EUROPEAN HEMATOLOGY ASSOCIATION

Acute myeloid leukemia - Section 1

Molecular diagnostics in acute myeloid leukemia

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Take-home messages

- There is a growing need to implement novel next-generation-sequencing (NGS) based gene panel diagnostic tools to rapidly capture inter- and intra-individual disease heterogeneity.
- Future technological developments will enable genome-wide comprehensive genomic, epigenomic and transcriptomic characterization of the disease (at single cell level), but for now these approaches are reserved for research questions.
- Molecular genomics have started to inform patient care with regard to improved disease classification and risk prediction (knowledge databases), MRD monitoring and guiding targeted therapeutic approaches.

Introduction

For many years, genomic aberrations have been known to play an important role in the pathogenesis of acute myeloid leukemia (AML) and have become well established diagnostic and prognostic markers.[1,2] Since the turn of the century, advances in microarray and next-generation sequencing (NGS)-based "omics" technologies have contributed to an exponential knowledge growth of the molecular aberrations underlying AML,[3,4] but only recently molecular diagnostics have begun to translate into improved disease classification and clinical care.[5,6] In this article, I will provide a brief overview of the heterogeneous genomic landscape of AML and its impact on molecular diagnostics, including recent advances in genomics-based AML classification and patient care.

Current state of the art

Genomic landscape

Following first comprehensive studies using high-throughput microarray technologies, AML was also the first tumor genome to be completely sequenced using the novel NGS technology.[7] Subsequent studies led to the identification of novel recurrent somatic mutations of biologic, prognostic, and therapeutic relevance, and they identified AML as complex and dynamic disease characterized by a high inter- and intra-individual heterogeneity. Genome-wide profiling of 200 *de novo* AML cases within the 'The Cancer Genome Atlas (TCGA)' project revealed an average of 13 coding mutations [single nucleotide variations (SNVs), and insertions/deletions (indels)] per adult AML as well as a median of one somatic copy-number variant (e.g., trisomies or monosomies) and an average of less than one gene-fusion event.[3] While the recurrently mutated genes included known candidates (such as *NPM1, FLT3, CEBPA, DNMT3A, IDH1*, and *IDH2*) as well as genes just recently implicated in leukemogenesis (including *EZH2, U2AF1, SMC1A*, and *SMC3*), the mutational patterns were non-random of co-occurrence and mutual exclusivity. Especially *NPM1*, *CEPBA*, and *RUNX1* mutations were mutually exclusive of transcription factor fusions, thereby indicating that these aberrations might be leukemia-initiating events similar to the fusion genes.

Clonal evolution

Analysis of the variant allele frequency (VAF) demonstrated that over half of the TCGA cases exhibited at least one subclone in addition to a founding leukemia clone (the clone showing the highest VAF values).[3] Together with other studies, these data support a clonal evolution concept in which epigenetic regulator mutations (e.g. *DNMT3A, TET2*, and *ASXL1* mutations) or splicing factor gene mutations (e.g. *SF3B1,* and *SRSF2* mutations) occur as early founder events in pre-leukemic progenitor cells prior to transforming leukemogenic events (e.g. *NPM1* or signaling molecule mutations). In accordance, recurrent mutations in epigenetic regulators and splicing factor genes can be found in the blood of mainly elderly patients,[8,9] and the term 'clonal hematopoiesis of indeterminate potential' (CHIP) was proposed to describe the presence of leukemia-associated somatic mutations in blood or bone marrow in the absence of conventional diagnostic criteria for a hematologic malignancy.[10] While the transformation rate of CHIP into a hematologic malignancy is 0.5-1% per year, in the future the role of persisting CHIP following leukemia treatment will have to be better understood by monitoring of minimal residual disease (MRD) for both pre-leukemic and leukemic markers.

Molecular diagnostics

Today, conventional cytogenetic analysis remains mandatory for the AML workup, although molecular testing by reverse transcriptase–polymerase chain reaction (RT-PCR) for recurring rearrangements can be useful if cytogenetic analysis fails and in the future whole genome sequencing approaches might fill in. Molecular genetic diag-

nostics, as recommended by the European LeukemiaNet (ELN),[6] should comprise at least screening for (i) disease defining mutations in *NPM1*, *CEBPA*, and *RUNX1* genes; (ii) prognostic and targetable mutations in *FLT3*, both tyrosine kinase domain mutations (at codons D835 and I836) mutations and internal tandem duplications [ITDs] (including data on the mutant–to–wild-type allelic ratio); and (iii) mutations in *TP53* and *ASXL1* that have consistently been associated with poor prognosis (Table 1). While it is time consuming and cost ineffective to capture these aberrations by conventional sequencing strategies, the list of molecular markers informing clinical practice is growing and testing will have to be replaced by gene panel diagnostics. Currently, there are already a number of commercial and custom designed gene panels available,[11] but it will be crucial to invest in flexible platforms and to develop diagnostic tools that can simultaneously test for both gene mutations and gene rearrangements.[12,13]

Genomic classification

Leukemia-associated chromosomal translocations and inversions opened the avenue towards the genetic AML classification reflected in the currently updated World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia,[5] however, during recent years NGS studies have also been informing disease classification.[3,4,14] Beyond currently defined classes (such as the balanced rearrangements, AML with mutated *NPM1*, or biallelic mutated *CEBPA*), three more heterogeneous classes emerged, i.e. '*AML with mutated chromatin, RNA-splicing genes, or both*', '*AML with TP53 mutations, chromosomal aneuploidy, or both*', and '*AML with IDH2^{R172} mutation*' (without other class-defining lesions). Using this classification scheme, at least 80% of AML could ambiguously be categorized in a single group based upon the underlying genetic abnormalities.[4]

Genomics informed patient care

Recent advanced proved also that novel genetic information can be successfully applied to inform clinical practice. For example, a large knowledge bank of matched genomic-clinical AML data could be devised to accurately predict likelihoods of remission, relapse and mortality with findings being validated on independent TCGA data.[15] Future models based on increased patient numbers will allow to further reduce the error rate of such personalized treatment predictions, and European initiatives like HARMONY (Healthcare Alliance for Resourceful Medicines Offensive against Neoplasms in HematologY) are currently capturing, integrating, and harmonizing patient data from large AML cohorts to gain valuable novel insights (https://www.ehaweb.org/news/eha-news/article/125). Similarly, genomic knowledge does now also facilitate follow-up monitoring of MRD, and highly sensitive digital PCR as well as targeted ultra-deep NGS approaches are valuable novel tools adding to quantitative

Table 1. 2017 European LeukemiaNet (ELN) risk stratification by genetics.[a]

Risk Category[b]	Genetic Lesion
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITDlow(c) Biallelic mutated *CEBPA*
Intermediate	Mutated NPM1 and *FLT3*-ITD$^{high(c)}$ Wild type NPM1 without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ (w/o adverse-risk gene mutations) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[d] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, *MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,[e] monosomal karyotype[f] Wild type *NPM1* and *FLT3*-ITD$^{high(c)}$ Mutated *RUNX1*[g] Mutated *ASXL1*[g] Mutated *TP53*[h]

Adapted from: Dohner et al. Blood 2017;129:424-47; with permission. Frequencies, response rates and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated; [b]prognostic impact of a marker is treatment-dependent and may change with new therapies; [c]low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5); semi-quantitative assessment of FLT3-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve (AUC) 'FLT3-ITD' divided by AUC 'FLT3-wild type'; recent studies indicate that AML with NPM1 mutation and FLT3-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic hematopoietic-cell transplantation; [d]the presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations; [e]three or more unrelated chromosome abnormalities in the absence of one of the WHO-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with BCR-ABL1; [f]defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML); [g]these markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes; [h]TP53 mutations are significantly associated with AML with complex and monosomal karyotype.

reverse-transcriptase polymerase chain reaction (qRT-PCR) and multiparameter flow cytometry (MFC) methods. The NGS-based identification of molecular markers in almost 100% of diagnostic AML cases provides a prerequisite for comprehensive and individualized MRD assessment to identify patients at high relapse risk at early time points and to detect persistent pre-leukemic hematopoiesis.[16,17] Finally, genomics knowledge will allow us to better guide the use of novel drugs such as protein kinase inhibitors, epigenetic modulators, immune checkpoint inhibitors and cellular immunotherapies.[2,6] However, selective inhibition may only address distinct leukemia subclones. Thus, future molecularly targeted treatment designs will have to take clonal relationships into account and treatment strategies should be adjusted based on longitudinal clonal monitoring.

Future perspectives

Given a growing list of disease-relevant genes in AML, NGS-based gene panel diagnostics have started to enter our daily clinical routine. Today, rapid technical NGS advances allow for more accurate MRD assessment and start to offer the possibility to capture leukemia heterogeneity at the single cell level.[18,19] In addition, future developments will ultimately allow genome-wide unbiased tests at high quality based on which individualized treatment approaches can be further advanced.

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