ring sideroblasts, the establishment of a diagnosis of MDS in many cases requires repeated bone marrow analyses and remains a difficult task even for experienced morphologists.

Differently from acute leukemias, where molecular diagnostics had been part of the diagnostic and prognostic workup since more than a decade, molecular markers so far played practically no role for diagnosis and prognostication in patients with MDS. This changed rapidly in the past years, as the introduction of the novel second or next-generation sequencing (NGS) techniques catalyzed the detection of a multitude of mutations in this heterogenous entity. Spliceosome gene mutations such as *U2AF1*, *SF3B1*, *SRSF2*, or *ZRSR2* were identified as recurrently mutated genes and were shown to be associated with distinct cytomorphology or MDS risk subgroups [4,5]. Investigating two independent large cohorts of patients with MDS by large-scale genomic approaches, Papaemmanuil et al. [6] and Haferlach and colleagues [7] detected and confirmed additional molecular mutations in 74% and 89.5% of patients, respectively. Moreover, a novel prognostic score for MDS patients based on molecular mutations only was reported in our study [7]. In its recent recommendations (2013), the European Leukemia Net (ELN) had already suggested to include mutation analysis of candidate genes in the diagnostic workflow for patients with MDS [8].

In sight of these achievements in the molecular pathogenetic understanding of MDS, the question arises whether and how these new diagnostic parameters can be integrated into clinical practice. This review article outlines the presently available molecular armamentarium and discusses indications for mutation analysis in patients with MDS.

Molecular techniques

Conventional PCR methods

For molecular PCR analyses, genomic DNA or cDNA following reverse transcription from RNA provides the basis. Standard PCR and visualization of the amplification products by gel electrophoresis or fragment analysis is used for example for detecting *FLT3*-ITD mutations as they consist of insertions of variable length spanning up to hundreds of base pairs [9]. Multiplex-PCR is performed by a single reaction mixture simultaneously carried out with multiple primer sets. Reverse transcription PCR (RT-PCR) is used for subsequent amplification of known RNA sequences and may be used e.g. for detection of reciprocal rearrangements. Melting-curve based PCR uses fluorescence-tagged gene probes. Quantitative real-time PCR allows to detect a distinct gene sequence, e.g. a distinct *NPM1* mutation subtype [10], and to exactly quantify the amount of amplification products. Mutations that are detected by these or other techniques were so far confirmed and subclassified by Sanger sequencing [11].

Next-generation sequencing (NGS)

Despite the great achievements of the Sanger sequencing technology, such as the completion of the initial reference human genome sequence, there were restrictions with respect to time and labor intensiveness and the limited sensitivity of mutation detection as being highly relevant for different hematological malignancies of myeloid or lymphatic origin [12]. Therefore, novel sequencing technologies were strongly desired [13]. The introduction of various second-generation sequencing assays in recent years revolutionized the field sequencing technology. NGS, and in particular amplicon deep-sequencing assays, were successfully introduced into diagnostics of hematological malignancies [14–16].

Template preparation, sequencing, imaging, and data analysis steps vary widely between different commercially available NGS platforms, of which only a few can be addressed in some detail [13]. The 454 Life Sciences Titanium chemistry (Roche Applied Science) represents a large-scale miniaturized