Chapter

Haematological Malignancies: Overview of the Recent Progresses in Genetics

Mounia Bendari, Sofia Sraidi and Nisrine Khoubila

Abstract

Genetic defects play a major role in pathogenesis of the most of haematological malignancies, including cytogenetic abnormalities, gene mutations, and abnormal gene expression. Our knowledge about the genetics of haematological disorders has been dramatically improved during the past decade, due to revolution of sequencing technologies which have played a crucial role. In this chapter, we describe the techniques commonly employed for elucidating chromosomal aberrations, prognostic impact of recurrent chromosomal abnormalities, and recently updated risk stratification systems. We will summarise the chromosomal abnormalities recently identified on many of haematological diseases such acute myeloid leukaemia, acute lymphoid leukaemia, myelodysplasic syndrome, multiple myeloma, meyloproliferative disease and clarify their impacts on clinical phenotype and prognosis, as well as their role in the pathogenesis of these diseases. The aim of this chapter is to provide a brief overview of the recent progresses in haematological diseases genetics.

Keywords: clonal evolution, cytogenetic, somatic mutation, chromosomal abnormality, WHO classification

1. Introduction

The few recent years seen revolution on genetic technic with a big improvement of new technology. Haematological malignancy benefits from this development. Geneticists cooperate closely with hemato-oncologists; this cooperation improves many aspect of hemato-oncologists practice. It s can be helpful for diagnostic tests but also the key its allow also a better knowledge of cancer genetics which helps the specialist assess prognosis of their patient, selection of the most appropriate anticancer therapy, and monitoring the response to treatment [1].

Cytogenetic techniques occupied an important place in haematological diagnostics. The first chromosomal aberration described was in 1960 by Nowel and Hungeford characteristic, it was about CML- Philadelphia chromosome, few years after, it was be proved that almost every haematological neoplasm possess was associated with karyotype abnormalities [2].

ON 1980s, new method was developed: fluorescence in situ hybridization (FISH), it s characterised by high sensitivity and specificity, this technic has an other major particularity, it can be performed rapidly, and can classified the nature of chromosomal abnormalities [3].

Over the last few years, more powerful technologies were developed, the most remarkable one is was the Next-generation sequencing (NGS) witch was a real revolution on haematology [4].

In this chapter, we describe the techniques commonly employed for elucidating chromosomal aberrations, prognostic impact of recurrent chromosomal abnormalities, and recently updated risk stratification systems.

We will summaries the chromosomal abnormalities recently identified on many of haematological diseases such acute myeloid leukaemia, acute lymphoid leukaemia, myelodysplasic syndrome, multiple myeloma, meyloproliferative disease and clarify their impacts on clinical phenotype and prognosis, as well as their role in the pathogenesis of these diseases.

The aim of this chapter is to provide a brief overview of the recent progresses in haematological diseases genetics.

2. Cytogenetic technic

Cytogenetic study plays a crucial role on haematology, its the main outil for making diagnosis for almost all haematological malignancies, and its have an important impact prognosis for those diseases, and cytogenetic abnormalities are included on almost all prognosis score and risk stratifications for haematological neoplasms.

The past several years' remarkable efforts were deployed for better understanding of genetics and genome biology, many new technologies were developed, and the old technic saw improved their sensibility and specificity.

Veritable revolution was seen thanks to NGS, this technic offers possibility of broad analysis of a genome by whole-genome sequencing (WGS), exome sequencing, transcriptome sequencing, and epigenomics [5].

2.1 Conventional cytogenetic

Cytogenetic analyse in hematologicl neoplasms is performed by bone marrow aspiration in sterile way with heparine filled probes.

Sometimes, katyotype can be realised by peripheral blood.

The Celle of aspirated bonne marrow are cultured in vitro, then microscopic slides with metaphases chromosomes and/or interphase nuclei is performed.

Karyotype needs many metaphases cell (20 to 30) to be significatif, so its required time [2].

Conventional cytogenetics still be the most frequently ordered genetic test for various leukaemias, most prominently chronic myelogenous leukaemia (CML) in a resource limited situation.

2.2 Fluorescence in situ hybridization (FISH)

FISH is the best alternative to karyotype, it is rapid technic, with high level of specificity and sensibility. It can be realised from bone marrow or peripheral blood, it can also be performed from fixed and sectioned tissue [3].

FISH constitues a big step for studying somatic chromosomal mosaicism and molecular cytogenetic detection of chromosomal variations in interphase nuclei [6, 7].

FISH is a molecular cytogenetic technique, it identifies chromosomal abnormalities using molecular technology.

Technic of FISH is based on A DNA probe is tagged with a fluorescent marker. The probe and target DNA are denatured, and the probe is allowed to hybridise with the target. The fluorescent tag is then detected with a fluorescent microscope.

2.3 Polymerase chain reaction (PCR)

PCR is a technique that involves amplification of a desired segment of DNA by using primers, nucleotides and enzymes like reverse transcriptase and DNA polymerases. It represents the most used molecular technique on haematology. Different types of PCRs exist. Reverse transcriptase PCR (RT-PCR), Real time PCR (RQ-PCR).

In clinical practice RQ PCR is commonly used for viral copies detection and specific gene detection like Bcr-abl and PML-RARA for assessment of treatment response [1] new technique was developted: Digital PCR it s used for DNA/RNA detection and quantification. It is emerging as an alternative to conventional RQ-PCR for quantification and low abundance mutation detection [8].

PCR have many applications in hematologic malignancies include. it s used for detection of fusion genes and mutations. Its also performed for analysing of post transplant chimerism, and can be realised for determination of lymphoid clonality.

2.4 Genome-wide arrays

Microarray based testing such as array comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNP) arrays are now more used in routine diagnostics for haematological malignancies.

The copy numbers of DNA sequences in the test and reference samples are quantified by assessment of relative fluorescence intensities detected by digital imaging systems.

2.5 Gene expression profiling

This technique is based on DNA microarray which utilises plates which have various complementary genetic sequence covalently attached to them.

At present availability of GEP is limited to few research centers only limiting its wide use in clinical practice.

2.6 New generation sequencing

Over the past few years, an important increasing of the use of NGS on haematology have been shown, new platforms are available and are very helpful to identify the genetic basis of haematological neoplasms and genome biology,

Next-generation sequencing (NGS) encompasses several different methodologies that allow the investigation of genomics, transcriptomics and epigenomics [4].

Application of NGS in hematologic malignancies has confirmed presence of a lot of mutation of certain genes like TP53, ATM, RAS etc. the inconvenient for NGS, its the cost, this technic still expensive and can not be used on large spectre today especially for limited resource's country.

3. Haematological malignancies applications

Haematological neoplasms benefits from progress of biological technology, the use of new platforms helps to approve the performance of identification of genetic abnormalities.

This knowledge is crucial and have clinical utility, it can also improve diagnostics, prognosis, monitoring of minimal residual disease and it can be helpful to target of dysregulated signalling pathways by specific therapeutic targets [4].

We will summaries the chromosomal abnormalities recently identified on many of haematological diseases such acute myeloid leukaemia, acute lymphoid leukaemia, myelodysplasic syndrome, multiple myeloma, chronic lymphoblastic leukaemia, meyloproliferative disease and clarify their impacts on clinical phenotype and prognosis, as well as their role in the pathogenesis of these diseases.

3.1 Acute myeloid leukaemia (AML)

Cytogenetic abnormalities are frequently reported in the literature describing the presence of chromosomal rearrangements in important cases of acute myeloid leukaemia (AML): the rate can reach 50–60% of cases of AML [9].

It has been proved that AML is a complex and evaluative disease [10, 11]. There are many leukaemia genes, most of which are infrequently mutated, and patients typically have more than one driver mutation. The AML evolved over time, with multiple competing clones coexisting at any time [10, 11].

Over the few recent years, genome biology have seen a veritable revolution of technology, including chromosome banding, with fluorescence/chromosome in situ hybridization, or other analyses like array comparative network genomic hybridization, genome breakpoints cloning and Sanger Sequencing of candidate genes and profiling of single nucleotide polymorphism, and even whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA sequencing have all contributed to incremental improvements in understanding the genetic basis of the AML.

The whole-genome sequencing for AML showed that it is an evaluative and complex disease. There are many leukemia genes, most of which are infrequently mutated, and patients typically have many driver mutations. The evolution is characterized by emergence of many competing clones which can coexist at any time.

In fact, it has been proved that different genes and clones coexisting in the same patient, **Figure 1** illustrates that clearly [12].

200 AML patients has been analysed by The Cancer Genome Atlas (TCGA) consortium, they use whole-genome or whole-exome sequencing and they identified 23 genes as "significantly mutated" at a higher-than-expected frequency [13].

Conventional cytogenetics is very important on AML, it identifies chromosomal abnormalities, it can be balanced translocations, inversions, insertions, monosomies, and trisomies, which are present in approximately 55% of adult cases and 80% of children with AML. These are the strongest prognostic factors for response to treatment and survival in multivariate analysis. The 2008 WHO classification categorized AML based on cytogenetic or molecular abnormalities [14, 15].

The WHO 2008 and 2016 classifications incorporated modifications that allowed for a greater number of patients to be classified into the category of AML [16, 17].

Even patient with normal karyotype AML, it has been proved recently with certitude that those patients constituted very heterogeneous group; new technology helps to identified many gene mutations in normal karyotype AML by cutting-edge next-generation sequencing NGS technology, like FLT3-ITD, NPM1, CEBPA, and other additional mutations.

The most important predictors of shorter overall survival in AML patients aged less than 60 years are represented by DNMT3A and RUNX1 mutations especially those with intermediate-risk cytogenetic.

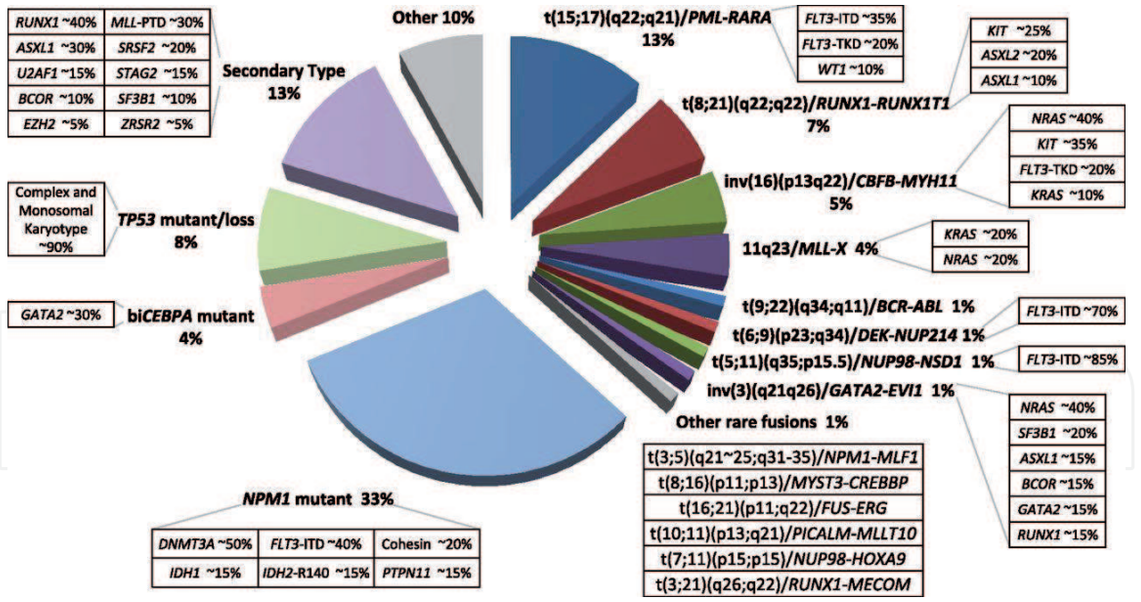

Figure 1.
Molecular classes of AML and concurrent gene mutations in adult patients up to the age of ~65 years. For each AML class denoted in the pie chart, frequent co-occurring mutations are shown in the respective boxes. Data on the frequency of genetic lesions are compiled from the databases of the British Medical Research Council (MRC) and the German-Austrian AML study group (AMLSG) and from selected studies. It indicates cohesin genes including RAD21 (10%), SMC1A (5%), and SMC3 (5%); inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11; and inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1), and TP53 mutations are found in 45% and complex karyotypes in 70% of this class.

NPM1 mutations were also considered as important molecular prognosticators of Overall Survivor, particularly in the absence of FLT3-ITD, mutated TP53, and bi-allelic CEBPA mutations.

Actually, for treating patient with AML, it is indispensable to perform the research of these gene mutations. It s important for diagnosis and it can be helpful as molecular marker of prognosis, and its necessary to predictive for response of treatment, and used also for disease monitoring.

Studies demonstrated that patients with cytogenetically normal AML or intermediate-risk abnormalities have more additional gene mutations than patients with favourable or unfavourable abnormal cytogenetic and especially those with balanced translocations [18].

Recent research showed that aged patients have more driver gene mutations than younger patients. Its seems that elderly patients have more alterations in specific genes including TET2, RUNX1, ASXL1, and SRSF2. All this gene has recently been implicated in age-related clonal haematopoiesis. These found participate to improve our understanding of knowledge in AML biology between younger and older patients [19].

The application for biological technology such NSG are multiple, for exemple, there are a number of FLT3 inhibitors at various stages of clinical development were produced, such as PKC412 (midostaurin), CEP-701 (lestaurtinib), or MLN518 (tandutinib).

TKIs are promising agents in the treatment of AML patients with an FLT3-ITD mutation, especially when they are combined with chemotherapy [20].

3.2 Acute lymphoblastic leukaemia (ALL)

Acute lymphoblastic leukaemia (ALL) is the most often childhood neoplasm occuring about 30% of all cancer.

Abnormalities in chromosome number as well as structural rearrangements (translocations) are detected in 60–80% of patients with ALL, whereas the remaining 20–40% have a normal karyotype [21, 22].

Improvement of cytogenetic, FISH, and reverse transcription polymerase chain reaction (RT-PCR) analyses permits to identify subgroups of acute lymphoblastic leukaemia with specific chromosome abnormalities and allow determining treatment strategy for childhood ALL, especially when specific aberrations are present [23, 24].

Recurrent genetic abnormalities have been identified on ALL, including balanced translocations and aneuploidies. Based on the World Health Organization (WHO) classification, BCP-ALL is categorized into ALL with hyperdiploidy (>50 chromosomes), ALL with hypodiploidy (<44 chromosomes), and ALL with translocation t(9;22) (q34;q11.2) encoding BCR–ABL1, t(12;21) (p13;q22) encoding TEL–AML1, t(1;19) (q23;p13.3) encoding E2A–PBX1, t(5;14) (q31;q32) encoding IL3–IGH, and rearrangement of MLL at 11q23, with a diverse range of partner genes [25, 26].

Concerning T- ALL, common alterations include rearrangement of the T-cell receptor gene loci to transcription factor genes including TLX1, TLX3, LYL1, TAL1, and MLL [27].

ALL genomes are not static but exhibit acquisition of new chromosomal abnormalities over time. Single-nucleotide polymorphism microarray profiling studies of matched diagnosis–relapse ALL samples show that most ALL cases exhibit changes in the patterns of structural genomic alterations from diagnosis to relapse and that many relapse-acquired lesions, including those targeting genes associated with high-risk ALL (IKZF1, IKZF2, CDKN2A, and CDKN2B), are detectable at the diagnosis [28, 29].

3.3 Myelodysplasic syndrome

Myelodysplasia syndromes (MDS) are defined by a heterogeneous group of myeloid malignancies characterised by peripheral blood cytopenia and dishematopoiesis and frequently progress to acute myeloid leukaemia.

The 2016 revision defines 10 MDS subtypesas follows:

- MDS with single lineage dysplasia (MDS-SLD),
- MDS with dysplasia in two or more myeloid lineages (MDS-MLD),
- MDS-SLD/MLD with ≥15% ring sideroblasts (RSs; MDS-MLD-RS),
- MDS with an excess of blasts of up to 9% in bone marrow and up to 4% in peripheral blood (MDSEB- 1),
- MDS with 10%–19% bone marrow and 5%–19% blood blasts (MDS-EB-2),
- MDS with isolated deletion of chromosome 5q [del(5q)]
- MDS unclassifiable (MDS-U) based on defining cytogenetic abnormality, MDS-U with SLD and pancytopenia and MDS-U with 1% blood blasts.

Conventional cytogenetic allow the identification of abnormalities in approximately 50% of MDS. Some of cytogenetic abnormalities are characteristic of MDS, they may be considered as specific to MDS if the clinical context is appropriate such del(5q).

Majority of of MDS (90%) presents somatic mutations, those mutations identify molecular pathways that drive the pathogenesis of MDS. Even low abundance mutations can have prognostic value as they identify emerging clones before they impact clinical parameters.

Recent studies demonstrated that 65% of MDS patients harboured mutations in RNA splicing (SF3B1, SRSF2, U2AF1, ZRSR2) [28], 47% harbouring mutations in DNA methylation genes (DNMT3A, IDH1/2, TET2) [29, 30] and 28% in histone modification genes (ASXL1,BCOR, EZH2).

Mutations in isocitrate dehydrogenase 1 or 2 (IDH1 and IDH2) are important to identify at the time of diagnosis of high- or very high-risk MDS. These particular mutations lead to abnormal leukemogenesis. Mutated IDH1 or IDH2 are not common and are only found in approximately 4% to 12% of patients with MDS. Those gene mutations have treatment impact. Recently, two IDH inhibitors, specifically ivosidenib targeting IDH1 and enasidenib for IDH2, are approved by the United States Food and Drug Administration (FDA) for use in AML, but not in MDS [30, 31].

At present, NGS is rarely incorporated into clinical guidelines although an increasing number of studies have demonstrated the benefit of using NGS in the clinical management of MDS patients [32].

3.4 Multiple myeloma

Multiple myeloma is a malignant disease characterised by proliferation of monoclonal plasma cells leading to clinical features that include hypercalcaemia, renal dysfunction, anaemia, and bone disease (frequently referred to by the acronym CRAB) which represent evidence of end organ failure.

Recent studies have confirmed that myeloma is an heterogeneous disease composed of multiple molecularly-defined subtypes each with varying clinicopathological features and disease outcomes [33].

Chromosomal translocations account for 40–50% of primary events in myeloma and strongly influence disease phenotype [34].

Karyotypes are complex, hyperploidy can be seen in2/3 of cases, karyotypes can change from normal to abnormal during evolution of multiple myoloma.

Fluorescence in situ hybridization (FISH) ssems to be more adequate for recognising specific chromosomal changes in quiescent cells and increases the proportion of detection of chromosomal abnormalities in MM up to more than 90% [35].

IG rearrangements: translocations involving 14q32 are found in at least 65–70% of patients, most of them result from short segments exchange and are detected quite exclusively by FISH.

The (4; 14) is present in 15% of myeloma cases and has been associated with a poor prognosis in a variety of clinical settings such as those receiving high dose therapy with autologous stem cell transplant (ASCT).

The (11; 14) is observed in approximately 17% of myeloma patients and also directly up regulates a cyclin D gene in the form CCND1.

The (6; 14) is a rare translocation occurring in 2% of myeloma patients which results in the direct up regulation of the CCND3 gene [36].

Other translocations with IGH involving are reported, but they are rare and it seems that they are secondary [37].

Like other haematological neoplasm, multiple myelom benefits from the development of molecular technic like NGS, the knowledge about pathogenesis and the progression of disease has been improved, with apparition of a new concept called subclonality. In fact, NGS characterised the the wide molecular heterogeneity of the disease and the frequent occurrence of some supposedly “driver” mutations only in subclones. Those found are important for the targeted future therapies [38].

3.5 Meyloproliferative neoplasm (MPN)

Myeloproliferative neoplasms (MPN) can be denifed by a group of diseases characterised by increased proliferation of erythroid, megakaryocytic, or granulocytic.

According to WHO (2008) MPN regroups clonal disorders of myeloid progenitor cells., MPN have been classified into 3 groups. Also called Philadelphia chromosome-negative (Ph –), myeloproliferative neoplasms (MPNs), include polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (both primary (PMF) and secondary (post-ET/PV MF)) [39].

The 3 MPN entities are characterised by their clinical heterogeinicity, establishment of precise diagnosis at disease onset can be a real challeng for physician. The World Health Organisation (WHO) established revision for diagnostic and defined new criteria for MPN on 2016, (**Figure 2**) [40].

The first gene mutation described in 2005, JAK2-V617F, turned out to be the most important and most frequently recurring somatic mutation in MPN [6–9]. The frequency ofJAK2-V617F is around 95% in PV and between 50% and 60% in ET and PMF [41].

MPN patients who do not present mutations in any of the aforementioned genes (so-named "triple-negative" MPN cases), but those patients seem to have hyperactive JAK2 signalling [42].

The *JAK2*V617F mutation arises in a multipotent haematopoietic progenitor, is present in all myeloid lineages.

The *JAK2*V617F is mainly restricted to classical MPNs with the exception of refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T). It can rarely be found in some other malignant hemopathies [43, 44].

Concerning MPL mutation, it have been demonstrated that two types of mutation exist: (*MPL*; the thrombopoietin [TPO] receptor [TPOR]) mutations located in exon 10, both have been reported on association with MPNs.

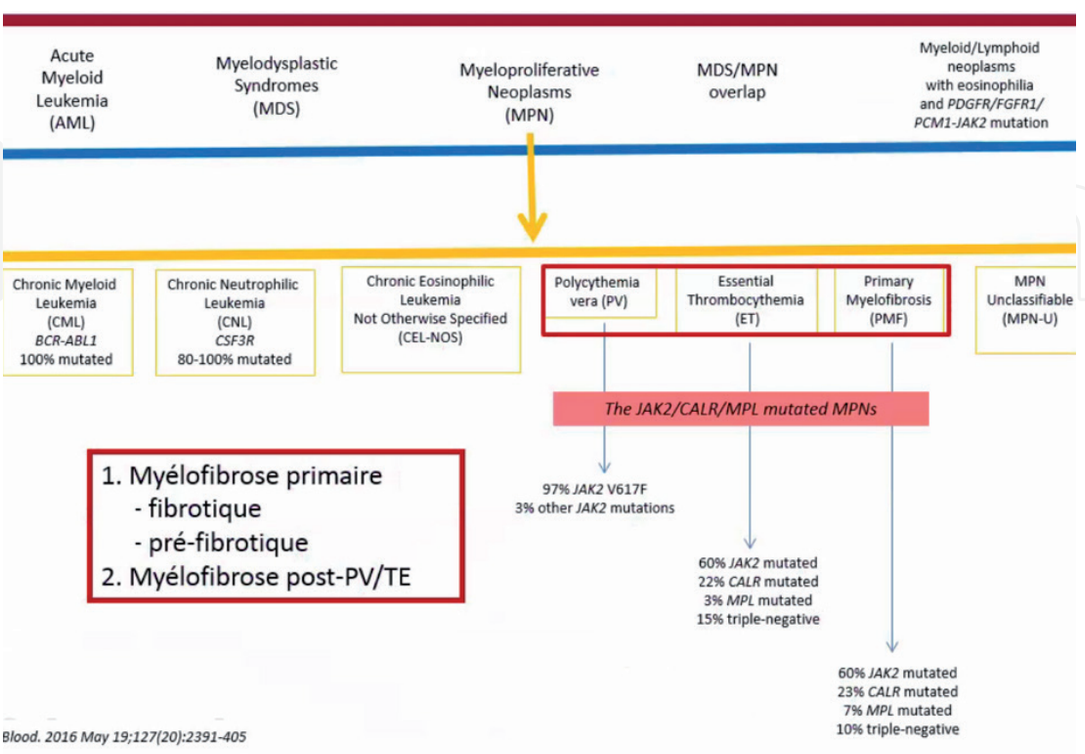

Figure 2.
2016 WHO classification of myeloid malignancies.

The most frequent are mutations on the tryptophan W515 located at the boundary of the transmembrane and the cytosolic domains of MPL, the most prominent mutations being MPLW515L and K [42].

Mutations on *MPL*W515 are restricted to ET (around 3%) and PMF (around 5%).

At the end of 2013, frameshift mutations in the *CALR* gene were identified in the majority of *JAK2*$^-$ and *MPL*$^-$ ET and PMF (50%–60% ET and 75% PMF).

There are great differences in the frequency between type 1 and type 2 mutations in ET and PMF.

CALR mutations reported are often heterozygous, only few cases of homozygous mutations have been reported, more particularly for type 2 mutations [43].

It has been also proved that CALR mutants gives a stronger clonal advantage when compared with *JAK2*V617F.

In 2010, somatic mutations in exon 2 of *LNK* (*SH2B3*), an adaptor protein which regulates JAK2 activation, were detected in 2 patients (PMF and ET).

The 3 MPN oncogenes are considered as true drivers of the disease phenotype with JAK2 exon 12 giving only an erythrocytosis phenotype, JAK2V617F giving rise to ET, PV, and MF, whereas CALR mutant and MPLW515L/K/A are associated with ET and MF, resembling the phenotype observed in patients.

The 3 main driver mutations do not explain the entire heterogeneity of the classical *BCR-ABL*$^-$ MPNs. The 3 main driver mutations do not explain the entire heterogeneity of the classical *BCR-ABL*$^-$ MPNs.

Like other hematologic malignancies, the mutations in epigenetic regulators can be observed, such as MDS and AML; and some of the gene mutations, such as TET2 and ASXL1, are more frequent in MDS than in MPN.

Some studies suggest that the presence of mutations in TET2, EZH2, and ASXL1 are associated with hight risk of secondary AML.

Few time after the discovery of the JAK2 V617F mutation, multiple small molecule inhibitors were developed for therapeutic use: ruxolitinib, is the first JAK1 and JAK2 inhibitor, approved in August 2011 for use in intermediate and high-risk PMF and post PV/ET myelofibrosis.

Selective JAK2 inhibitors, (SAR302503 and BMS911543), combination JAK2/JAK3 inhibitor (CEP701), and combination JAK2/TYK2 inhibitor (pacritinib) have shown clinical efficacy in phase I/II trials.

4. Conclusion

Major progress has been achieved in understanding the molecular pathogenesis of haematological malignancies in a very short period of 10 years.

While the clinical utility of this genetic and epigenetic revolution, novel therapeutic agents aimed at the aberrant underlying processes are more and more included rational combination therapies. This knowledge increase outcome for haematological diseases patients, and are helpful to develop therapies based on insights into the genetic basis of these haematological neoplasms.

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