Review Series

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PRECISION HEMATOLOGY

The relative utilities of genome-wide, gene panel, and individual gene sequencing in clinical practice

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Advances in technology that have transpired over the past 2 decades have enabled the analysis of cancer samples for genomic alterations to understand their biologic function and to translate that knowledge into clinical practice. With the power to analyze entire genomes in a clinically relevant time frame and with manageable costs comes the question of whether we ought to and when. This review focuses on the relative merits of 3 approaches to molecular diagnostics in hematologic malignancies: indication-specific single gene assays, gene panel assays that test for genes selected for their roles in cancer, and genome-wide assays that broadly analyze the tumor exomes or genomes. After addressing these in general terms, we review specific use cases in myeloid and lymphoid malignancies to highlight the utility of single gene testing and/or larger panels. (*Blood*. 2017;130(4):433-439)

Introduction

The use of Southern blots to assess the clonality of antigen receptor loci to aid the diagnosis of lymphoma in the 1980s marked the beginning of the era of cancer molecular diagnostics.[1] Polymerase chain reaction[2] (PCR) emerged in the 1990s, and its ease of use and versatility helped transition molecular diagnostics from a specialty service that was only available in research centers to standard practice performed in most clinical labs. Gone were the days of freezing or rapidly processing fresh samples to preserve long genomic DNA. PCR enables tests to be performed on DNA isolated from formalin-fixed, paraffin-embedded samples with <100 ng of input DNA (and, increasingly, considerably less) and completed within several hours or a few days of initiation by using automated systems with clinical-grade quality control.[3,4] Alterations in DNA or RNA present in a small fraction of cells in a heterogeneous specimen can be selectively amplified with high fidelity and identified. During the first decade of the 21st century, as our knowledge about the linkage of specific mutations with disease entities grew, so, too, did the number of individual tests designed, with the use of a broad variety of methods, to detect these alterations (henceforth, referred to as single gene assays). By 2010, it was common for a molecular diagnostic laboratory in an academic medical center to offer ≥15 single gene assays. Adding and maintaining these tests has become increasingly burdensome. The introduction of next-generation sequencing[5,6] (NGS) technology in the last decade quickly captured the attention of molecular diagnosticians. The ability to perform simultaneous analysis of scores to thousands of genetic alterations opened numerous possibilities, from testing a panel of selected targets in a multiplex fashion (henceforth, referred to as gene panel assay) to sequencing the exome (all coding exons of all known genes) or even genome (referred to as genome-wide assay herein). Medical professionals, public health administrators, and payers alike are faced now not with the question of whether testing can be done, but rather when and which tests are clinically relevant. Although in the world of "omics" knowing more seems better, finding the optimal formula to deploy limited resources and balance it with the desire for scientific and medical advancement can be a challenge. We summarize the trade-offs when considering among these testing strategies (Table 1).

Single-gene assays

The single-gene assays have been the mainstay of cancer molecular diagnostics for 3 decades. They generally involve amplification of the target of interest (DNA or RNA) from samples and normal controls followed by one of a number of analysis techniques, including direct sequencing (Sanger or pyrosequencing), size assessment (electrophoresis), restriction digestion, or hybridization with sequence-specific probes; modern single gene assays often combine amplification and analysis in a single step. The main advantages of single gene assays are that they are relatively quick to perform, inexpensive, and straightforward to develop, validate, run, and interpret. Different tests typically share relatively inexpensive equipment that is well established and familiar to use and maintain, and do not require sophisticated mathematical processes to interpret. They are highly customizable and can be tailored to the specific needs of different clinical scenarios, including the need for linear and precise quantitative assays (eg, BCR-ABL1), ultrasensitive assays (eg, *KIT* D816V), analysis of genomic regions that are chemically challenging for multiplexed methods (eg, *CEBPA*), and analysis of genomic regions that are computationally challenging for NGS methods (eg, *FLT3*).

The major drawback of single gene assays is their limited scope. Each individual test requires time, resources, and sample. As the number of assays rises, the feasibility of efficiently performing all of them diminishes. Eventually, the complexity of performing many individual assays exceeds the benefit of customization and the ability to deliver all of the necessary results efficiently and cost effectively.

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Additionally, many single gene assays have limited sensitivity, particularly methods such as Sanger sequencing, and cannot reliably detect subpopulations or work with limited samples. Moreover, developing single gene assays, although each is relatively simple, still takes considerable time and resources, and it is becoming infeasible for an individual clinical laboratory to keep deploying new assays at the same pace with which medical science has been evolving in the past 10 years. On the other hand, for scenarios where frequent monitoring of a small number of targets is needed, single-gene assays may still be the format of choice.

Gene panel assays

The availability of clinical-grade, fast, inexpensive, benchtop next generation sequencers, with prepacked analysis software and reagents, has fueled the rapid growth and spread of this disruptive technology (NGS) into clinical laboratories in recent years. NGS can simultaneously sequence millions of DNA molecules in parallel. These DNA molecules can be random, unselected fragments derived from the whole genome of an organism, or enriched for genetic regions of interest. Although many different enrichment methods have been devised, 2 target enrichment strategies have been employed for clinical applications. "Hybrid-capture" enrichment requires synthesized oligonucleotide "baits" (typically 60-100 bp oligonucleotides) specific for sequences of interest and using them in hybridization reactions to capture corresponding DNA from samples for subsequent amplification and sequencing. This design can be optimized to improve copy number quantitation and to include introns for fusion breakpoint detection. The PCR-based "amplicon" enrichment method relies on synthesized PCR primers flanking the sequences of interest and amplification of intervening sequences from samples for sequencing. As such, it requires higher-quality DNA, is subject to variation of amplification efficiency across different amplicons, and cannot detect structural alterations where no flanking primers can be designed a priori. Compared with hybrid-capture, amplicon-based library preparation is faster, costs less, and can provide better sensitivity for subclonal populations or heterogeneous samples.

Today, the majority of the hybrid-capture–based gene panel assays test for several hundred genes, mostly from coding exons with or without a smaller number of selected introns. The amplicon-based panels tend to be smaller (<100 genes) and target the coding exons of the tumor suppressor genes and selected mutation hotspots of oncogenes. NGS panels have a very low incremental cost for additional genes and, therefore, one of the benefits of these panels is their capacity to also include a large number of investigational genes with potential clinical utility at little added cost.

The limitations of gene panel assays are the upfront investment in equipment and the cost of the sequencing reagents, which makes it only financially viable to run the samples in batches and thus impractical when the overall specimen volume is too small; however, given an adequate sample volume, the cost savings of a panel may be realized when compared with relatively few single gene tests, depending on scale and methodology. Despite the wider availability of the technology in recent years, experienced personnel, both technical and professional, are still in short supply. The lack of expertise leads to uneven quality in analysis and interpretation of the complex data generated by these tests, and the results are difficult to incorporate into medical records in a structured format, making it a challenge to follow patient results over time. The regulatory and reimbursement frameworks for clinical laboratories that have been in practice for 40 years is largely inappropriate for gene panel assays, and the lack of clarity about how to validate, control, and bill for these tests has limited their deployment in hospital laboratories.

Tumor exome sequencing

Tumor exome sequencing refers to a special form of hybrid capture gene panel assay that includes all (or nearly all, because some genes cannot be reliably captured or disambiguated from homologous regions) of the known human genes. It is technically identical to other gene panel assays except that the amount of data generated is more than an order of magnitude greater. It eliminates the need to select the content of the panel and does not need to be revised so it is easier to maintain. However, this approach increases the computational requirements, including data storage costs, and the majority of the additional genetic data provided exceeds our ability to understand and apply it. Despite the significant increase in data, because most of the commercially available bait sets do not capture introns, it cannot detect most structural variants with intron breakpoints.

Genome-wide assays

Analysis of an entire genome, once a massive multicenter project that cost billions and took decades, is now feasible in 1 laboratory, in a few days, for around $1000. Because there is no enrichment step, the test is simpler to perform, although a massive amount of data is generated, and the limiting part of the procedure switches from chemistry to computation. The main advantage of genome-wide analysis is the inclusion of "everything": fusions and copy number changes are more comprehensively assessed than in smaller panels, whereas the single nucleotide variants are still detectable. One particularly promising application is "low-pass" whole-genome analysis, which enables copy number analysis and structural variant detection from fragmented DNA that compares well with cytogenetic analysis.[7] The main drawback of genome-wide analysis is also, however, the inclusion of "everything": millions of interindividual variations that are unique to each human and, therefore, nigh impossible to interpret, not to mention the lack of coverage depth associated with generating so much sequence data per patient, thus limiting the ability to interrogate subclones or heterogeneous samples.

With such an intense computational requirement and low throughput, few clinical laboratories have embraced genome-wide sequence analysis outside of specialty centers, primarily working on constitutional genetics rather than cancer applications.

Utility in myeloid neoplasms

Myeloid neoplasms are diseases of the hematopoietic stem/progenitor cells. There are significant overlaps in the mutational profiles among different entities as currently defined in the latest World Health Organization (WHO) classification.[8] Certain genes may be more prevalent in one diagnostic entity than others, but none is exclusive to any one entity. The *JAK2* V617F mutation, for example, is found in most patients with polycythemia vera,[9] but is also found in patients with other myeloid neoplasms.[10,11] Furthermore, patients with myeloid

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neoplasms commonly have mutations in >1 gene, each with independent prognostic implications. Thus, it is more practical, time-saving, and cost-effective to perform gene panel assays than multiple single gene assays for patients with suspected or newly diagnosed myeloid neoplasms. The majority of myeloid gene panel assays are amplicon based to meet the short turnaround time needed for patients with acute leukemia. Although the list of genes varies among the panels performed at different institutions, they share a core set of 20 to 25 genes that are frequently mutated in myeloid neoplasms.[12] These include *NPM1*, *CEBPA*, and *RUNX1* (to classify acute myeloid leukemia [AML] according to the 2016 WHO classification[8] and 2017 European LeukemiaNet recommendations[13]); *FLT3*, *KIT*, *ASXL1*, *WT1*, and *TP53* (to stratify patients into prognostic groups[14-21]); *JAK2*, *MPL*, *CSF3R* and *CALR* (to diagnose patients with myeloproliferative neoplasms [MPNs][9,22-26]); *IDH1* and *IDH2* (to identify patients for targeted therapy[27-29]); *TET2* and *DNMT3A* (to evaluate clonal hematopoiesis of indeterminate potential and prognosis[30-32]); *NRAS*, *KRAS*, and PTPN11 (to identify RAS pathway alterations[33]); and *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, and *STAG2* (to assess risk for myelodysplastic syndrome [MDS][21,34-36]). Pappaemanueil et al[37] recently applied a gene panel to further classify AML, identifying distinct categories with different prognoses, independent of traditional prognostic stratification, including *TP53*-mutated/aneuploid AML, *IDH1/2*-mutated AML, *NPM1*-mutated AML, and AML with mutations in any of a number of genes involved with splicing, chromatin, or transcription.

Beyond the initial testing at diagnosis, the value or the optimal interval of repeated testing with gene panel assays remains to be established.[34] However, when MDS or MPN patients progress to AML, they frequently acquire secondary alterations that can be therapeutic targets and, therefore, repeated gene panel testing may be warranted.[36] Panel analysis enables a richer assessment of the subclonal architecture of each patient's disease and changes in the relative abundance of clones during therapy.

The currently available gene panel assays lack the sensitivity to be used to detect minimal residual disease (MRD). In patients with AML treated with induction chemotherapy, there may still be value in ultrasensitive single gene assays to monitor MRD status with genes such as *NPM1*[38] or *IDH1*,[39] but it is difficult to generalize such an approach to genes where mutations do not occur in a single location (eg, *DNMT3A*[39]). The *KIT* D816V mutation in mastocytosis also requires a single gene assay, particularly for blood testing, because of the extremely low burden of circulating cells bearing the mutation. This is most typically performed by allele-specific PCR[40,41] or an emerging technology for ultrasensitive detection, droplet digital PCR.[42]

Although *FLT3* and *CEBPA* are included in many gene panel assays, they offer unique challenges for NGS. Internal tandem duplications (ITD) in *FLT3*,[43] which are associated with a poor prognosis in AML patients with a normal karyotype,[44,45] have variable boundaries and lengths, which poses a challenge for NGS assays that amplify short-read (<150 bp) sequences that are aligned and assembled with bioinformatics algorithms. Depending on the length and position of the duplication relative to the DNA fragments sequenced, a duplicated fragment may be misinterpreted by these algorithms as identical to the original and escape detection.[46,47] Novel informatics approaches may be considered to improve the sensitivity for these duplications, such as the FLT3 tandem finder algorithm published by McKerrell et al,[48] or by targeted de novo assembly of sequence reads. However, given this limitation in sensitivity, a single gene assay (ie, PCR-electrophoresis with or without Sanger sequencing) may be required to ensure adequate detection of these important alterations. Sanger sequencing is insensitive to minor populations, however, and this approach may fail to detect diversity of duplications in the same sample. *CEBPA* is a single-exon gene on chromosome 19 with a very high content of G:C nucleotides. This very high content of G:C nucleotides affects reaction chemistry, making it difficult to amplify properly in a multiplexed PCR reaction where conditions are optimized for sequences with average G:C content. However, this can readily be overcome in a single gene assay for which PCR conditions are tailored specifically for that gene.[49]

Although chromosomal rearrangements can be detected in hybrid-capture–based NGS gene panel assays,[48,50] these designs involve a significant increase in the target regions to be sequenced and lack the sensitivity and linearity to be useful as a monitoring tool. RNA-based NGS approaches are promising and likely to overcome some of these limitations in the future.[50] Currently, quantitative amplification of BCR-ABL1 transcripts remains an essential single gene assay for the management of chronic myeloid leukemia (CML) patients, with the advent of targeted ABL1 inhibitors.[51] The precise, linear quantitation, over 4.5 log range of concentration, needed today to monitor response to ABL1 inhibitor therapy requires a dedicated single gene assay, which is typically performed by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR).[52] Similarly, for CML patients who progress on an ABL1 inhibitor, a single gene assay for acquired mutations in *ABL1* is required to select a new inhibitor.[53,54] The presence of abundant wild-type *ABL1* sequence in admixed benign cells necessitates a single gene assay capable of amplifying a long nucleic acid molecule that contains *BCR* and the downstream sequence in *ABL1* where resistance mutations occur.

However, despite these few exceptions, the power of NGS to assess multiple genetic targets at once, in smaller samples, is transforming the practice of molecular diagnostics in hematologic malignancies as single gene tests give way to larger panels. This will likely progress further, and tumor exome or genome-wide assays may eventually be inexpensive and fast enough to perform on a routine basis at various stages of testing for myeloid neoplasms. Similarly, advances in single-cell genomic analysis, droplet digital partitioning technology, and molecular barcoding strategies will drive analytic sensitivity even lower, potentially enabling reliable MRD detection or, in the case of CML, combining MRD monitoring with the detection of secondary resistance mutations.

Utility in lymphoid diseases

Unlike the myeloid neoplasms where disease entities (ie, MDS or AML) represent a spectrum of a single process that originates from hematopoietic stem/progenitor cells, lymphoid malignancies represent more distinct entities with different pathogenesis and origins.[55] Thus, although one could compile a list of genes needed for a lymphoma panel, in practice, the diagnoses rendered by morphology and immunophenotyping can typically narrow the list of relevant genes to a smaller number. In this regard, the value of the gene panel testing is more for operational simplification within the laboratory, maintaining fewer tests overall, than for clinical utility.

In addition, ascertaining the diversity of the rearranged immunoglobulin or T-cell receptor alleles in neoplastic cells is often useful in diagnosis, but has a very different design and analysis from mutation testing. Although clonality testing is still typically performed

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Table 1. Clinical utility of different assay designs

Clinical utility	Single gene	Gene panel	Whole exome	Genome-wide
Cancer genome profile	No	Target-gene approach	Yes	Yes
Prognostic	One at a time	Yes	Yes	Yes
Identification of target-therapeutics	One at a time	Yes	Yes	Yes
Immuno-oncology	No	Maybe	Maybe	Maybe
Cancer predisposition	One at a time	If germ line included	Yes	Yes
Diagnostic (classification)	One at a time	Comprehensive	Yes	Yes
Monitoring	Ultrasensitive	Comprehensive	Comprehensive	Comprehensive
Minimal disease detection	Ultrasensitive	Comprehensive	No	No
Detecting unexpected findings	No	Limited	yes	yes

by dedicated PCR-electrophoresis assays,[56] NGS methods are also available.[57] NGS-based clonality tests offer superior resolution and depth, enabling not only the establishment of clonality, but also the monitoring of minimal residual disease[58] and, by analyzing the entire repertoire of immunoglobulin and T cell receptor loci in a specimen, the potential to reveal new relationships between the immune response and cancer. Lastly, although chromosomal rearrangements involving the immunoglobulin or T-cell receptor genes are common in lymphomas, they typically cause overexpression of the rearranged oncogenes and not a chimeric transcript; RNA-based assays aimed at the detection of fusion transcripts would be suboptimal methods, whereas DNA-based assays would need to identify the actual breakpoints that often spread across several hundred kilobases of DNA, which is impractical for most NGS assay designs short of whole-genome sequencing. NGS-based gene fusion tests can theoretically detect these, and low-pass whole-genome sequencing is particularly promising in this regard, but cytogenetic assays, either fluorescence in situ hybridization (FISH) or karyotype, remain important complementary and confirmatory assays.

For patients with B acute lymphoblastic leukemia (B-ALL), chromosomal rearrangement and karyotypic findings, including *BCR-ABL1*, *ETV6-RUNX1*, and *KMT2A (MLL)*, are essential for establishing prognosis and therapy. With the inclusion of *BCR-ABL1*-like ALL as a provisional entity in the 2016 WHO classification,[8] the number of fusions that need to be tested may exceed 200. Although these rearrangements can be tested by multiplexed FISH or RT-PCR assays, the time and resource requirements are substantial. *MLL*, for example, has >100 known fusion partners,[59] making RT-PCR impractical, and thus break-apart FISH probes are typically used. Although *ETV6-RUNX1* and *MLL* fusions are typically detected by cytogenetics, *BCR-ABL1* fusions could also be tested by RT-PCR. *BCR-ABL1*-like ALL,[60] characterized by a similar expression pattern to Philadelphia chromosome-positive (Ph^+) ALL, but lacking the *BCR-ABL1* fusion, has been described in 15% to 20% of childhood B-ALL. These patients have an adverse prognosis, and preclinical data and small case series have suggested that they may be amenable to targeted therapy,[61,62] and such targeted approaches are being evaluated in open clinical trials in pediatric ALL. More than 60 fusions have been described for *BCR-ABL1*-like ALL with a variety of 5′ and 3′ fusion partners. A targeted RNA sequencing assay for fusion oncoproteins that involves capture of known 3′ fusion partners and sequencing into unknown 5′ partners may be a better option. Many of these patients have an intragenic deletion of the *IKZF1* gene,[63-65] which can be detected by NGS panels, but is more commonly detected by a single gene multiplexed ligation probe amplification assay.

Several other genes have prognostic and/or diagnostic significance for ALL patients, including *PAX5, JAK1, JAK2, FBXW7*, and *IKZF1*. The evidence supporting the utility of these in clinical practice is emerging rapidly, and the need to perform each of these favors a panel.

Only a handful of substitution mutations have demonstrated clinical utility in lymphoid neoplasia, although many frequently mutated genes are emerging biomarkers (Tables 2 and 3). The activating L265P mutation in *MYD88* is present in <30% of many types of B-cell lymphomas, but in nearly all cases of Waldenstrom's macroglobulinemia (WM)/lymphoplasmacytic lymphoma (LPL)[66]; the lack of an *MYD88* mutation in an apparent LPL is reason to reconsider the diagnosis. This test is not only useful for the diagnosis of WM/LPL, but it is currently being evaluated as a marker for monitoring disease burden, for which an ultrasensitive test is required, necessitating a single gene assay, typically allele-specific PCR. This is particularly important for the use of peripheral blood samples in disease monitoring.

Table 2. Utility of molecular alterations in myeloid diseases

	Gene	Disease	Drug
Diagnosis	KITD816V	Mastocytosis	
	JAK2; MPL; CALR; CSF3R	MPN/MDS	
Prognosis	TP53	Most	
	NPM1; CEBPA; RUNX1; FLT3; ASXL1	AML	
	SF3B1	MDS	
Therapeutic target	ABL1	CML	Tyrosine kinase inhibitor
	IDH1; IDH2;	AML	IDH inhibitor
	FLT3-ITD,TKD	AML	Midostaurin
	JAK2V617F	MPN	JAK2 inhibitor
	PML-RARA	APL	ATRA and ATO
Other recurrent alterations with unclear clinical utility	TET2;DNMT3A; SRSF2; U2AF1; EZH2; ZRSR2; STAG 2; CBL; NRAS; SETBP1; ETV6; WT1; BCOR; BCORL1	Myeloid neoplasms	

ATO, arsenic trioxide; ATRA, all-trans retinoic acid.

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Table 3. Utility of molecular alterations in lymphoid diseases

	Gene	Disease	Drug
Diagnosis	BRAF V600E	Hairy cell leukemia, Langerhans cell histiocytosis	
	MAP2K1	Variant hairy cell leukemia	
	MVD88 L265P	Waldenstrom macroglobulinemia	
	STAT3, STAT5B	T-cell large granular lymphocyte leukemia	
	RHOAG17V	Angioimmunoblastic T-cell lymphoma	
	TCF3, ID8	Burkitt Lymphoma	
Prognosis	IKZF1 deletion	B-cell ALL (poor)	
	ERG deletion	B-cell ALL (good)	
	TP53	Most (poor)	
	NOTCH 1	CLL	
	SF3B1	CLL	
	EZH2; ARID1A; EP300; FOX 01; MEF2B; CREBBP; CARDl1	Follicular lymphoma (M7-FLIPI)	
	NOTCH 2	Splenic marginal zone lymphoma	
Therapeutic target	BRAF V600E	Hairy cell leukemia, Langerhans cell histiocytosis	BRAF inhibitors
	BCR-ABL	B-cell ALL	Tyrosine kinase inhibitors
	ABL1 class Ph$^+$-like fusions	B-cell ALL	Tyrosine kinase inhibitors
	JAK2; CRLF2	B-cell ALL	Ruxolitinib (trials)
	ABL1 kinase mutations		Tyrosine kinase inhibitors
	BTK C451S; PCLG2; CXCR4		Ibrutinib
Other recurrent alterations with unclear clinical utility	ATM; BCL2; BCL6; PIMl; S0CS1; STAT6; PTEN; S1PR2; CD28; GNA13; CD79B; TNFAIP3; PRDM1; CDKN1B; CDKN2A/B;NT5C2; PAZ5; PHF6; PLCG1; JAK1; JAK3; NRAS; KRAS; PTPN11; PRKCB; DDX3X; SETD2; WHSC1		

The *MYD88* L265P mutation leads to activation of the NF-κB pathway via *BTK*, prompting treatment with the BTK inhibitor ibrutinib.[67] A subset of LPL patients who were resistant to ibrutinib treatment harbored C-terminal truncation mutations of *CXCR4*.[68] Other known causes for ibrutinib resistance include the acquisition of *BTK* C418S and *PLCG2* R665W mutations.[69] Although these genes have not yet been incorporated into standard clinical practice, they are often included in NGS panels, anticipating future need.

Like MYD88, >95% of *BRAF* gain-of-function mutations occur in 1 hotspot, at codon V600. *BRAF* mutations are found in a variety of solid tumors, but are relatively uncommon in hematolymphoid neoplasms with 2 notable exceptions: hairy cell leukemias (HCLs)[70] and histiocytic neoplasms, including Langerhans cell histocytosis (LCH)[71] and Erdheim–Chester disease.[72] Variant forms of HCL may have mutations in other genes in the same signaling pathway, with *MAP2K1* most commonly reported.[73,74] Testing in these diseases is primarily used to assist in establishing diagnosis, as the absence of *BRAF* mutations in these diseases is grounds to reconsider the diagnosis.

Other genes have emerging clinical utility and may be included in multiple gene panels for lymphomas, although their application is often limited to atypical disease presentations and, as such, few laboratories offer these as single gene assays. For example, activating mutations in the *TCF3 (E2A)* transcription factor and/or loss-of-function mutations in its inhibitory partner *ID3* are seen in 30% and 60% to 70% of Burkitt lymphoma cases, and are rare in other lymphoid neoplasms.[75,76] Another mutation with diagnostic utility in a specific context is *RHOA* G17V, which is seen in ~70% of angioimmunoblastic T-cell lymphoma and 15% to 20% of peripheral T-cell lymphoma, not otherwise specified, but is almost never found in other T- or B-cell lymphomas or myeloid neoplasia.[77-79]

Prognostic mutations in lymphoid neoplasia include C-terminal PEST domain mutations in *NOTCH1*, which are seen in ~15% of chronic lymphocytic lymphoma (CLL) patients and are associated with poor outcomes.[80,81] Mutations in *SF3B1*, diagnostic of RARS in patients with MDS, also have utility as adverse prognosticators in CLL, where it is seen in 15% to 20% of cases.[82] Loss-of-function mutations in *TP53* and *ATM*, both involved with DNA repair, are also adverse prognostic indicators, in a variety of lymphoid malignancies.[83] The Eastern Cooperative Oncology Group has recently proposed a combined follicular lymphoma (FL) risk model called m7-Follicular Lymphoma International Prognostic Index (FLIPI) based on the mutation status of 7 genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP*, and *CARD11*) and the performance status according to the FLIPI.[84] The m7-FLIPI model stratifies patients into high-risk and low-risk groups that, in a retrospective analysis, showed improved prognostication compared with FLIPI alone.

Targeted therapies are being developed in lymphoid malignancies. Along with the use of BTK inhibitors for malignancies with activation of NF-KB signaling, such as the *MYD88* mutant LPL, activating *STAT3* mutations are being targeted for patients with large granular lymphocyte leukemia in preclinical and early-stage trials.[85]

Conclusions

The role of molecular diagnostics in the management of hematologic malignancies has expanded dramatically in the past decade. Although some of the targets that are needed for patient care today are still tested by traditional single gene assays, either because of a need for precise quantitation (*BCR-ABL1* in CML) or ultrasensitive detection (*KIT* D816V in mastocytosis, *MYD88* L265P in WM), are particularly challenging for NGS methods (*FLT3* ITD, *CEBPA*), or because they require a unique type of assessment (*IGH* and *TCR* clonality assays), NGS-based gene panel assays have gained wide clinical acceptance for a larger number of genetic alterations that are amenable to such an approach. Genome-wide assays, although providing even more scientific information than panels, have not proven utility beyond the targeted panels and, therefore, remain investigational tools at this time. However, the history of cancer

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genomic testing has taught us that as technology improves and cost lowers, the trend is toward more comprehensive testing for more targets. As our capacity to interrogate the genome continues to advance, our ability to interpret and use these data has to keep pace in order to maximize the benefit to patients.

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References

1. Cleary ML, Chao J, Warnke R, Sklar J. Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. *Proc Natl Acad Sci USA*. 1984;81(2):593-597.
2. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 1985;230(4732):1350-1354.
3. Sheikine Y, Kuo FC, Lindeman NI. Clinical and technical aspects of genomic diagnostics for precision oncology. *J Clin Oncol*. 2017;35(9): 929-933.
4. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19(1):4-23.
5. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456(7218):53-59.
6. Metzker ML. Sequencing technologies - the next generation. *Nat Rev Genet*. 2010;11(1):31-46.
7. Dong Z, Zhang J, Hu P, et al. Low-pass whole-genome sequencing in clinical cytogenetics: a validated approach. *Genet Med*. 2016;18(9): 940-948.
8. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2406.
9. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352(17):1779-1790.
10. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med*. 2011;364(26):2496-2506.
11. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med*. 2009;361(11): 1058-1066.
12. Kuo FC, Dong F. Next-generation sequencing-based panel testing for myeloid neoplasms. *Curr Hematol Malig Rep*. 2015;10(2):104-111.
13. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
14. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. *Br J Cancer*. 2009;100(8):1343-1346.
15. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. *Oncotarget*. 2015;6(10):8388-8396.
16. Care RS, Valk PJM, Goodeve AC, et al. Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. *Br J Haematol*. 2003;121(5):775-777.
17. Tang JL, Hou HA, Chen CY, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. *Blood*. 2009;114(26):5352-5361.
18. Schneider F, Hoster E, Unterhalt M, et al. The FLT3ITD mRNA level has a high prognostic impact in NPM1 mutated, but not in NPM1 unmutated, AML with a normal karyotype. *Blood*. 2012;119(19):4383-4386.
19. Liersch R, Müller-Tidow C, Berdel WE, Krug U. Prognostic factors for acute myeloid leukaemia in adults–biological significance and clinical use. *Br J Haematol*. 2014;165(1):17-38.
20. Bejar R, Stevenson KE, Caughey BA, et al. Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. *J Clin Oncol*. 2012;30(27):3376-3382.
21. Bejar R, Stevenson KE, Caughey B, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J Clin Oncol*. 2014;32(25):2691-2698.
22. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013;369(25):2379-2390.
23. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med*. 2013; 369(25):2391-2405.
24. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006; 108(10):3472-3476.
25. Maxson JE, Gotlib J, Pollyea DA, et al. Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. *N Engl J Med*. 2013;368(19): 1781-1790.
26. Cui Y, Li B, Gale RP, et al. CSF3R, SETBP1 and CALR mutations in chronic neutrophilic leukemia. *J Hematol Oncol*. 20147:77.
27. Yaqub F. Inhibition of mutant IDH1 in acute myeloid leukaemia. *Lancet Oncol*. 2015;16(1):e9.
28. Wang F, Travins J, DeLaBarre B, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*. 2013;340(6132): 622-626.
29. Inoue S, Li WY, Tseng A, Nolan GP, Cairns RA, Mak TW. Mutant IDH1 downregulates ATM and alters DNA repair and sensitivity to DNA damage independent of TET2. *Cancer Cell*. 2016:30(2): 337-348.
30. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26): 2488-2498.
31. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. *Blood*. 2015;126(21):2355-2361.
32. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009;360(22):2289-2301.
33. Malcovati L, Papaemmanuil E, Ambaglio I, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. *Blood*. 2014;124(9):1513-1521.
34. Duncavage EJ, Tandon B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. *Int J Lab Hematol*. 2015;37(Suppl 1):115-121.
35. Wen XM, Hu JB, Yang J, et al. CEBPA methylation and mutation in myelodysplastic syndrome. *Med Oncol*. 2015;32(7):192.
36. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9): 1367-1376.
37. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23): 2209-2221.
38. Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016;374:422-433.
39. Debarri H, Lebon D, Roumier C, et al. IDH1/2 but not DNMT3A mutations are suitable targets for minimal residual disease monitoring in acute myeloid leukemia patients: a study by the Acute Leukemia French Association. *Oncotarget*. 2015; 6(39):42345-42353.
40. Arock M, Sotlar K, Akin C, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia*. 2015;29(6):1223-1232.
41. De Matteis G, Zanotti R, Colarossi S, et al. The impact of sensitive KIT D816V detection on recognition of indolent Systemic Mastocytosis. *Leuk Res*. 2015;39(3):273-278.

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42. Mochizuki H, Thomas R, Moroff S, Breen M. Genomic profiling of canine mast cell tumors identifies DNA copy number aberrations associated with KIT mutations and high histological grade. *Chromosome Res*. 2017;25(2):129-143.

43. Nakao M, Yokota S, Iwai T, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia*. 1996;10(12):1911-1918.

44. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079-1089.

45. Gale RE, Green C, Allen C, et al; Medical Research Council Adult Leukaemia Working Party. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood*. 2008;111(5):2776-2784.

46. Spencer DH, Abel HJ, Lockwood CM, et al. Detection of FLT3 internal tandem duplication in targeted, short-read-length, next-generation sequencing data. *J Mol Diagn*. 2013;15(1):81-93.

47. Rustagi N, Hampton OA, Li J, et al. ITD assembler: an algorithm for internal tandem duplication discovery from short-read sequencing data. *BMC Bioinformatics*. 2016;17(1):188.

48. McKerrell T, Moreno T, Ponstingl H, et al. Development and validation of a comprehensive genomic diagnostic tool for myeloid malignancies. *Blood*. 2016;128(1):e1-e9.

49. Behdad A, Weigelin HC, Elenitoba-Johnson KSJ, Betz BL. A clinical grade sequencing-based assay for CEBPA mutation testing: report of a large series of myeloid neoplasms [published correction appears in *J Mol Diagn*. 2015;17(2):206]. *J Mol Diagn*. 2015;17(1):76-84.

50. Cheng DT, Cheng J, Mitchell TN, et al. Detection of mutations in myeloid malignancies through paired-sample analysis of microdroplet-PCR deep sequencing data. *J Mol Diagn*. 2014;16(5):504-518.

51. Zhen C, Wang YL. Molecular monitoring of chronic myeloid leukemia: international standardization of BCR-ABL1 quantitation. *J Mol Diagn*. 2013;15(5):556-564.

52. Kalmanti L, Saussele S, Lauseker M, et al. Safety and efficacy of imatinib in CML over a period of 10 years: data from the randomized CML-study IV. *Leukemia*. 2015;29(5):1123-1132.

53. Soverini S, Colarossi S, Gnani A, et al; GIMEMA Working Party on Chronic Myeloid Leukemia. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin Cancer Res*. 2006;12(24):7374-7379.

54. Nicolini FE, Corm S, Lê Q-H, et al. Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukemia patients: a retrospective analysis from the French intergroup of CML (Fi(phi)-LMC GROUP). *Leukemia*. 2006;20(6):1061-1066.

55. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. 2016;127(20):2375-2391.

56. van Dongen JJM, Langerak AW, Brüggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003;17(12):2257-2317.

57. Ladetto M, Brüggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia*. 2014;28(6):1299-1307.

58. Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. *Blood*. 2015;125(22):3501-3508.

59. Meyer C, Kowarz E, Hofmann J, et al. New insights to the MLL recombinome of acute leukemias. *Leukemia*. 2009;23(8):1490-1499.

60. Roberts KG, Mullighan CG. Genomics in acute lymphoblastic leukaemia: insights and treatment implications. *Nat Rev Clin Oncol*. 2015;12(6):344-357.

61. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014;371(11):1005-1015.

62. Iacobucci I, Li Y, Roberts KG, et al. Truncating erythropoietin receptor rearrangements in acute lymphoblastic leukemia. *Cancer Cell*. 2016;29(2):186-200.

63. Clappier E, Grardel N, Bakkus M, et al. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia Group study 58951. *Leukemia*. 2015;29(11):2154-2161.

64. Kuiper RP, Waanders E, van der Velden VHJ, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia*. 2010;24(7):1258-1264.

65. van der Veer A, Waanders E, Pieters R, et al. Independent prognostic value of BCR-ABL1-like signature and IKZF1 deletion, but not high CRLF2 expression, in children with B-cell precursor ALL. *Blood*. 2013;122(15):2622-2629.

66. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med*. 2012;367(9):826-833.

67. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med*. 2015;372(15):1430-1440.

68. Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of *CXCR4* WHIM-like mutations in Waldenström Macroglobulinaemia. *Br J Haematol*. 2016;172(5):735-744.

69. Xu L, Tsakmaklis N, Yang G, et al. Acquired mutations associated with ibrutinib resistance in Waldenstrom macroglobulinemia. *Blood*. 2017;129(18):2519-2525.

70. Falini B, Martelli MP, Tiacci E. BRAF V600E mutation in hairy cell leukemia: from bench to bedside. *Blood*. 2016;128(15):1918-1927.

71. Badalian-Very G, Vergilio J-A, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. *Blood*. 2010;116(11):1919-1923.

72. Hervier B, Haroche J, Arnaud L, et al; French Histiocytoses Study Group. Association of both Langerhans cell histiocytosis and Erdheim-Chester disease linked to the BRAFV600E mutation. *Blood*. 2014;124(7):1119-1126.

73. Waterfall JJ, Arons E, Walker RL, et al. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. *Nat Genet*. 2014;46(1):8-10.

74. Mason EF, Brown RD, Szeto DP, et al. Detection of activating *MAP2K1* mutations in atypical hairy cell leukemia and hairy cell leukemia variant. *Leuk Lymphoma*. 2017;58(1):233-236.

75. Richter J, Schlesner M, Hoffmann S, et al; ICGC MMML-Seq Project. Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nat Genet*. 2012;44(12):1316-1320.

76. Love C, Sun Z, Jima D, et al. The genetic landscape of mutations in Burkitt lymphoma. *Nat Genet*. 2012;44(12):1321-1325.

77. Yoo HY, Sung MK, Lee SH, et al. A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. *Nat Genet*. 2014;46(4):371-375.

78. Sakata-Yanagimoto M, Enami T, Yoshida K, et al. Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. *Nat Genet*. 2014;46(2):171-175.

79. Palomero T, Couronné L, Khiabanian H, et al. Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. *Nat Genet*. 2014;46(2):166-170.

80. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011;475(7354):101-105.

81. Rossi D, Rasi S, Fabbri G, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. *Blood*. 2012;119(2):521-529.

82. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet*. 2011;44(1):47-52.

83. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127(17):2122-2130.

84. Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol*. 2015;16(9):1111-1122.

85. Lamy T, Moignet A, Loughran TP Jr. LGL leukemia: from pathogenesis to treatment. *Blood*. 2017;129(9):1082-1094.

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