Current Role of Genetics in Hematologic Malignancies

Gaurav Prakash¹ · Anupriya Kaur² · Pankaj Malhotra¹ · Alka Khadwal¹ ·
Prashant Sharma³ · Vikas Suri¹ · Neelam Varma³ · Subhash Varma¹

Received: 10 August 2015 / Accepted: 17 August 2015 / Published online: 16 September 2015
© Indian Society of Haematology & Transfusion Medicine 2015

Abstract Rapidly changing field of genetic technology and its application in the management of hematological malignancies has brought significant improvement in treatment and outcome of these disorders. Today, genetics plays pivotal role in diagnosis and prognostication of most hematologic neoplasms. The utilization of genetic tests in deciding specific treatment of various hematologic malignancies as well as for evaluation of depth of treatment response is rapidly advancing. Therefore, it is imperative for practitioners working in the field of hemato-oncology to have sufficient understanding of the basic concepts of genetics in order to comprehend upcoming molecular research in this area and to translate the same for patient care.

Keywords Hematology · Oncology · Genetics · Molecular tests

Introduction

Hematologic malignancies are amongst the top 10 malignant disorders with respect to the incidence as well as cause of death in patients suffering from cancers. Collectively, they constitute approximately 9 % of all cancer cases diagnosed in a year [1]. Annual incidence rates of some of these cancers are consistently increasing. However, a trend of significant

decline in mortality due to many hematological malignancies has been observed [1]. This positive trend is being observed predominantly because of the numerous advancements in their diagnosis and management over last decade or so. Many of these advancements have been due to the developments in genetic technology and their applications in the field of hemato-oncology. Today, geneticists are providing hemato-oncologists with not only diagnostic tests but also the key 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. This has established a close relationship between the fields of genetics and hemato-oncology. Therefore, it is imperative for practitioners working in this field to have sufficient understanding of the basic concepts of genetics in order to comprehend upcoming molecular research and to translate the same in practice.

In this review we describe some frequently used genetic tests in the management of hematologic malignancies. We also discuss their applications for the major hematologic malignancies. Genetics of lymphoma is an extensive topic and it should be addressed in a separate article, therefore lymphomas are excluded from the discussion of this article. A glossary of the genetic terms for a better understanding of the subject and the text in this article is also provided with this review.

Frequently Used Genetic Techniques in Hemato-Oncology

Conventional Cytogenetics (Karyotyping)

Cytogenetics is the study of chromosomes. Human chromosomes were first reported in 1882 by Flemming in the

✉ Anupriya Kaur
anukaur.genetics@gmail.com

¹ Clinical Hematology and BMT Division, Department of Internal Medicine, PGIMER, Chandigarh, India
² Medical Geneticist, Sarai Building, Government Medical College, Chandigarh 160030, India
³ Department of Hematology, PGIMER, Chandigarh, India

dividing corneal epithelial cells [2]. Since then, chromosomal studies have advanced significantly. From its initial use in inherited disorders related to aneuploidy, cytogenetics has now become frequently used genetic investigation in various malignancies. In contrast to the epithelial carcinoma, genetic aberrations in hematological malignancies frequently involve balanced or reciprocal chromosomal translocations [3]. Therefore techniques useful in detecting chromosomal aberrations (like cytogenetics) are more relevant in hemato-oncology than in other spheres of oncology.

Conventional cytogenetics continues to be the most frequently ordered genetic test for various leukemias, most prominently chronic myelogenous leukemia (CML) in a resource limited situation. Conventional cytogenetics also enjoys the tag of flag bearer of genetics in the field of hemato-oncology as it was the first genetic test used in clinical practice for diagnosis of CML. In the year 1960 Philadelphia chromosome (named after the city of its discovery Philadelphia) was discovered in the University of Pennsylvania USA [4] (Fig. 1).

A karyotype is prepared from living cells (most commonly leukocytes in peripheral blood or bone marrow). It detects numerical and large structural chromosomal aberrations (structural abnormalities of ≥ 3 –5 Mb of DNA) in the sampled tissue. Generally peripheral blood lymphocyte culture is used for diagnosis of non-malignant genetic disorders. The cells are cultured in a favorable media using T lymphocyte mitogens like phytohemagglutinin (PHA), so as to enhance the mitotic division, thereby abundant metaphases are obtained. However malignant cells from bone marrow or peripheral blood are used for identification of cytogenetic abnormalities in

hematological malignancies, wherein the yield of metaphases may be sub-optimal. Therefore various methods are employed to improve the mitotic index and morphology of chromosomes. Mitosis inhibitor (like colchicine) is used to arrest cells in metaphase so that the chromosomes can be studied in their most condensed form. Slide preparation is done to get metaphase spreads and the cells are then subjected to G banding (G stands for Giemsa). The slides are then studied with an image analyzer for various detectable numerical or structural chromosomal aberrations. Cytogenetic analysis of chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) patients is often difficult because of the low proliferating rate of the malignant cells and the presence of normal cells in bone marrow which may interfere with their growth. In such cases, TPA (12-O-tetradecanoylphorbol 13-acetate) stimulated cultures are used to improve the yield of metaphases. Some common applications of conventional karyotyping are illustrated in Table 1.

Another advantage of conventional cytogenetics is its ability to detect additional cytogenetic abnormalities which cannot be detected by locus specific investigations like fluorescent in-situ hybridization (FISH) or polymerase chain reaction (PCR) (described ahead in the article). A conventional karyotype cannot detect small deletion of few kilo base pairs length and cryptic translocations. For detection of such small genetic changes more advanced genetic techniques are employed. Cytogenetic analysis of hematological malignancies is much more difficult, complex and labour intensive, as compared to non-malignant genetic disorders; main reasons being need to analyze multiple types of samples/ cultures and difficulty in standardizing the methodology in order to obtain good metaphase yield and chromosome morphology. In the Indian context, the discipline of cytogenetics has not grown as much as that of molecular genetics. Although both are complementary to each other, cytogenetic analysis remains the gold standard for most numerical and large structural chromosomal abnormalities.

Fluorescent In-Situ Hybridization (FISH)

FISH is a molecular cytogenetic technique i.e. it identifies chromosomal abnormalities using molecular technology. Commercially available DNA probes (single stranded DNA labeled with fluorochrome) are hybridized to a patient's sample and the hybridization is visualized using a fluorescence microscope [5] (Fig. 2). This technique doesn't essentially require living cells and can be performed both on dividing as well as on non-dividing inter-phase cells. FISH has several advantages over karyotyping.

Fig. 1 Karyogram shows diagnostic cytogenetic abnormality, t(9, 22) in a patient with chronic myelogenous leukemia (CML)

Table 1 Clinical utility and tissue source for some key genetic investigations in hematologic neoplasm

Disease	Genetic abnormality	Current genetic technology	Clinical utility	Sample to be collected
CML	Philadelphia chromosome study	Conventional Karyotyping (cytogenetics)	To diagnose CML To assess depth of response to therapy	PB or BM
	Bcr-abl gene rearrangement	FISH or PCR	To diagnose CML To assess depth of response to therapy	PB or BM
	Bcr-abl transcripts	Real time PCR	For monitoring response of therapy on oral TKI	PB or BM
	Kinase domain mutations	PCR	To detect tyrosine kinase drug resistance	PB/BM
AML	Chromosomal aberrations	Conventional karyotyping	Prognostication of AML patient Selection of optimal therapy	PB or BM
	Selective gene mutation analysis	PCR	Selection of optimal therapy of AML	PB or BM
MM	Chromosomal aberrations	By Conventional karyotyping	For prognostication at baseline	BM
		By FISH	For prognostication at baseline	BM
CLL	Chromosomal aberrations (deletion/translocation)	FISH	Prognostication	PB or BM
ALL	Chromosomal aberrations (translocation)	Conventional Karyotype/FISH	Prognostication at baseline	PB or BM
	Genetic MRD detection	PCR	Prognostication and decision on therapy	BM
MDS	Various Chromosomal deletion and translocations	FISH	Diagnosis of MDS and assessment of prognosis	Bone marrow
APL	PML-RARA transcripts	RQ PCR	For diagnosis and monitoring of response to therapy	PB or BM
	t(15, 17)	FISH	Diagnosis	PB or BM

ALL Acute Lymphoblastic Leukemia, *AML* Acute Myeloid Leukemia, *APL* Acute Promyelocytic Leukemia, *CLL* Chronic Lymphocytic Leukemia, *CML* Chronic Myelocytic Leukemia, *MDS* Myelodysplastic syndrome, *MM* multiple myeloma

Fig. 2 Interphase FISH with a *BCR/ABL* D-FISH probe shows two fusion signals (arrows) in a Ph + cell. Fusion is represented by yellow color dot while non fused alleles are in green and red colors. (Color figure online)

It can be performed on cells with low in vitro mitotic index such as in indolent lymphoid and plasma cell malignancies where conventional karyotyping has limitations. Greater

number of cells can be analyzed in a given case by FISH, providing a more representative assessment of the proportion of abnormal cells. Also, when karyotype analysis is hampered by considerable karyotypic variability and complexity, FISH analysis provides a rapid and reliable method for determination of specific abnormalities. At present FISH is one of the most commonly used genetic techniques in evaluation and prognostication of various hematologic malignancies like acute and chronic leukemia and multiple myeloma (Table 1).

Comparative genomic hybridization (CGH) [6] and spectral karyotyping (SKY) [7], which are also based on the hybridization technique, are relatively recent and improved techniques of chromosomal analysis.

Spectral karyotyping (SKY) is also known as multicolor FISH. This technique uses specific whole chromosome paint probes to paint each of the 24 (22 pairs of autosomes and 2 sex chromosomes) human chromosomes with different colors [8]. This tool has proved invaluable to identify origin of a derivative chromosome and the chromosome of origin in a translocation. This has also improved the accuracy of cytogenetic testing. However,

due to limited availability of this technique and expensive probes, at present, clinical usability of these tests remains limited.

Polymerase Chain Reaction (PCR) and its Modifications

PCR is the most commonly used molecular assay in the management of hematological malignancies. Basics of most PCR techniques involve amplification of a desired segment of DNA by using primers, nucleotides and enzymes like reverse transcriptase and DNA polymerases. Various mutations can be studied using sequencing or restriction enzymes (RFLP-Restriction fragment length polymorphism) in the amplified DNA. There are various types of PCRs. Reverse transcriptase PCR (RT-PCR) is the commonest type of PCR used in practice today. The reverse transcriptase PCR uses the enzyme reverse transcriptase to synthesize what is called as cDNA (complementary DNA) from mRNA (messenger RNA). Final amplified product is assessed by agarose gel electrophoresis. Thus with the help of RT-PCR one is able to study the expression of genes in a qualitative manner. The acronym “RT-PCR” usually denotes reverse-transcriptase PCR and not real-time PCR; however some scientific communications do not adhere to it [9]. Real time PCR (RQ-PCR) is the second most common technique based on PCR. In this technique, amplified DNA is tagged with non-specific fluorescent dyes that intercalate with any double-stranded DNA [10] the thermo-cycler (machine to carry out PCR reactions) contains sensors for measuring the fluorescence emitted from the fluorophore after it has been excited at certain wavelength. Generation rate of fluorescence is measured and real time values of DNA amplification are provided, thus the name real time PCR. This technique can quantify copy numbers of a given DNA sequence in a sample. In clinical practice RQ PCR is commonly used for viral copies detection and specific gene detection like bcr:abl1 and PML-RARA for assessment of treatment response. A type of PCR such as Amplification-refractory mutation system (ARMS-PCR) is used to detect any mutation involving single base changes or small deletions [11]. Digital PCR is a new approach to DNA/RNA detection and quantification. It is emerging as an alternative to conventional RQ-PCR for quantification and low abundance mutation detection [12]. This is a chip based assay in which several samples can be tested simultaneously. After replication of genetic material dye-labeled probes are used to detect sequence specific targets in a given sample. Table 3 describes key differences in various PCR techniques.

PCR is usually performed on DNA/RNA extracted from fresh blood/tissue samples. But, owing to exquisite

sensitivity of this technique it can be used to analyze paraffin fixed tissues or bone marrow aspiration slides as well.

The applications of PCR in hematologic malignancies include [13]

1. Detection of fusion genes
2. Detection of mutations
3. Post transplant chimerism analysis
4. Determination of lymphoid clonality

The best example of fusion gene is BCR-ABL1 in CML (Fig. 3). The fusion gene produces mRNA transcript which can be quantified using real time RT-PCR thus allowing determination of response to treatment (*Major molecular response—as defined by >3 log reduction in bcr-abl transcript*), monitoring of residual disease and treatment failure (2-5 fold increase in BCR-ABL1 transcripts). Other examples include detection of PML-RARA fusion gene in APL and bcl2-IgH in follicular lymphoma.

Very often subtle mutations rather than new fusion genes are the genetic abnormalities in hematological malignancies. Detection of these mutations provides important diagnostic and prognostic information. Table 2 provides a list of such mutations and their corresponding malignancies.

Apart from diagnosis and prognostication, another important use of PCR in Hematology is in the field of bone marrow transplant and minimal residual disease (MRD) detection. After an allogeneic bone marrow or peripheral blood stem cell transplant there is a phase of crossover between host's bone marrow and grafted bone marrow. Thus a temporary state of chimera is created in this initial phase of transplant. Ultimately grafted bone marrow takes over and all the hematopoietic cells are of donor's stem

Fig. 3 Gel photograph shows results of multiplex RT-PCR performed for BCR-ABL hybrid transcripts. Lanes P1 and P2 show positivity for BCR-ABL transcripts; PC positive control (b2a2); NC negative control; M marker (100-bp ladder)

Table 2 Genetic alterations in major haematologic malignancies and their prognostic significance

Disease	Genetic alteration	Remarks
AML (18)	Favorable risk	3 years survival
	1. CBF mutation positive	66 %
	t(8;21)(q22;q22)RUNX1-RUNX1T1	
	inv(16)(p13.1q22)CBFB-MYH11	
	t(16;16)(p13.1q22)CBFB-MYH11	
	2. NPM1 mutation positive/without FLT3 ITD mutation (commonest)	
	CEBPA mutation positive	
	Intermediate 1	3 years survival
	1. CNAML (commonest) with	28–45 %
	NPM1 gene wild type/without FLT3 ITD mutation	
APL	NPM1 gene wild type with FLT3 ITD mutation	
	NPM1 gene mutated and FLT3-ITD mutation	
	Intermediate 2	
	1. t(9;11)MLL gene rearrangement	
	2. delY, del(9q)	
	3. <i>Cytogenetic abnormalities not qualifying for poor risk</i>	
	Poor risk	3 years survival
	1. del(5q), del(7q),	12 %
	2. Inv(3q), t(3;3)	
	3. t(9;22)	
ALL	4. t(6;9)	
	5. t(v,11q) MLLgene rearranged	
	6. Complex karyotype (commonest)	
	1. t(15;17)(q22;21) PML-RARA APL	1. Favorable
	2. t(5;17)(q32;q21) NPM-RARA APL	2. Poor
ALL	3. t(11;17)(q23;q21) PLZF-RARA APL	3. Poor
	4. t(11;17)(q13;q21) NuMA-RARA APL	4. Poor
	1. Hyperdiploidy (trisomies for chromosomes 4, 10, and 17)	Low risk
	2. High hyperdiploidy (51–67 chromosomes)	
	3. (Note: ChromosomeD 56–67 better than 51–55)	
CML [47]	4. t(12;21)/TEL-AML1	
	5. del(9p)	
	t(1;19)/E2X-PBX1	High risk
	1. t(9;22)/BCRABL	Very high risk
	2. Chromosome 11q23/MLL gene rearrangements	
	3. Hypodiploidy/near triploidy,	
	4. t(4;11)(q21;q23), t(8;14)(q24;q32), and	
	5. Complex karyotype (five or more chromosomal abnormalities)	
	1. Cytogenetics or FISH-Philadelphia chromosome or t(9,22)	Diagnostic tests
	2. PCR: bcr-abl fusion gene and its variants on the basis of break points	As well as for treatment monitoring
CML [47]	Major BCR (M-bcr) p210 (commonest variant >95 %)	
	Minor BCR (m-bcr)p190 (rare, marked monocytosis)	
	Micro BCR (mu-bcr) p230 (rare, marked thrombocytosis)	
CLL	In order of prognosis (poor to good) FISH analysis	Median survival

Table 2 continued

Disease	Genetic alteration	Remarks
MM [48, 49]	1. del17p (resistance to purine based therapy)	1. 32 months
	2. del11q (more lymphadenopathy, poor outcome)	2. 6.5 years
	3. del6q	3. 9.5 years
	4. Trisomy12 (atypical morphology, intermediate outcome)	4. 9.2 years
	5. Normal karyotype (favourable prognosis)	5. 9.2 years
	6. del 13q (favourable prognosis)	6. 11 years
	Standard-risk	Median OS
	Hyperdiploidy	Standard-risk 6–7 years
	t (11;14)	Intermediate-risk 4–5 years
	t (6;14)	High-risk 2–3 years
	Intermediate-risk	
	t (4;14)	
	<i>Deletion 13</i> or hypodiploidy	
	High-risk	
	17p deletion	
	t (14;16)	
	t (14;20)	
	High-risk gene expression profiling signature	
	Genetic alterations Frequency (%)	
	1. All trisomies only 57 %	
	2. Translocations 46 %	
	t(11;14)(q13; q32.3)	
	t(14;16)(q32.3; q23)	
	t (4;14)(p16.3;q32.3)	
	3. Monosomy 49 %	
	Monosomy 13/14/16	
	del(13)(q14.3)	
	P53 abnormalities 13 %	
	del(17)(p13.1)	
	Monosomy 17	
	4. Normal karyotype 3 %	
	5. Others <1 %	
MDS [43, 44]	Very good karyotype	IPSS-R score
	–Y or del(11q)	Very good = 0
	Good Karyotype	Good = 1
	del (5q), del(12p), del (20q)	Intermediate = 2
	Intermediate karyotype	Poor = 3
	del(7q), +8, +19, I(17q)	Very poor = 4
	Poor karyotype	
	del(7q), trisomy8, trisomy19, I(17q)	
	Very poor karyotype	
	Complex karyotype, monosomy7	

BCR-ABL breakpoint cluster region-Abelson gene, *CBF* core binding factor, *CEBPA* CCAAT/enhancer binding protein alpha, *CN_AML* (cytogenetically Normal AML), *E2X-PBX1* pre-B-cell leukemia homeobox, *NPM1* Neucleophosmin1, *FLT3ITD* FMS-like tyrosine kinase 3 gene producing internal tandem duplications, *MLL* mixed lineage leukemia also termed ALL-1, HRX, and TRX1, *TEL-AML1* TEL oncogene-Acute myeloid leukemia1 gene: also known as ETV6-RUNX1 results from a t(12;21(p12,q22), *OS* Overall survival

Table 3 Comparative study of various PCR techniques

	Reverse transcriptase PCR (RT-PCR)	Real time PCR (RQ-PCR)	Digital PCR
Qualitative assessment of a genetic sequence	Yes	Yes	Yes
Quantitative assessment of a genetic sequence	No (can be semi quantitative)	Yes	Yes
Technical complexity	++	+++	+++
Principle	Amplification of target gene and then its detection with gel electrophoresis against a standard	Amplification of target gene and real time quantification using SYBR green dye	Direct counting of target genetic sequence and quantification using difference in negative and positive dye labeled nucleotide sequences
Need of gel electrophoresis	Required	Not required	Not required
Uses	Identification of presence or absence of a particular nucleic acid	Quantification of a particular nucleic acid sequence	Quantification of a particular nucleic acid sequence Rare allele detection

cells origin. Assessment of fraction of cells derived from host bone marrow versus donor's bone marrow is commonly called as chimerism study. The assessment of chimerism helps predict graft failure, disease relapse and graft versus host disease. This is studied using PCR based amplification of short tandem repeats (STR) and variable nucleotide tandem repeats (VNTR) markers followed by capillary gel electrophoresis. The level of chimerism is assessed by the allele size pattern. Single nucleotide polymorphisms or insertion/deletion polymorphisms and real-time PCR are more sensitive techniques to quantify chimerism status in a post transplant patient [14].

Genome-Wide Arrays

Cancer cells have complex genetic alterations. Very small genomic changes remain undetectable by the above mentioned techniques and require more sensitive technology for identification. Microarray based testing such as array comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNP) arrays are making their way in routine diagnostics for hematological malignancies.

In array CGH equal quantities of DNA from a reference (control) and a test (case) sample are differentially labeled with two different fluorophores. They are then co-hybridized competitively onto an array platform. An array platform is a commercially available chip which is spotted with many thousands evenly spaced cloned DNA fragments or oligonucleotides. 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. This technique can detect very

small copy-number aberrations with high accuracy [15]. SNP arrays have an additional ability to detect copy-neutral Loss of Heterozygosity (also called uniparental disomy). At present wide use of genome wide arrays studies is limited by its complexity and lack of availability.

Gene Expression Profiling

Technique of gene expression profiling (GEP) enables study of thousands of genes expressed in a tumor simultaneously. In the context of hematologic malignancies, GEP has been used to classify tumors into various genetic subtypes according to their distinct genetic signature. This technique is based on DNA microarray which utilizes plates which have various complementary genetic sequence covalently attached to them. In the field of Hematology GEP has been best utilized for diffuse large B cell lymphoma, which is classified in two novel classes of germinal center B cell-type, or activated B cell type, the latter of which carries distinctly less favorable prognosis [16].

Now GEP is being used for stratification of cases of various hematologic neoplasm like plasma cell disorders, chronic lymphoproliferative disorders and lymphoma, into high and low risk groups. However, at present availability of GEP is restricted to few research centers only limiting its wide use in clinical practice.

Next Generation Sequencing (NGS)

The term “next generation” reflects the revolutionary advancement in field of sequencing. Sanger sequencing

Table 4 Glossary of genetic terms used in hematology oncology

Genetic technique	Genetic terminology	Brief description
Cytogenetics	Metaphase	The stage of cell division at which the chromosomes line up on the equatorial plate. The chromosomes are most condensed at this stage. Hence during karyotyping cells are arrested in metaphases
	Ploidy	The number of sets of chromosomes in a cell
	Haploid	The haploid number (N) is the number of chromosomes in a gamete (N = 23 in humans)
	Tripoidy/tetraploidy	A cell with three times the haploid number of chromosomes (i.e. 3N)/A cell with four times the haploid number of chromosomes (i.e. 4N)
	Polyploidy	State where all cells have multiple sets of chromosomes beyond the basic set, usually 3 or more
	Aneuploid	A chromosome number that is not an exact multiple of the haploid number (i.e. $2N-1$ or $2N+1$), where N is the haploid number of chromosomes (N = 23 in humans)
	Trisomy	The presence of a chromosome additional to the normal chromosomal complement (i.e. $2N+1$). It is therefore a type of aneuploidy
	Translocation	The transfer of genetic material from one chromosome to another
	Reciprocal translocation	If there is an exchange of genetic material between two chromosomes then this is referred as a reciprocal translocation
	Balanced translocation	If the exchange of parts of chromosomes is such that no genetic material is either lost or gained then this translocation is referred as a balanced translocation
	G-Banding	It is the most commonly used chromosome staining method. G stands for Giemsa which is a DNA binding dye and gives each chromosome a characteristic and reproducible pattern of light and dark bands
FISH	Interphase	The stage between 2 successive cell divisions during which DNA replication occurs
	Probe	A labelled ssDNA fragment that hybridizes with, thereby detects and locates,complementary sequences
	Hybridization	Process of binding of DNA probes to the complementary DNA sequence in the sample
	Spectral karyotyping	A modification of FISH in which the result is a karyotype with homologous pairs of chromosomes having distinctive colors. The SKY technique makes it easier to detect chromosomal abnormalities, as compared with a conventional karyotype
PCR	Thermo cycler	PCR machine or DNA amplifier
	Primer	A strand of short nucleic acid sequences that serves as a starting point for DNA synthesis during a PCR
	dNTPs	Deoxy Nucleotide Triphosphates (dATP,dCTP,dGTP,dTTP)
	cDNA	Double-stranded DNA synthesized from a messenger RNA (mRNA) template in a reaction catalysed by the enzyme reverse transcriptase
	Reverse transcription polymerase chain reaction(RT-PCR)	RT-PCR is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA
	Real time PCR	Used to amplify and simultaneously detect or quantify a targeted DNA molecule
	RFLP	A polymorphism resulting from the presence/absence of a particular restriction site. A commonly used method for mutation detection
	Gene	A part of the DNA molecule of a chromosome that directs the synthesis of a specific polypeptide chain
	Oncogene	A gene affecting cell growth or development that can cause cancer
	Tumor suppressor gene	A gene that appears to prevent the development of certain types of cancer
	Allele	Alternative forms of a gene. Each individual has two alleles of a gene. One paternal other maternal

Table 4 continued

Genetic technique	Genetic terminology	Brief description
Gene, mutation, polymorphisms	Mutation	A change in genetic material, either of a single gene or in the number or structure of the chromosomes
	Somatic mutation	A mutation that occurs in the somatic cells. As against a mutation that occurs in gametes it is NOT inherited
	Point mutation	A single base pair change
	Substitution	If a single nucleotide is replaced by another it is called as a substitution
	Mis-sense mutation	A single base pair substitution when results in coding for a different amino acid and the synthesis of an altered protein
	Non-sense mutation	A single base pair substitution when results in coding for a stop codon leading to premature termination of translation of a peptide chain
	Deletions	A deletion involves loss of one or more nucleotides
	Polymorphism	The occurrence in a population of two or more genetically determined forms in such frequencies that the rarest of them could not be maintained by mutation alone
	SNPs	Single nucleotide DNA sequence variation that is polymorphic
	Microsatellite/STR	A type of polymorphism consisting of a tract of repetitive DNA (2–5 base pairs repeated, 5–50 time. Individuals vary with respect to the number of repeats among each other
	Gene Expression Profiling (GEP)	Measurement of the activity (the expression) of thousands of genes at once, to create a global picture of cellular function
	GWAS	GWA studies identify SNPs and other variants in DNA which are associated with a disease
	Microsatellite/STR	A type of polymorphism consisting of a tract of repetitive DNA (2–5 base pairs repeated, 5–50 time. Individuals vary with respect to the number of repeats among each other

(now called first generation sequencing) could sequence only a single DNA fragment at a time by capillary electrophoresis. In NGS millions of DNA fragments can be sequenced simultaneously. Thus, it is a high throughput parallel sequencing technique and allows thousands of genomes to be studied in a short time.

Through NGS one can study not only the genome but also the transcriptome (from RNA) and epigenome (DNA methylation sites). The commonly used NGS methods include:

- A. Genomics—whole genome sequencing, exome sequencing, targeted sequencing
- B. Transcriptomics—mRNA sequencing
- C. Epigenomics—CHIP sequencing (chromatin immunoprecipitation) to study DNA–protein interactions.

Application of NGS in hematologic malignancies has confirmed presence of mutation of certain genes like TP53, ATM, RAS etc. which were earlier known to be mutated in various solid malignancies. Besides that NGS is unraveling several novel mutations in genes involved in hematologic malignancies. The list of such mutated genes like IDH1, IDH2, DNMT3A, and SF3B1 is continuously increasing [17]. Future directions are to incorporate this detailed genomic

information into the existing prognostic scoring systems and to provide optimum therapeutic plans based on an individual's specific genomic background. Table 4 enumerates glossary of commonly used genetic terms and their definitions.

Interpretation of Various Key Genetic Changes in Hematologic Malignancies

Our understanding of prognostic or predictive value of various genetic parameters is rapidly changing. Accumulation of data on long term outcome of large number of patients in future will definitely help us to validate newer specific genetic abnormalities. As of today, majority of genetic tests are used to determine prognosis in a given patient of a hematologic malignancy. In the times to come genetic information will be incorporated in treatment decisions more frequently and that will be another step towards personalized medicine in today's era. Wide range of prognostic markers including, clinical features, radiological, cytochemical, immunophenotypic and genetic alterations are available for hematologic neoplasms. For this review, we have focused only on the currently available genetic tests in clinical use and their interpretation.

Acute Myelogenous Leukemia (AML)

In WHO 2008 classification of hematologic neoplasm AML has been reclassified into 4 types and one of the types is AML with recurrent cytogenetic abnormalities. Molecular understanding of AML is rapidly advancing. At present we stratify these cases according to various known karyotypic and molecular genetic aberrations into good, intermediate and poor risk subtypes. This stratification will surely expand to accommodate newer prognostic markers in the years to come.

European Leukemia Net has included the newer prognostic markers in AML risk stratification and suggest to stratify AML cases in 4 subtypes viz. favorable, intermediate I, intermediate II, and adverse prognosis group in decreasing order of overall survival [18].

Mutation in core binding factor (CBF), Nucleophosmin1 (NPM1), and CCAAT/enhancer binding protein alpha (CEBPA) genes are associated with favorable outcome in AML patients. Table 2 shows various translocations which are associated with these genetic mutations. Some studies have also shown that mutation in KIT gene and Wilms Tumor 1 (WT1) gene are associated with poorer outcome in AML patients [19, 20].

Mutations in the FMS-like tyrosine kinase 3 gene producing internal tandem duplications (FLT3-ITD) lead to constitutive activation of the FLT3 tyrosine kinase. FLT3-ITD has emerged as one of the most potent poor prognostic markers in cytogenetically normal AML (CN-AML) patients. In CN-AML cases presence of FLT3ITD mutation imply poor outcome similar to the patients in the poor risk group. As shown in Table 2, FLT3ITD gene mutation has potential of nullifying good prognostic value of NPM1 gene mutation if both of them are expressed together [21]. FLT3ITD has also emerged as a predictive marker for benefit with multi-kinase inhibitor sorafenib in AML patients [22].

Acute Lymphoblastic Leukemia (ALL)

For ALL conventional karyotype is ordered to see the ploidy level in leukemic cells as it is now known that hyperdiploidy carries a much better prognosis than hypodiploidy.

The translocation t(12;21)(p13;q22) is the most frequent genetic abnormality in precursor B cell acute lymphoblastic leukemia (ALL) cases. It is seen in approximately 25 % cases. This translocation results in the fusion of the 5' region of the gene TEL (ETV6), to the AML1 (RUNX1) locus and is associated with favorable prognosis [23]. Apart from providing important prognostic information certain genetic aberrations have emerged as predictive markers. The translocation t(12;21) involving TEL/AML1

genes is associated with good outcome and these patients show high cellular sensitivity to L asparaginase [24]. Similarly patients with t(1;19) have a better response with high dose methotrexate containing regimen [25]. However conventional karyotyping has limitation that it cannot detect many translocations in ALL because they are cryptic in nature therefore FISH is a better tool to employ for cytogenetic study of ALL patients.

The latest development in the field of ALL is use of minimal residual disease (MRD) after induction therapy for prognostication as well as treatment planning. MRD is defined by residual leukemic cells following the achievement of complete hematologic remission, but are below the limits of detection by microscopic assessment of blood and bone marrow. The detection of residual disease is usually based on either molecular or immune-phenotypical markers present in malignant cells. Variety of techniques can be used for detection of MRD, including immunophenotyping, cytogenetics, FISH, Southern blotting, spectral karyotyping, PCR, and deep sequencing [26–28].

It is agreed upon that to be effective, MRD detection technique should be able to detect one malignant cell out of 1000 cells [28]. At present, FISH, spectral karyotyping, PCR and gene sequencing fulfill these criteria. ALL is a clonal disorder and malignant cells have unique immunoglobulin gene rearrangements in B-lineage ALL and T cell receptor gene rearrangements in T-lineage ALL. For MRD study by molecular technique, the specific rearrangements must be sequenced in each case at the time of diagnosis. Presence of the same sequence is tested after finishing a particular phase of treatment to assess amount of MRD [29].

In some cases ALL cells carry a specific genetic abnormalities like translocation leading to gene fusion. The most common example of such gene fusion in ALL is *BCR-ABL1*. This finding makes estimation of MRD easier as PCR studies targeting gene fusions do not require the laborious sequencing or patient-specific primers required in antigen-receptor gene rearrangements studies because fixed sets of primers can be applied to all cases with a given gene fusion like BCR-ABL1. [30].

Chronic Myelogenous Leukemia (CML)

Role of genetics in monitoring and changing treatment in hematologic malignancies is exemplified by CML. Most patients of CML show remarkable response to tyrosine kinase inhibitors and achieve complete hematologic response relatively early in the treatment rendering the hematologic parameters suboptimal for monitoring of treatment response thereafter. It is evident from Table 1 that either conventional Cytogenetics showing Philadelphia chromosome or FISH for translocation t(9;22) is sufficient

Table 5 Comparative study of commonly used genetic tests in CML

Parameter	Conventional cytogenetics	FISH	PCR
Detection of aneuploidy	+++	–	–
Detection of a particular chromosomal translocation	+ But only for major translocations (>3–5 Mb of DNA)	+	+
Detection of cryptic translocations	–	+	–
Detection of a fusion gene	–	+	++
Quantification of gene expression	–	–	++
Detection of additional cytogenetic abnormality	++	–	–
Detection of MRD	–	+ (very select group of disorders)	++

Table 6 Classification of type and depth of treatment response in CML

Response	Definition
On the basis of cytogenetics/FISH	
Complete cytogenetic response	No Ph + cells detectable
Major cytogenetic response	1–35 % Ph + cells detectable
Minor cytogenetic response	36–65 % Ph + cells detectable
Minimal cytogenetic response	66–95 % Ph + cells detectable
No cytogenetic response	>95 % Ph + cells detectable
On the basis of RQ PCR	
Major molecular response, MR3.0	3 log reduction in bcr-abl transcript on international scale from baseline
Major molecular response, MR3.5	3.5 log reduction in bcr-abl transcript on international scale from baseline
Major molecular response, MR4.0	4 log reduction in bcr-abl transcript on international scale from baseline
Complete molecular response, MR4.5 to Not detectable	4.5 log reduction or more in bcr-abl transcript on international scale from baseline
Undetectable molecular transcripts	bcr-abl not detectable

for diagnosis of a case of CML. These tests can also be effectively used for monitoring of treatment response at specific time intervals and a patient can be classified into various categories of response depending up on proportion of remaining Philadelphia chromosome positive cells viz. [1] No response (>95 % Ph+ cells), [2] minimal (66 to 95 % Ph+ cells), [3] minor (36 to 65 % Ph+ cells), [4] major (1 to 35 % Ph+ cells), and [5] complete response (no Philadelphia chromosome positive cell). However better quantification techniques were desirable and it can be achieved by using RQ-PCR to detect levels of bcr-abl gene transcripts. Comparative advantages and disadvantages of various genetic tests for CML response monitoring are illustrated in Table 5.

It is important to understand two terms in relation to CML response monitoring, [1] International scale (IS) [2] Log reduction. The International Scale is defined by the ratio of BCR-ABL1 transcripts to normal ABL1 transcripts on a logarithmic scale. Log reduction is the level of reduction of bcr-abl transcript from the baseline value

expressed on a log scale. Therefore, mathematically, one log reduction is equal to 10 fold reduction in the bcr-abl transcripts. With the increased sensitivity of PCR technique, molecular responses are now “graded” as MR3.0, MR3.5, MR4.0, and MR4.5 whereby suffix number denotes level of log reduction. MR4.5 states that there is ≥ 1000 fold reduction in bcr-abl transcript or they are not detectable. This desirable state is also called as “complete molecular response” [31]. Table 6 illustrates various levels of response in CML on the basis of cytogenetics and PCR respectively.

In clinical practice, cytogenetic response is routinely checked at 6, 12 and 18 months of starting treatment and thereafter annually for follow-up. Similarly, molecular response is assessed at 3,6,12 and 18 months and thereafter periodically for follow-up. It is evident from the literature that the patients who have suboptimal cytogenetic or molecular response at a given point of time fare poorly in the long run [32–34]. Interpretation of these tests and alteration in treatment for management of suboptimal

response is out of purview of this article. However in suboptimal responders, genetics can again contribute by detecting molecular reason of poor response in an otherwise compliant patient. Mutational analysis by RTPCR for various known mutations in tyrosine kinase ATP binding domain can identify a specific mutation [35, 36] which eventually helps oncologists to alter the management of a patient accordingly.

Current research is focusing on using these ultra sensitive PCR techniques to select appropriate patients for cessation of tyrosine kinase therapy who have achieved deepest molecular response. A positive outcome of such study may lead to second revolutionisation in the field of CML after the development of TKI imatinib.

Chronic Lymphocytic Leukemia (CLL)

CLL is a clonal disorder and 40–50 % patients have clonal abnormalities identifiable with conventional cytogenetics. However as discussed earlier, cytogeneticist may face a problem in obtaining metaphase karyotype owing to low mitotic index of leukemic lymphocytes in CLL. This brings in the role of FISH in the analysis of CLL patients. For example del17p leads to loss of TP53 gene which is regarded as poorest prognostic marker in CLL with median survival of less than 3 years. This deletion imparts relative resistance to conventional antineoplastic agents. It is therefore indicated that CLL patients with del17p should be treated with newer targeted agents or they should be encouraged to participate in a clinical trial. At present, specific agents like ibrutinib (bruton tyrosine kinase inhibitor), idelalesib (Phosphatidylinositol-4,5-bisphosphate 3-kinase inhibitor) and flavopiridols have shown promising results in CLL with del17p [37]. Short of these newer agents, CLL patients with del17p are eligible candidate for allogeneic stem cell transplant for long term survival.

On the basis of immunoglobulin heavy chain variable region (IGHV) gene rearrangement CLL cases are divided into two subtypes, viz. IGHV mutated and unmutated. These two subtypes have different outcome with IGHV unmutated patients having shorter survival and higher rate of disease recurrence after therapy. Approximately 50 % of the patients of CLL fall in mutated and unmutated group each. These mutational studies have robust prognostic value however their availability is not universal due to complex nature of the investigation.

MicroRNAs (miRNAs) are small non-coding RNAs and they are involved in post transcription regulation of genes. Mutations in miRNA are frequently seen in cases of CLL. miRNA29c, miR-223, and miRNA 155 are linked with outcome of CLL and could predict treatment—free survival (TFS) and overall survival (OS) [38, 39].

Multiple Myeloma (MM)

Multiple myeloma arises from plasma cells which are the most differentiated form of B cell. Conventional karyotype reveals cytogenetic abnormality in one-fourth of the patients. Myeloma is a genetically heterogeneous disorder. With the help of conventional and modern cytogenetic techniques MM cases can be classified into two groups: non-hyperdiploid and hyperdiploid myeloma cases. Non-hyperdiploid myeloma is seen in about 40 % of the cases and is characterized by translocations involving the immunoglobulin heavy chain gene at chromosome 14q32, resulting in transcriptional activation of CCND1, CCND3, MAF, MAFB, or FGFR3/MMSET. On the other hand hyperdiploidy in myeloma is seen due to trisomies of multiple odd chromosomes (3, 5, 7, 9, 11, 15, 19, and 21) and is believed to have a better prognosis [40]. However like mature lymphocytes, plasma cells also have a low growth fraction which makes decisions on conventional karyotyping unreliable.

More readily used genetic tests for multiple myeloma are based on FISH to detect key translocations as mentioned in Table 2.

The most common partner in chromosomal translocations in MM is chromosome 14q32, the site of the immunoglobulin heavy chain gene. These include: t(11, 14), t(4, 14), t(8, 14), and t(14, 16).

Modern genetic techniques such as microarray based DNA testing are coming to aid in such situations. For example—recently University of Arkansas for Medical Sciences developed a 70 gene signature microarray called MyPRS (Myeloma Prognostic Risk Score). This gene expression profiling analysis allocates scores on the basis of tested genes which is presented as either low (70-gene score < 45.2) or high (≥ 45.2) risk for relapse [41, 42]. However such techniques remain expensive as well as sparsely available.

Myelosplastic Syndrome (MDS)

MDS is unique due to protean morphologic features in blood and BM examinations. At times dysplastic changes in various cell lines are subtle and beyond the limits of human eyes. Detection of key genetic changes in such patients may clinch the diagnosis in time and can assist in selection of treatment as well. International prognosis scoring system (IPSS, IPSS-R) is used for prognostication of MDS patients and cytogenetic abnormalities are one of the 5 components of IPSS [43, 44]. Good karyotype includes normal karyotype, $-Y$, del(5q), or del(20q). Poor karyotype includes complex karyotype (≥ 3 abnormalities) or abnormal chromosome 7. Other cytogenetic abnormalities are clubbed together as intermediate karyotype. Cytogenetic abnormalities are found at baseline in 20 to 70 % of patients with MDS. The highest frequencies were

found in patients with refractory anemia with excess blasts (RAEB)-1 and (RAEB)-2.

Deletion involving chromosome 5, del(5q), is one of the most common cytogenetic abnormality in patients with MDS. Approximately 5 % of cases are classified as the myelodysplastic syndrome with isolated del(5q) [45]. The prognostic value of del(5q) is variable in published literature, however emerging data suggested that immunomodulating drug lenalidomide is more efficacious in patients with del(5q) MDS. Perhaps this is explained by modulation of aberrant signaling pathways caused by haplosufficiency of specific genes in a commonly deleted region on chromosome 5 by lenalidomide [46].

Conclusions

Induction of genetics in clinical practice may appear intimidating due to sheer complexities of the techniques involved. However, genetics has provided a completely new perspective with which we approach hematologic neoplasm today. With time these complex investigations are becoming not only more refined, but also more affordable and easily accessible. Future looks more promising in the field of genetics in relation to oncology. At present The Cancer Genome Atlas is being developed. Team of geneticists and oncologists are now working together to map the cancer cell genome to try to learn precise mechanism behind oncogenesis. The results of this project will further improve our understanding of the genetic basis of cancer. In the times to come hemato-oncologists, hematopathologists and medical geneticists will be an integral part of a team which can deliver optimal and personalized care to patients suffering from various hematologic malignancies on the basis of genetic profile of their illness.

Acknowledgments We acknowledge Department of Hematology, PGIMER, Chandigarh for providing images for this publication.

Compliance with Ethical Standards

Conflict of interest There are no potential conflicts of interest of authors writing this article.

Research involving human participants and/or animals This is a review article and does not involve any human or animal intervention or experiment.

Informed consent No human subjects or biologic material was used for this review article.

References

1. Siegel R, Ward E, Brawley O, Jemal A (2011) Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 61(4):212–236
2. Harper PS (2006) The discovery of the human chromosome number in Lund, 1955–1956. *Hum Genet* 119(1–2):226–232
3. Rowley JD (1998) The critical role of chromosome translocations in human leukemias. *Annu Rev Genet* 32:495–519
4. Nowell PC, Hungerford DA (1960) Chromosome studies on normal and leukemic human leukocytes. *J Natl Cancer Inst* 25:85–109
5. Trask BJ (1991) Fluorescence in situ hybridization: applications in cytogenetics and gene mapping. *Trends Genet TIG* 7(5):149–154
6. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F et al (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258(5083):818–821
7. Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA et al (1996) Multicolor spectral karyotyping of human chromosomes. *Science* 273(5274):494–497
8. Imataka G, Arisaka O (2012) Chromosome analysis using spectral karyotyping (SKY). *Cell Biochem Biophys* 62(1):13–17
9. Logan J, Edwards K, Saunders N (eds) (2009) Real-time PCR: current technology and applications. Caister Academic Press, Norfolk, p 284
10. Watson JD (ed) (2004) Molecular biology of the gene. San Francisco, Pearson/Benjamin Cummings [u.a.], p 732
11. Little S (2001) Amplification-refractory mutation system (ARMS) analysis of point mutations. In: Haines JL, Korf BR, Morton CC, Seidman CE, Seidman JG, Smith DR (eds). Current protocols in human genetics [Internet]. Wiley & Sons, Inc., Hoboken [cited 2015 Aug 8]. Available from <http://doi.wiley.com/10.1002/0471142905.hg0908s07>
12. Sedlak RH, Jerome KR (2013) Viral diagnostics in the era of digital polymerase chain reaction. *Diagn Microbiol Infect Dis* 75(1):1–4
13. Bench AJ (2012) The role of molecular genetic analysis within the diagnostic haemato-oncology laboratory. *Int J Lab Hematol* 34(1):21–34
14. Khan F, Agarwal A, Agrawal S (2004) Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. *Bone Marrow Transplant* 34(1):1–12
15. Lichter P, Joos S, Bentz M, Lampel S (2000) Comparative genomic hybridization: uses and limitations. *Semin Hematol* 37(4):348–357
16. Read JA, Koff JL, Nastoupil LJ, Williams JN, Cohen JB, Flowers CR (2014) Evaluating cell-of-origin subtype methods for predicting diffuse large B-cell lymphoma survival: a meta-analysis of gene expression profiling and immunohistochemistry algorithms. *Clin Lymphoma Myeloma Leuk* 14(6):460
17. Braggio E, Egan JB, Fonseca R, Stewart AK (2013) Lessons from next-generation sequencing analysis in hematological malignancies. *Blood Cancer J* 3(7):e127
18. Mrózek K, Marcucci G, Nicolet D, Maharry KS, Becker H, Whitman SP et al (2012) Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *J Clin Oncol Off J Am Soc Clin Oncol* 30(36):4515–4523
19. Renneville A, Boissel N, Zurawski V, Llopis L, Biggio V, Nibourel O et al (2009) Wilms tumor 1 gene mutations are associated with a higher risk of recurrence in young adults with acute myeloid leukemia: a study from the Acute Leukemia French Association. *Cancer* 115(16):3719–3727
20. Paschka P, Marcucci G, Ruppert AS, Mrózek K, Chen H, Kittles RA et al (2006) Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv (16) and t(8, 21): a Cancer and Leukemia Group B Study. *J Clin Oncol Off J Am Soc Clin Oncol* 24(24):3904–3911

21. Schlenk RF, Döhner K, Krauter J, Fröhling S, Corbacioglu A, Bullinger L et al (2008) Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med* 358(18):1909–1918
22. Ravandi F, Cortes JE, Jones D, Faderl S, Garcia-Manero G, Konopleva MY et al (2010) Phase I/II study of combination therapy with sorafenib, idarubicin, and cytarabine in younger patients with acute myeloid leukemia. *J Clin Oncol Off J Am Soc Clin Oncol* 28(11):1856–1862
23. Zelent A, Greaves M, Enver T (2004) Role of the TEL-AML1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. *Oncogene* 23(24):4275–4283
24. Stams WAG, den Boer ML, Beverloo HB, Meijerink JPP, van Wering ER, Janka-Schaub GE et al (2005) Expression levels of TEL, AML1, and the fusion products TEL-AML1 and AML1-TEL versus drug sensitivity and clinical outcome in t(12, 21)—positive pediatric acute lymphoblastic leukemia. *Clin Cancer Res Off J Am Assoc Cancer Res* 11(8):2974–2980
25. Garg R, Kantarjian H, Thomas D, Faderl S, Ravandi F, Lovshe D et al (2009) Adults with acute lymphoblastic leukemia and translocation (1, 19) abnormality have a favorable outcome with hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and high-dose cytarabine chemotherapy. *Cancer* 115(10):2147–2154
26. Campana D (2003) Determination of minimal residual disease in leukaemia patients. *Br J Haematol* 121(6):823–838
27. Faham M, Zheng J, Moorhead M, Carlton VEH, Stow P, Cousstan-Smith E et al (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood* 120(26):5173–5180
28. van Dongen JJ, Szczepański T, de Bruijn MA, van den Beemd MW, de Bruin-Versteeg S, Wijkhuijs JM et al (1996) Detection of minimal residual disease in acute leukemia patients. *Cytokines Mol Ther* 2(2):121–133
29. Campana D (2010) Minimal residual disease in acute lymphoblastic leukemia. *Hematology* 2010(1):7–12
30. Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N et al (2003) Standardization and quality control studies of “real-time” quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. *Leukemia* 17(12):2318–2357
31. Cross NCP, White HE, Müller MC, Saglio G, Hochhaus A (2012) Standardized definitions of molecular response in chronic myeloid leukemia. *Leukemia* 26(10):2172–2175
32. Marin D, Milojkovic D, Olavarria E, Khorashad JS, de Lavallade H, Reid AG et al (2008) European LeukemiaNet criteria for failure or suboptimal response reliably identify patients with CML in early chronic phase treated with imatinib whose eventual outcome is poor. *Blood* 112(12):4437–4444
33. Alvarado Y, Kantarjian H, O’Brien S, Faderl S, Borthakur G, Burger J et al (2009) Significance of suboptimal response to imatinib, as defined by the European LeukemiaNet, in the long-term outcome of patients with early chronic myeloid leukemia in chronic phase. *Cancer* 115(16):3709–3718
34. Hehlmann R, Müller MC, Lausker M, Hanfstein B, Fabarius A, Schreiber A et al (2014) Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. *J Clin Oncol Off J Am Soc Clin Oncol* 32(5):415–423
35. Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G et al (2011) BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood* 118(5):1208–1215
36. O’Hare T, Eide CA, Deininger MWN (2007) Bcr-Abl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood* 110(7):2242–2249
37. Hallek M (2015) Chronic lymphocytic leukemia: 2015 Update on diagnosis, risk stratification, and treatment. *Am J Hematol* 90(5):446–460
38. Lin TS, Ruppert AS, Johnson AJ, Fischer B, Heerema NA, Andritsos LA et al (2009) Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. *J Clin Oncol Off J Am Soc Clin Oncol* 27(35):6012–6018
39. Ferrajoli A, Shanafelt TD, Ivan C, Shimizu M, Rabe KG, Nouraei N et al (2013) Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. *Blood* 122(11):1891–1899
40. Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, Winkler JM et al (2003) The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. *Blood* 102(7):2562–2567
41. Broyl A, Hose D, Lokhorst H, de Knecht Y, Peeters J, Jauch A et al (2010) Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. *Blood* 116(14):2543–2553
42. van Laar R, Flinchum R, Brown N, Ramsey J, Riccitelli S, Heuck C et al (2014) Translating a gene expression signature for multiple myeloma prognosis into a robust high-throughput assay for clinical use. *BMC Med Genom* 7:25
43. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* 89(6):2079–2088
44. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F et al (2012) Revised international prognostic scoring system for myelodysplastic syndromes. *Blood* 120(12):2454–2465
45. Tefferi A, Vardiman JW (2009) Myelodysplastic syndromes. *N Engl J Med* 361(19):1872–1885
46. Giagounidis A, Mufti GJ, Fenaux P, Germing U, List A, MacBeth KJ (2014) Lenalidomide as a disease-modifying agent in patients with del(5q) myelodysplastic syndromes: linking mechanism of action to clinical outcomes. *Ann Hematol* 93(1):1–11
47. Melo JV (1997) BCR-ABL gene variants. *Baillieres Clin Haematol* 10(2):203–222
48. Rajkumar SV (2012) Multiple myeloma: 2012 update on diagnosis, risk-stratification, and management. *Am J Hematol* 87(1):78–88
49. Kumar S, Fonseca R, Ketterling RP, Dispenzieri A, Lacy MQ, Gertz MA et al (2012) Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics. *Blood* 119(9):2100–2105