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providing the latest information

Facts About **Biomarker Testing**

Introduction

Cancer therapies have always leveraged the differences between normal and neoplastic cells, and over recent years our understanding of precisely what is different has expanded exponentially. As DNA sequencing technology has uncovered more and more genetic and molecular defects driving blood cancers, therapies have evolved away from "one-size-fits-all" to more targeted approaches, all with a goal of improving clinical outcomes. Molecular profiling helps identify the precise molecular changes associated with malignancy and frequently guides diagnosis, treatment, and prognosis for blood cancers. There are numerous targeted therapies on the market for hematologic malignancies and many more in clinical trials.

While advances in genetic analysis have shed welcome light on precise molecular changes driving malignancies, incorporating molecular profiling into routine practice is not without challenges. DNA sequencing reports can deliver overwhelming amounts of difficult-to-interpret information, leaving clinicians uncertain of which mutations are clinically relevant to determine the best course of action. It is challenging to keep pace with the extremely rapid development of newly identified targets or indications for approved therapies. There are access and reimbursement difficulties, particularly for targeted therapies not FDA-approved for a particular malignancy that may harbor a druggable aberration. Finally, patients need to be educated about molecular profiling in order to better inform their decision-making and allow them to be fully engaged partners in their care.

This Fact Sheet provides a broad overview of the basics of molecular profiling and how results can inform care for patients with hematologic malignancies. Additional resources for clinicians and patients, providing detail beyond the scope of this Fact Sheet, are provided. Highlighted terminology in **blue** is defined in the glossary found on pages 13-14.

Highlights

- The goal of molecular profiling is to identify biomarkers associated with malignancies that can serve as therapeutic targets, inform diagnosis or prognosis, or gauge response to therapy.
- Standard techniques such as chromosome banding, fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and flow cytometry have been used for years to investigate hematologic malignancies. These techniques are limited by low resolution, and they can miss genome-wide changes.
- Chromosomal microarrays (CMAs) enable whole genome "molecular karyotyping" in a single assay that does not require viable cells.
- CMAs have become routine for identifying copy number losses or gains of whole or segmented chromosomes.
- Next generation sequencing (NGS) technologies have allowed the detection of multiple DNA aberrations across multiple genes.
- Targeted mutation panels sequence only genes suspected or known to be associated with a malignancy. There are many commercially available gene panels for hematologic malignancies.
- Resources are emerging to assist clinicians in identifying the right test for the right patient at the right time.
- Molecular tumor boards and online resources are available to assist in the interpretation of data generated from molecular investigations of hematologic malignancies.

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Molecular Profiling:

The Basics

The goal of molecular profiling is to identify **biomarkers** associated with malignancy. This can include the identification of molecular targets of approved therapies (or those in clinical trials), or identification of biomarkers that can inform prognosis, disease progression, or gauge response to therapy. Molecular pathology analysis is rapidly becoming a critical tool for uncovering the tumor biology that can drive optimal therapeutic decisions.[1]

Types of genetic alterations

Biomarkers associated with cancer most frequently arise from **mutations** resulting in changes in the expression patterns or activities of genes or proteins.[2] Mutations can be either **somatic** (acquired during a person's lifetime), or **germline** (mostly inherited). Somatic mutations are present only in certain cells, while germline mutations are present in virtually every cell in the body.

Common somatic mutations that have been identified in hematologic malignancies include[3,4]:

- **Substitutions**, in which one base (or more) in the DNA sequence is replaced by a different base
- **Insertions/deletions (indels)**, small insertions or deletions of 1-1,000 bases[5]
- **Copy number alterations (CNAs)**, in which large segments of DNA (from a few kilobases [kb] to entire chromosomes) are gained or lost[6]
- **Gene fusions**, created by joining 2 different genes, commonly from 2 different chromosomes
- **Inversions**, in which a segment of a chromosome breaks off and reattaches in the opposite direction
- **Translocation**, where a piece of one chromosome breaks off and attaches to another chromosome

Tests used to identify biomarkers

Karyotyping

Conventional cytogenetics, including chromosome analysis via karyotyping, has been used for decades to identify chromosome abnormalities in hematologic malignancies.[4] Karyotyping is a technique that produces an image of an individual's chromosomes. **Chromosome banding** refers to light and dark regions along the length of a chromosome after staining with a dye. This technique provides a low-resolution, whole genome scan that can identify many recurrent structural chromosome abnormalities, fusion gene translocations, and inversions common in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL).[4]

Karyotyping is an important part of the standard investigation of many hematologic malignancies, although due to its low resolution many aberrations may be missed.[7] In fact, abnormal karyotypes are seen in only 20–40% of patients with multiple myeloma (MM), 60% of those with ALL, 40% of those with CLL and 40%–50% of those with AML.[7] (The yield in CLL can be low due to the poor growth of these cells in culture).

Fluorescence in situ hybridization (FISH)

FISH is another standard technique that uses fluorescently-labeled DNA probes to detect aberrations at the gene level, including rearrangements, translocations and CNAs.[1,7] It has a higher resolution than karyotyping and can be performed on fresh or paraffin-embedded tissue and interphase cells from liquid tumor cells.[8] FISH is typically used to probe genomic regions from 100-600kb, not the entire genome, and is commonly used to complement karyotyping.[7]

In hematologic malignancies, FISH is capable of detecting **druggable** fusion genes (PML-RARA, BCR-ABL1, PDGFRA/PDGFRB) in addition to cytogenetic aberrations of diagnostic or prognostic value.[9] Most laboratories use commercially available probes targeted to specific regions known to be important for certain hematologic malignancies (i.e., FISH panels for AML or ALL). FISH is recommended as a priority test in MM and CLL, as it has been shown to identify up to 95% of patients with MM, 80% of those with CLL, and ≈89% of ALL cases.[7]

While karytope and FISH are standard practice for investigating hematologic malignancies, studies have demonstrated that they can miss genome-wide changes, highlighting the need for techniques capable of higher resolution investigation across the entire genome.[7]

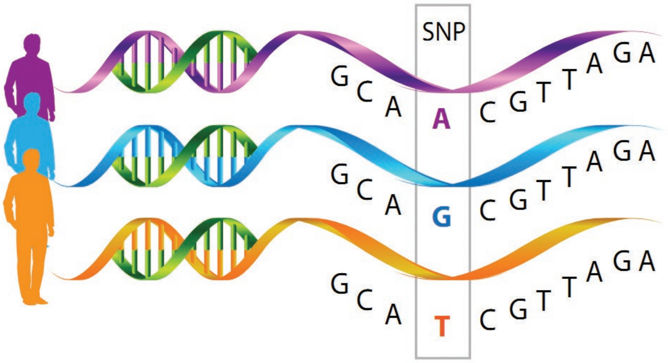
Chromosomal Microarrays (CMAs)

These techniques, including **array comparative genomic hybridization (aCGH)** assays and **single nucleotide polymorphism (SNP)** arrays, enable whole-genome molecular karyotyping in a single assay that does not require viable cells.[4,8,9] They can be used for identifying copy number losses or gains of whole (or segmental) chromosomes.

aCGH-based assays compare genomic DNA from a tumor sample with that of a normal reference DNA sample. Each set of DNA is fragmented, labeled (a different fluorescent label is used for patient vs control DNA), and allowed to hybridize to DNA probes that have been immobilized on a solid surface (typically a glass chip).[8,10] Following hybridization, an imaging system scans the chip to measure the relative fluorescence intensity associated with each probe, which is proportional to the copy numbers present.[9] Depending on the number of probes and their distribution, aCGH assay can provide a genome-wide analysis at a resolution of ≈50kb.[4] Microrrays can detect CNAs such as **aneuploidies**, microdeletions and duplications, **chromothripsis** and amplifications.[9]

A **SNP array** is similar to aCGH but uses DNA probes from regions in the genome that show differences between individuals at a single base pair (a "**single nucleotide polymorphism**" or SNP). SNP arrays can detect a specific genotype at the location of the probe.[9] As opposed to aCGH, which measures the difference in fluorescence intensity between DNA from a tumor vs a control sample, SNPs measure the absolute probe intensities between the tumor DNA and a set of normal control samples that have been analyzed separately, normalized, and combined into a reference set (this is referred to as an *in silico* comparison).[9,10]

Single Nucleotide Polymorphism (SNP)



SNP arrays can reveal copy number information as well as **copy neutral loss of heterozygosity (CN-LOH)**, often referred to as uniparental disomy (UPD)[44], (identified in myeloid malignancies[11]) and polyploidies.[9] Since identification of balanced rearrangements and fusion genes lend important prognostic information in hematologic malignancies, SNP array analysis should be accompanied by FISH or targeted sequencing panels.[7]

The resolution of a specific array depends on the size and types of probes used and their distribution across the genome. Potential indications for CMAs for certain hematologic malignancies are found in **Table 1.**

List of Abbreviations

aCGH: Array comparative genomic hybridization
ALL: Acute lymphoblastic leukemia
AML: Acute myeloid leukemia
B-ALL: B-cell acute lymphoblastic leukemia
CLL: Chronic lymphocytic leukemia
CMA: Chromosomal microarray
CML: Chronic myeloid leukemia
CN-LOH: Copy neutral loss of heterozygosity
CNA: Copy number alterations
FISH: Fluorescence in situ hybridization
FL: Follicular lymphoma
IHC: Immunohistochemistry
Indel: Insertion/deletion
MCL: Mantle cell lymphoma
MDS: Myelodysplastic syndrome
MM: Multiple myeloma
MPN: Myeloproliferative neoplasm
MRD: Minimal (or measurable) residual disease
MTB: Molecular tumor board
NGS: Next generation sequencing
PCR: Polymerase chain reaction
Ph+: Philadelphia chromosome-positive
qPCR: Quantitative PCR
SLL: Small lymphocytic lymphoma
SNP: Single nucleotide polymorphism
T-ALL: T-cell acute lymphoblastic leukemia
VUS: Variants of uncertain significance
WES: Whole exome sequencing
WGS: Whole genome sequencing

Table 1. Microarray Studies in Select Hematologic Neoplasms[8] (adapted from Peterson JF, et al. 2018)

Neoplasm	Indication
Myelodysplastic syndromes (MDS)	• To detect recurrent copy number alterations such as del(5q), del(7q)/-7,+8, del(13q)/-13, del(11q), del(17p), del(20q) • Following karyotype or FISH results – To resolve complex results – To confirm normal results • If no metaphase cells are available for karyotype
B-lymphoblastic leukemia/lymphoma	• To distinguish hyperdiploidy from pseudohyperdiploidy • To detect amplification of chromosome 21 (or to distinguish from polysomy 21) • To detect small gene deletions not detectable by karyotype or FISH • To confirm normal karyotype or FISH results • Potential routine use for all newly diagnosed patients
Chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL)	• To detect recurrent CNAs (e.g., del(11q), +12, del(13q), del(17p), complex karyotypes • Potential routine use for all newly diagnosed patients
Burkitt-like lymphoma with 11q aberrations	• To detect 11q aberrations in cases that resemble Burkitt lymphoma but lack *MYC* rearrangements

Polymerase chain reaction (PCR)

PCR is a technique that rapidly produces large quantities of a precise DNA segment from minute quantities of starting material. PCR is an automated technique that involves denaturing, or separating, the 2 DNA strands, using DNA polymerase to synthesize 2 new DNA strands, then repeating the process as many as 30 or 40 times. **Quantitative PCR** (**qPCR** or **real-time PCR**) can determine the absolute or relative quantities of a known sequence in a starting sample, typically by quantitating fluorescence from a dye that binds to double-stranded DNA.

Because hematologic malignancies can be driven by single genetic aberrations that make good PCR targets, qPCR is an excellent method for quantifying **Measurable Residual Disease (MRD)**.[12,35]

Next Generation Sequencing

Advances in **next generation sequencing (NGS)** technology have allowed molecular profiling of hematologic malignancies to evolve to detection of multiple DNA aberrations across multiple genes, including inversions/deletions, copy number variations, translocations, and gene fusions.[1,3] NGS has long been invaluable in advancing research through the discovery of novel mutations in hematologic malignancies and has now become an integral part of clinical diagnostics, particularly in conditions like MDS and AML.[4]

In NGS, millions of small DNA fragments are immobilized on a solid surface, amplified (copied), and sequenced simultaneously. During sequencing, a signal (i.e., light, pH change) is generated and detected when each base is incorporated. The resulting "reads" are then aligned to a reference sequence and analyzed. NGS requires intense computational analysis and generates voluminous data.[13] A continuing challenge has been developing the tools and protocols that optimally translate the resulting information into better patient care.[1]

NGS analyses can range from the testing of multiple genes in **targeted gene panels** to **whole exome** or **whole genome sequencing** of cancer cell DNA.[14]

Whole exome sequencing (WES) captures the DNA sequence of only the protein-coding regions of the genome, which represents only ≈1% of the genome. WES is less costly than whole genome sequencing (WGS), but cannot identify any mutations that lie outside the coding regions, nor can it identify chromosomal translocations or inversions with breakpoints located outside coding regions.

Whole genome sequencing (WGS) captures the sequence of the entire genome. While many biomarkers now known to be important in hematologic malignancies were discovered with WGS (e.g., *DNMT3A, IDH1/2*), and the information it yields is not limited to preselected targets, it is not routinely performed in the clinical setting.[13] WGS is time consuming, expensive, and results can be difficult to interpret, particularly when there is a low tumor burden.[4,13]

In addition, for both WGS and WES, a non-tumor germline control tissue sample from each patient must be analyzed simultaneously to identify acquired mutations that may be drivers of malignancy.[13] To date, WES and WGS are largely limited to the research space.[1]

Targeted gene panels (mutation panels)

This NGS approach sequences only genes or areas suspected or known to be associated with a particular malignancy ("hotspot" regions known to contain mutations).[1] There are scores of available gene panels for hematologic malignancies (with largely overlapping target genes/mutations). Examples include:

- **GeneDX:** https://www.genedx.com
- **University of Chicago:** https://dnatesting.uchicago.edu
- **Prevention Genetics:** https://www.preventiongenetics.com
- **Illumina:** https://www.illumina.com/products/selection-tools/gene-panel-finder.html
- **Foundation Medicine:** https://www.foundationmedicine.com/resources
- **Blueprint Genetics:** https://blueprintgenetics.com/tests/panels
- **Invitae:** https://www.invitae.com/en/physician/panelsgenes
- **Neogenomics:** https://neogenomics.com/test-menu?search=NGS&methodology=61&diseases=All
- **TEMPUS|ONCOLOGY:** https://www.tempus.com/oncology

Table 2 is an overview of the types of cytogenomic tests currently used in clinical practice.

Table 2. Overview of Cytogenomic Tests in Current Clinical Practice.[7,8]

Technique	Detects	Resolution	Whole genome?	Requires dividing cells?
Karyotype	Chromosome structural abnormalities, translocations (fusion genes), inversions, aneuploidies, balanced rearrangements	Very low	Yes	Yes
Fluorescence in situ hybridization (FISH)	Changes in targeted genomic regions with diagnostic or prognostic value for a particular malignancy. Can detect fusion genes, balanced rearrangements and CNAs[7,9]	≈100kb-300kb	No	Varies depending on application
SNP microrarray analysis	CNAs, copy neutral loss of heterozygosity (CN-LOH), uniparental disomy (UPD), polyploidies.[9] Does not identify balanced rearrangements or fusion genes	≈3kb-10kb	Yes	No
Next Generation Sequencing				
Targeted gene panels	Changes at the DNA level in selected areas of interest (hotspots with known mutations)	< 1kb	No	No
Whole exome sequencing (WES)	Changes at the DNA level in protein coding region and immediately surrounding regions	< 1kb	Yes	No
Whole genome sequencing (WGS)	Changes at the DNA level in **both coding and non-coding regions**, including point mutations (single-nucleotide variants, SNVs), indels, translocations, gene fusions, CNAs and unexpected structural changes	< 1kb	Yes	No

For all NGS approaches, when seeking to identify genetic alterations that result in a druggable target, it is important to distinguish **driver mutations**, which have been established to play a direct role in the development or maintenance of malignancy, from **passenger mutations**, which are commonly found in most cancers but do not confer a selective advantage and are not actionable.[2] An individual malignancy may have more than one driver mutation.[2]

Immunohistochemistry (IHC) and Flow Cytometry: Protein-level Information

Along with karyotyping, IHC is another laboratory technique with variable sensitivity and specificity for detecting actionable biomarkers that has been in routine use for years.1 IHC is a technique in which a biomarker-specific antibody is applied to a tissue or blood sample, which is then linked to an enzyme or fluorescent tag that permits visualization with fluorescence microscopy. IHC can detect changes at the protein level resulting from any number of genetic aberrations including gene amplifications, DNA rearrangements and point mutations.1 Flow cytometry is based on the same principles but is performed on a liquid sample and the signal is read on a flow cytometer.

What to Test for

Multiple molecular diagnostic tests and testing platforms are available from both hospital and commercial labs. Clinicians can be hard pressed to determine the right test for the right patient at the right time, and discern which tests will provide information that can be used to guide treatment decisions.[14] Specific guidance for which molecular tests are warranted during the course of diagnosis, treatment, and disease monitoring for every hematologic malignancy is beyond the scope of this Fact Sheet.

As an example of testing guidance, European guidelines for the cytogenomic investigation of hematologic malignancies were recently published.[15] The testing recommendations in **Table 3** have been adapted from this resource. For testing recommendations for ALL, which vary by age, see **Table 4**.

Table 3. Recommendations for Testing[15] (adapted from Rack KA, et al. 2019)

Disease	Test	Requirement	Suggested Method
Chronic myelogenous leukemia (CML)	• Karyotype	Mandatory	Chromosome banding
	• *BCR-ABL1* gene fusion	Mandatory	FISH (at diagnosis only), PCR
	• *ABL1* mutation (when resistant to therapy)	Mandatory	PCR, NGS
	• *MRD* at baseline	Indicated	PCR
Myeloproliferative neoplasms (MPN)	• *JAK2, CALR, MPL* mutations • Rule out *BCR-ABL1* gene fusion	Mandatory	PCR, NGS
	• Karyotype	Indicated	Chromosome banding
Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions	• Recurrent gene fusions: *PDGFRA, PDGFRB, FGFR1, PCM1-JAK2*	Strongly recommended	FISH, PCR, NGS
	• Karyotype	Indicated	Chromosome banding, WGS
Myelodysplastic syndromes (MDS)	• Karyotype	Mandatory	Chromosome banding
	• Targeted chromosome abnormalities: -5/-5q, -7/-7q, inv3/3q (MECOM); extended panel: +8, -20q, 17p (TP53)	Recommended (if karyotype fails or where there is morphological suspicion of specific abnormality)	FISH, SNP array, PCR, NGS (all not routinely done)
	• High resolution chromosome analysis, acquired CN-LOH	Recommended	SNP array (not necessarily routine)
	• Mutation analysis of candidate genes	Recommended	PCR, NGS

Table 3. CONTINUED. Recommendations for Testing[15] (adapted from Rack KA, et al. 2019)

Disease	Test	Requirement	Suggested Method
Acute myeloid leukemia (AML)	• Karyotype	Mandatory	Chromosome banding, WGS
	• Gene mutations: *NPM1, DNMT3A, IDH1, IDH2, CEBPA, RUNX1, FLT3, TP53, ASXL1* (among others)	Mandatory	PCR, NGS
	• Recurrent gene fusions: *PML-RARA* (if there is clinical suspicion), *CBFB-MYH11, RUNX1-RUNX1T1, KMT2A* rearrangement, *DEK-NUP214, MECOM, KAT6A::CREBBP*	Mandatory (to inform prognosis)	FISH, NGS, including *DNMT3A, IDH1, IDH2*, and other genes, is standard.
	• Enriched in pediatric *AML: NUP98* rearrangements, *CBFA2T3-GLIS2, RBM15::MRTFA, UBTF*		
	• Other Gene Mutations: *BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*[36]	Recommended (to inform prognosis)	NGS
Acute lymphoblastic leukemia (ALL) (pediatric recommendations found in Table 4)	• Recurrent gene fusions (age-related priority), including *ETV6-RUNX1* and *TCF3-PBX1* in pediatrics. *KMT2A* rearrangements, *BCR-ABL1*, and *BCR-ABL1*-like across the age spectrum. • Ph-ALL	Mandatory	FISH: • for 12;21 in pediatrics • for 4;11 in all patients • for *BCR-ABL* in all patients PCR, NGS • for *KMT2Ar* for ALL regardless of age
	• Hypodiploidy/Hyperdiploidy	Recommended	Chromosome banding, SNP array, FISH
	• Recurrent microdeletions	Recommended in pediatric ALL	Multiplex Ligation-dependent Probe Amplification (MLPA), array, PCR, NGS
	• Karyotype (may not be required for all pediatric patients)	Mandatory	Chromosome banding
	• MRD at baseline • MRD upon completion of initial induction • MRD at end of consolidation	Indicated	PCR, NGS, or flow cytometry
Chronic lymphocytic leukemia (CLL)	• Deletion 13q14, *ATM, TP53*, trisomy12	Mandatory	FISH, SNP array, NGS
	• *TP53* mutation/IGHV mutational status	Mandatory	PCR, NGS
	• Karyotype	Indicated	Chromosome banding
Multiple myeloma (MM)	• t(4;14), t(14;16), deletion *TP53* gain 1q/del(1p)	Recommended	FISH for gene rearrangements
	• t(11;14), t(14;20), ploidy status (extended panel)	Recommended	FISH or array, MLPA for copy number gains/losses
Other mature B-cell neoplasms	• Recurrent gene rearrangements (depending on differential diagnosis)		FISH
	• MYC rearrangements (prognostic)		FISH

Table 4. Age-dependent Recommendations for Fusion Gene investigations in ALL[15]
(adapted from Rack KA, et al. 2019)

Diagnosis	Age	Recommended	Optional
B-cell acute lymphoblastic leukemia (B-ALL)	< 1 yr	*KMT2A*	ETV6-RUNX1, BCR-ABL1
	>1 yr, < 25 yr	*ETV6-RUNX1, BCR-ABL1, BCR-ABL1*-like, *DUX4* rearranged, *ZNF384*, then *KMT2A* and *TCF3*	–
	Adult	*BCR-ABL1, BCR-ABL1*-like, then *KMT2A* and *TCF3*	*ETV6-RUNX1*
T-cell acute lymphoblastic leukemia (T-ALL)	Childhood and adult	*KMT2Ar*	*TLX3, TLX1, KMT2A, TAL1, LMO2, ABL1, PICALM-MLLT10*

Identification of Druggable Targets

Importantly, molecular profiling permits the identification of patients more likely to benefit from targeted therapies and experience fewer toxicities. The number of identified, druggable tumor-specific targets has grown exponentially in recent years.[1] **Table 5** lists targeted drugs that are FDA-approved or have demonstrated activity against hematologic malignancies.[1]

As noted above, a malignancy may have more than one driver mutation, explaining in part why a single targeted therapy may not be effective – combination approaches with multiple targeted agents is an area of active investigation. Additionally, the oncogenic target may be necessary for normal cells to function, resulting in on-target, off-tumor effects.

Table 5. Targeted Drugs for Hematologic Malignancies[1] (adapted from Rack KA, et al. 2019)

Diagnosis	Therapy	Malignancy
***BCL-2* protein expression, *BCL-2* amplification/ translocation**	Venetoclax (Venclexta®)[16]	CLL, SLL, AML, CML*
***BCR/ABL1* fusion**	Imatinib (Gleevec®)[17]	Ph+CML, Ph+ALL
		MDS/myeloproliferative syndromes resulting from PDGFR gene rearrangements
	Dasatinib (Sprycel®)[18]	Ph+CML, Ph+ALL
	Nilotinib (Tasigna®)[19]	Ph+CML, Ph+ALL
	Bosutinib (Bosulif®)[20]	Ph+CML
	Ponatinib (Iclusig®)[21]	CML, Ph+ALL,
		T315I+CML, T315I+Ph+ALL
	Asciminib (Scemblix®)[37]	Ph+CML
***BRAF V600E/K* mutations**	Vemurafenib (Zelboraf®)[22]	Hairy cell leukemia*
***PDGFRB* rearrangement**	Imatinib (Gleevec®)[17]	MDS/myeloproliferative syndromes
***FLT3* mutations**	Midostaurin (Rydapt®)[23]	AML, MCL
	Gilteritinib (Xospata®)[24]	AML
	Quizartinib (Vanflyta®)[38]	AML
***IDH1/2* mutations**	Ivosidenib (Tibsovo®)[25]	AML
	Enasidenib (Idhifa®)[26]	AML
	Olutasidenib (Rezlidhia™)[39]	AML
***PI3K* mutation (PI3K alpha, delta)**	Copanlisib (Aliqopa™)[27]	FL
***PI3K* mutation (PI3K delta and gamma)**	Duvelisib (Copiktra®)[28]	CLL, SLL, FL
***PI3K* mutation (PI3K delta)**	Idelalisib (Zydelig®)[29]	CLL, FL, SLL
JAK2 V617F	Ruxolitinib (Jakafi®)[30]	MPNs: polycythemia vera (PV), myelofibrosis (MF), essential thrombocythemia (ET)*
	Fedratinib (Inrebic®)[40]	
	Pacritinib (Vonjo®)[41]	
	Momelatinib (Ojjaara)[42]	
***ALK* rearrangement**	Crizotinib (Xalkori®)[31]	Anaplastic large cell lymphoma*
***PML-RARA* fusion**	All-*trans* retinoic acid (Vesanoid®)[32]	Acute promyelocytic leukemia (APL)
	Arsenic trioxide (Trisenox®)[33]	
***KMT2A* rearrangement, NPM1, NUP98**	SNDX-5613 (Syndax)[43]	AML*, ALL*, MPAL/ALAL (Refer to Table 3. PMID: 39086307)
***KMT2A* translocation**	Revumenib (Revuforj®)[45]	R/R acute leukemia (AML, ALL, or MPAL/ALAL)

*Not FDA-approved for this indication

Turning Results into Actionable Information

As NGS technology becomes more efficient and less expensive and genomic analyses become more widely available, the amount and complexity of information clinicians must sift through and interpret to discern what is actionable becomes more challenging. Data provided from commercial NGS labs are not uniform, with some including many **variants of uncertain significance (VUS)** or ambiguous copy number alterations.[14] In addition, with near daily publication of new information, it is very difficult for busy clinicians to keep pace with the development of newly identified targets, indications for new or approved therapies, and available clinical trials.

Molecular Tumor Boards

Practices across the country have adopted a **molecular tumor board (MTB)** model to assist in interpreting molecular profiling data and guide personalized therapy.[14] Members of an MTB may include medical and radiation oncologists, surgeons, pathologists, molecular biologists, geneticists, genetic counselors, bioinformaticians, and a clinical-trial referral team. The goal of the team is to come together and determine treatment steps and best options for care, which could include clinical trials.[14,34]

Online Resources

Numerous up-to-date online resources are available to help interpret the results of data generated from molecular investigations of malignancies, including:

- OncoKB.org: A precision oncology knowledge base containing information about the effects and treatment implications of specific cancer gene alterations. It is developed and maintained by the Knowledge Systems group in the Marie Josée and Henry R. Kravis Center for Molecular Oncology at Memorial Sloan Kettering Cancer Center (MSK). OncoKB contains detailed information about specific alterations in 676 cancer genes curated from various sources, such as guidelines from the National Comprehensive Cancer Network (NCCN), or American Society of Clinical Oncology (ASCO), ClinicalTrials.gov and the scientific literature.
- My Cancer Genome®: A precision cancer medicine knowledge resource for physicians, patients, caregivers and researchers providing up-to-date information on mutations, available therapies and clinical trials. Managed by the Vanderbilt-Ingram Cancer Center.
- CIViCdb.org: Clinical Interpretation of Variants in Cancer: An open-access, open-source community-driven resource with the goal of enabling precision medicine by providing a forum for dissemination of knowledge and active discussion of cancer genome alterations and their clinical significance. Managed by the McDonnell Genome Institute at Washington University School of Medicine.
- The Cancer Genome Atlas Program (TCGA): A joint effort of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) with an overarching goal of improving the diagnosis, treatment, and prevention of cancer. TCGA is a coordinated effort to accelerate understanding of the molecular basis of cancer through the application of genome analysis technologies.

Evolution of Clinical Trial Design

With the goals of facilitating the approval of new drugs and more efficiently matching patients with appropriate targeted therapies based on results from genomic screens, traditional clinical trial design has undergone a shift to include **master trials** and **basket trials**.

Master Trials (or Umbrella Trials) involve multiple parallel arms operating under one overarching protocol. The Beat AML® Master Trial is an example. The trial sponsor is The Leukemia & Lymphoma Society (LLS), who brought together academic researchers, industry partners and clinical research organizations to collaborate on the trial. Patients diagnosed with AML are given a genomic screen (NGS) upon enrollment and are assigned a personalized therapy in a substudy based on the results. The study design is flexible, with treatment arms adjusted as clinical data become available. To date, over 900 patients have been enrolled across 16 clinical trial sites.

Another example is PedAL (Pediatric Acute Leukemia), a master clinical trial, developed as an international collaboration to transform care for children with acute leukemia. The bold concept behind PedAL is to run the same trial around the world, with the same protocol, testing mechanisms, definitions, tracking, and methodology. Launched in June 2022, it is the first integrated, global, acute leukemia master clinical trial to test new, safer therapies on children, who will be matched to treatments based on their unique tumor biology.

Basket Trials enroll patients on the basis of the underlying actionable genetic mutation, not on the basis of a particular type of cancer. The NCI-MATCH trial is an example, with 40 treatment arms currently exploring numerous genetic abnormalities and matched drugs in a large number of cancer types including leukemia, lymphoma and myeloma.

Resources for patients

Molecular profiling is complex, and patients need accurate, up-to-date, understandable information in order to be full partners in their care.[14] The LLS has a host of resources available here, including:

Understanding Genetics (Booklet)

https://www.LLS.org/booklet/understanding-genetics

Biomarker Testing for Cancer Treatment (Fact Sheet)

https://www.LLS.org/booklet/biomarker-testing-cancer-treatment

Minimal/Measurable Residual Disease (MRD)

https://www.LLS.org/booklet/minimalmeasurable-residual-disease-mrd

This publication is designed to provide accurate and authoritative information about the subject matter covered.

It is distributed as a public service by The Leukemia & Lymphoma Society (LLS), with the understanding that LLS is not engaged in rendering medical or other professional services.

Glossary

A

Aneuploidy: The presence of an abnormal number of chromosomes in a cell, for example, a human cell having 45 or 47 chromosomes instead of the usual 46. It does not include a difference of one or more complete sets of chromosomes. A cell with any number of complete chromosome sets is called a euploid cell.

Array comparative genomic hybridization (aCGH): Used to compare genomic DNA from a tumor sample to that from a normal reference DNA sample. DNA to be analyzed is fragmented, tagged with a fluorescent label (a different label is used for each set of DNA), and allowed to hybridize to DNA probes that have been spotted to a gene chip surface.[9,10] An imaging system scans the chip to measure the relative fluorescence intensity associated with each probe, which is proportional to the copy numbers present.[9]

B

Basket trial: Enrolls patients with different types of cancers or tumor histology, assigning targeted therapy based on specific mutations or biomarkers without regard to anatomical location of the tumor or its histology.[34]

Biomarker: A measurable biological molecule whose presence can indicate either a normal or pathological biological process or predict response or resistance to treatment. Biomarkers can consist of DNA, RNA, or protein.

C

Chromosomal microarrays (CMAs): A technique used to analyze many genes at once. It involves placing thousands of known gene sequences in known locations on a "gene chip." A sample containing DNA or RNA is applied to the gene chip, and complementary base pairing between the sample and the gene sequences on the chip (indicating the sequences are complementary) produces light that is measured.

Chromosome banding: Refers to light and dark regions along the length of a chromosome after staining with a dye.

Chromothripsis: Literally "chromosome shattering." Refers to chromosomes that have fragmented into many pieces and have been rejoined in random order by DNA repair processes. Chromothripsis was first discovered in CLL. NGS technology has now identified chromothripsis in many types of cancer.

Copy neutral loss of heterozygosity (CN-LOH): Refers to duplication of the maternal or paternal chromosome (or chromosome region) with concurrent loss of the other allele. CN-LOH has been identified in myeloid malignancies.[11]

Copy number alterations (CNAs): Mutations in which large segments of DNA (from a few kilobases [kb] to entire chromosomes) are gained or lost.[6]

D

Driver mutation: A genomic mutation that falls within a cancer gene or its regulatory regions, and alters the cancer gene's function or activity such that it plays a role in the development and/or maintenance of the malignant phenotype. An individual tumor can harbor more than one driver mutation.[2]

Druggable: Amenable to treatment with drugs or susceptible to alteration or manipulation with drugs.

F

Flow cytometry: A technique in which a biomarker-specific antibody is applied to a liquid sample, which is then linked to an enzyme or fluorescent tag that can be detected by a flow cytometer.

Fluorescence in situ hybridization (FISH): A laboratory technique in which a fluorescently-labeled DNA probe is applied (or hybridized) to chromosomal DNA and visualized under fluorescence microscopy.

G

Gene fusions: Created by the fusion of 2 different genes from 2 different chromosomes.

Genetic mutation: A mutation in a specific or limited number of genes

Genome: The complete DNA content contained within a person's chromosomes

Germline mutation: A heritable DNA mutation present in egg and sperm cells. A germline mutation will be present in virtually every cell of the body.

I

Immunohistochemistry (IHC): A laboratory technique in which a biomarker-specific antibody is applied to a tissue or blood sample, which is then linked to an enzyme or fluorescent tag that permits visualization with fluorescence microscopy.

Insertions/deletions (indels): DNA mutations that involve small insertions or deletions of 1-1,000 bases.[5]

Inversions: Created when a segment of a chromosome breaks off and reattaches in the opposite direction.

K

Karyotyping: A laboratory technique that produces an image of an individual's chromosomes to look for abnormal numbers or structures of chromosomes.

M

Minimum (or measurable) residual disease (MRD): Describes a very small number of cancer cells that remain in the body during or after treatment. MRD can be found only by highly sensitive methods (like qPCR) capable of detecting 1 cancer cell among 1 million normal cells. (qPCR is becoming even more sensitive as the technology improves). MRD is also measured using flow cytometry.

Molecular profiling: The process of identifying specific biomarkers involved in the biology of cancer cells that can inform diagnosis and/or prognosis, or drive therapeutic decisions. Multiple technologies are used to assess DNA, RNA, and protein expression.

Molecular tumor board: Multidisciplinary tumor board that deals with modern molecular diagnostic tests in addition to classical radiological, clinical, and standard biological data. Members of an MTB may include medical and radiation oncologists, surgeons, pathologists, molecular biologists, geneticists, genetic counselors, bioinformaticians, and a clinical-trial referral team. The goal of the team is to come together and determine treatment steps and best options for care, which could include clinical trials.[14,34]

N

Next generation sequencing (NGS): Refers to a number of DNA sequencing technologies capable of rapidly analyzing multiple DNA sequences in parallel. Also called massively parallel sequencing (MPS).

P

Passenger mutations: A somatic mutation within either a coding or noncoding region of the genome that does not confer a selective advantage under a given set of selective pressures. Passenger mutations do not play causative roles or directly drive cancer initiation. Hundreds to thousands of passenger mutations are seen in most cancers.

PCR: Polymerase chain reaction (PCR) is an automated technique that produces large quantities of a precise targeted DNA segment from minute quantities of starting material. It involves denaturing, or separating two DNA strands in a sample, using DNA polymerase to synthesize two new DNA strands, and repeating the process as many as 30 or 40 times. In a PCR reaction, the target DNA segment can be amplified from just one or a few copies (not measurable) to billions (measurable).

Q

Quantitative PCR (qPCR or real-time PCR): PCR technique that can determine the absolute or relative quantities of a known sequence in a starting sample, typically by quantitating fluorescence from a non-specific dye that binds to double-stranded DNA. Among other uses, qPCR is used to quantify minimal residual disease (MRD) in hematologic malignancies.

S

Single nucleotide polymorphism (SNP) array: A SNP array is similar to aCGH but uses DNA probes from regions in the genome that show differences between individuals at a single base pair (a "single nucleotide polymorphism" or SNP). SNP arrays measure the absolute fluorescence probe intensities of the tumor sample DNA compared to normal control samples that have been analyzed separately and combined into a reference set (this is referred to as an *in silico* comparison).[10]

Somatic mutation: A mutation that is acquired, not inherited. Somatic mutations can result randomly from mistakes during DNA replication or from exposure to environmental factors. Somatic mutations are present only in certain cells, not in every cell of the body.

Substitutions: DNA mutations in which one or more bases in the DNA sequence is replaced by a different base.

T

Targeted gene panel (mutation panel): Uses next-generation sequencing to test multiple genes simultaneously. Also called multi-gene test, multiplegene test or gene panel.

Translocation: Where a piece of one chromosome breaks off and attaches to another chromosome.

W

Whole exome sequencing: To determine the sequence of only the protein-coding region of the genome. The human exome represents approximately 1% of the whole genome.

Whole genome sequencing: Reveals the identity of each of the more than 3 billion DNA bases in the entire genome using an NGS platform.

V

Variants of uncertain significance (VUS): A variation in genetic sequence for which the association with disease risk is unclear. Also called "unclassified variant."

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We're Here to Help

LLS is the world's largest voluntary health organization dedicated to funding blood cancer research, education and patient services. LLS has regions throughout the United States and Canada. To find the region nearest to you, visit our website at www.LLS.org/ChapterFind or contact

The Leukemia & Lymphoma Society
1201 15th Street N.W., Suite 410,
Washington, D.C. 20005
Phone Number: (800) 955-4572
(M-F, 9 a.m. to 9 p.m. ET)
Website: www.LLS.org

LLS offers free information and services for patients and families touched by blood cancers as well as for healthcare professionals. The resources listed below are available to you and your patients and are meant to be a compliment to the HCP team and an additional source of support.

Consult with an Information Specialist. Information Specialists are highly trained social workers and nurses who assist through treatment, financial, and social challenges. They offer up-to-date disease and treatment information. Language services are available. For more information, please:

- Call: (800) 955-4572 (M-F, 9 a.m. to 9 p.m. ET)
- Visit: www.LLS.org/IRC
- Email or Live chat: www.LLS.org/InformationSpecialists

Clinical Trials Support Center (CTSC). Work one-on-one with an LLS clinical trial nurse navigator who will personally assist throughout the entire clinical trial process. A nurse navigator will help identify potential clinical trials and overcome the barriers to enrollment (navigators help HCPs and patients). For more information about this free service, please:

- Call an Information Specialist: (800) 955-4572 to be referred to the CTSC
- Visit: www.LLS.org/CTSC
- Complete a referral form for your patient at: www.LLS.org/CTSCreferral

Nutrition Consultations. Nutrition Education Services Center (NESC) provides one-on-one *free* nutrition education and consultations to patients and caregivers of all cancer types with registered dietitians who have expertise in oncology nutrition.

- Visit: www.LLSnutrition.org

Free Information Booklets. LLS offers free education and support publications that can either be read online or downloaded. Free print versions can be ordered. For more information, please:

- Visit: www.LLS.org/booklets

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- Visite: www.LLS.org/espanol

LLS Community. LLS Community is an online social network and registry for patients, caregivers, and healthcare professionals. It is a place to ask questions, get informed, share your experience, and connect with others. To join:

- Visit: www.LLS.org/community

LLS Regions. LLS offers community support and services in the United States and Canada including the *Patti Robinson Kaufmann First Connection Program* (a peer-to-peer support program), in-person support groups, and other great resources. For more information about these programs or to contact your region, please:

- Call: (800) 955-4572
- Visit: www.LLS.org/ChapterFind

Patti Robinson Kaufmann First Connection® Program. A free peer-to-peer support program that connects patients and their loved ones to a trained peer volunteer who has gone through a similar experience.

- www.LLS.org/FirstConnection

Resources for Healthcare Professionals: Webinars, Podcasts, In-person Education Programs, Videos, and Fact Sheets:

- www.LLS.org/CE (free accreditation)
- www.LLS.org/HCPpodcast
- www.LLS.org/HCPvideos
- www.LLS.org/HCPbooklets

Resources for your Patients:

- www.LLS.org/programs
- www.LLS.org/EducationVideos
- www.LLS.org/podcast

Additional Resources

The National Cancer Institute (NCI)

www.cancer.gov
(800) 422-6237

The National Cancer Institute, part of the National Institutes of Health, is a national resource center for information and education about all forms of cancer. The NCI also provides a clinical trial search feature, the PDQ® Cancer Clinical Trials Registry, at www.cancer.gov/clinicaltrials, where healthcare professionals and patients can look for clinical trials.

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