Review article

A Narrative Review of the Clinical Utility of Next-Generation Sequencing and the Diagnosis of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a cancer affecting the blood and bone marrow progenitors due to heterogeneous genetic aberrations. The standard diagnostic testing regimen for AML includes cytologic and molecular techniques to determine the presence of abnormal myeloid cells and identify the genomic anomalies contributing to disease. These methods are beneficial for diagnosing AML but possess drawbacks in recognizing specific mutations within individual patient samples. AML mutations are being revealed with recent advancements in next-generation sequencing (NGS). Combining NGS-based approaches with standard hematopathology methods could significantly benefit physicians and patients by expediting AML diagnoses, contributing information about the disease, and supporting the development of tailored treatment.

Key words: Next Generation Sequencing, Acute Myeloid Leukemia, Molecular Diagnostics

Introduction

Clinical Overview of Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a bone marrow and blood disease that affects hematopoietic stem cells, or the precursors of blood cells including red blood cells, white blood cells, and platelets.[1,2] When bone marrow progenitor cells develop chromosomal changes or genetic alterations in the genes controlling cell division and cell death, the cells become leukemic myeloblasts which multiply rapidly and do not function properly.[3] Myeloblasts limit the number of circulating healthy blood cells, which can present phenotypically in the patient as easy bruising or unusual bleeding, persistent fever, abnormal cell counts in blood, and frequent infections.[3] Signs and symptoms of AML include bone pain, clotting problems, and abdominal swelling.[3] Factors associated with an increased risk of developing AML include age, history of other blood cancers, previous cancer treatments, genetic disorders, smoking, and gender.[3] Upon experiencing symptoms, patients may seek medical attention. AML cases are usually discovered following physician evaluation but may also be detected during routine blood work for asymptomatic patients.[4]

AML is primarily characterized by genetic mutations and epigenetic changes, for which

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most cases do not have clear etiology. Disease severity is determined through hemato-pathology examination of blood and bone marrow specimens.[1] Considering that AML is defined through the discovery of genetic anomalies, it is critical that physicians have access to a standardized classification system to characterize and understand the severity of disease for each patient to specifically define the diagnosis, aid in treatment plan development, and provide prognostic clarity. Evidence-based guidelines have been developped by the World Health Organization (WHO) to explicate cancer grading.[5] The latest WHO classification system categorizes AML into two major groups: AML with defining genetic abnormalities and AML defined by differentiation.[5] The category for AML with defining genetic abnormalities maintains the established AML diagnostic criteria outlined in previous WHO update versions and encompasses disease with recognizable genetic alterations.[5] The differentiation category includes cases that are not defined by genetic abnormalities to allow for classification of rare or novel disease presentations.[5] These groups simplify the classification structure for AML and encompass the six subgroups, while still allowing for prognostic risk group placement as favorable, intermediate, or adverse based on molecular and cytogenetic anomalies.[1,5]

The prognostic group assignment, which considers a patient's specific genotypic abnormalities, can be used to predict the patient's response to standard therapeutics, assist primary care providers to decide if clinical trial enrollment could benefit a patient on a case-by-case basis, and determine the likelihood of remission and overall survival.[1,5] A core set of mutations has been identified as precursors for AML with direct diagnostic, therapeutic, and prognostic significance. The most common genes identified in this group are *FLT3*, *NPM1*, *CEBPA*, *IHD1/2*, *DNMT3A,* and *TET2*, which produce functionally diverse proteins involved in signal transduction, ribosomal biogenesis, transcription, and DNA methylation.[2,6]

In AML, genetic mutations and epigenetic modulations are important differences to identify when building patient diagnoses. Genetic mutations are often easier to distinguish when comparing diseased and normal specimen. Epigenetic modifications are heterogeneous, have fewer mutations, and affect gene expression by interrupting DNA modulation and disrupting cellular differentiation.[5] AML cases with epigenetic abnormalities are more difficult to identify and study than cases with mutations in commonly identified genes.[5] By understanding the mutagenic basis of AML, important prognostic information can be used to determine targeted therapeutic approaches for patients.

Mutations in the *FLT3*, *NPM1*, and *DNMT3A* genes make up around 27% of identified mutations in AML patients. When treated with therapies targeting specific mutations, like midostaurin for *FLT3* and crenolanib for *DNMT3A,* patients show significant improvement in clinical response and overall survival.[2,7,8] *TET2* mutations have been identified in 20-25% of AML cases, and patients with these mutations are predicted to have favorable clinical outcomes with high response to standard chemotherapy combined with hypomethylating agents like azacitidine.[7,9,10] *CEBPA* and *IHD* mutations are less common and have been identified in 6-10% of AML cases, but typically predict good outcomes with chemotherapy.[2,10] The clinical implication for AML patients with these genetic abnormalities and the groundbreaking research which led to the novel therapeutic regimens would not be possible without the development and improvement of cytogenetic methods and advancements in molecular testing, like NGS.

Next-Generation Sequencing (NGS)

NGS, also referred to as massively parallel sequencing, is a nucleic acid sequencing technology which involves extracting deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from a patient specimen. The nucleic acids are fragmented into smaller portions and amplified to make millions of

copies in a library. The fragment library is sequenced, and the data is analyzed using bioinformatic workflows to identify the differences in patient samples compared to normal controls.[11]

Several sequencing methods can be classified as NGS, including whole exome sequencing to look for point mutations commonly found in AML samples and transcriptome sequencing to identify sequence mutations and fusion genes.[11] NGS is becoming an increasingly important tool for effectively pinpointing mutational differences in AML and other myeloid cancers. Within the last 10 years, NGS has been utilized to elucidate the specific genetic abnormalities associated with AML.[12] Through these advancements, a multitude of NGS arrays have been developed and optimized for nucleic acid library construction depending on the input material of the specimen. Standardized kits from companies like New England Biolabs, Illumina, Qiagen, and more are commercially available for both investigational and diagnostic use.[13]

NGS can provide detailed qualitative information for a patient's unique mutations which may influence how AML treatments are prescribed on a patient-specific level.[13] Since NGS can simultaneously detect more than 100 genes in a single assay, the approach enables the recognition of targeted aberrations.[7,14] NGS-based methods can also identify mutations in less common leukemogenic genes, providing further insight into the mutational effects of AML.[2]

Background

Standard AML Diagnostic Strategies

The leading method for screening and diagnosing AML is through evaluation of patient specimen. Blood samples, biopsies and fluid aspirations are critical to accurately diagnosing AML. Specimens provide critical information including the presence of genetic variations, the stage or severity of the cancer diagnosis, and other prognostic information.[15] Hematopathology specimens can be obtained through fine needle aspirations from the bone marrow or from bodily fluids like blood or cerebrospinal fluid.[15]

After sample collection, specimens are initially processed and analyzed in the clinical laboratory. Specimen preparation for biopsies involves stabilizing the structural integrity of the sample by chemical fixation followed by paraffin wax embedding.[15] The paraffin-embedded tissue sample undergoes histologic examination. The tissue is cut into microscopically thin sections, placed onto microscope slides, and stained with dyes that selectively intercalate into specific tissue structures and cellular components.[15] Blood smears are subject to similar staining methods, but do not require paraffin embedding or cutting. The prepared slides are evaluated first by medical laboratory professionals in the clinical laboratory to confirm the presence of aberrant cells or tumor markers, and abnormal specimens are referred to a pathologist for the final evaluation.[3,15]A pathology report is generated that includes a visual description of the specimen, a microscopic evaluation, and the final diagnosis.[15]

Clinical diagnostic laboratories have established molecular testing protocols to identify relevant genetic variations in patient samples, including Sanger sequencing, polymerase chain reaction (PCR) fragment analysis, and fluorescence *in situ* hybridization (FISH).[2,16] The molecular-based methods can provide insight into the genetic factors involved in a patient's disease. Molecular testing is valuable in diagnosing AML. Combining molecular techniques with hematopathology-based methods like cytogenetic testing, immunehistochemistry, and flow cytometry to identify the presence of AML-specific markers are important to develop a thorough diagnostic report.[2,6,16] While current testing platforms are essential for establishing AML diagnoses, the limitations of the approaches hinder the specificity of the data provided by testing. Recent advancements in whole-genome sequencing approaches, like NGS, can further elucidate genomic variants and provide greater

detail for diagnosing a patient's specific cancer subtype.

Diagnostic Implications for Treatment

Though chemotherapy and stem cell transplantation remain the best treatment regimens for AML patients, a "one-size-fits-all" approach for treating AML does not ensure success. This is due to the variable nature of genetic mutations and the inconsistency of abnormal genes identified in AML patients.[1] AML is considered a medical emergency for which the genetic factors associated with the disease must be identified as soon as possible to begin proper treatment regimens to improve patient outcomes.[4] Studies have shown that although the time from diagnosis to treatment does not have a negative effect on overall survival, the sooner that leukemic blasts can be eliminated in the patient the better chances a patient will have to reach a minimal measurable residual disease (MRD) or remission status.[2,4] NGS could be a beneficial tool for obtaining quick, qualitative diagnoses and determining the most applicable and tailored treatment regimen on a case-by-case basis.

Next-Generation Sequencing for Cancer Diagnostics

The recent development of NGS has drastically improved the speed and efficiency of diagnosing cancer. NGS methods have been used to identify cancerous tumors and annotate germline mutations, providing valuable information to guide molecular-targeted treatment plans for patients.[14] Various panel-based NGS tests have been approved for implementation, including the National Cancer Center Oncopanel test which recognizes 114 tumor-associated genes in patient specimens and can detect actionable gene mutations for therapeutic targets. Another diagnostic device, the ClonoSEQ manufactured by Adaptive Biotechnologies, quantifies MRD by differentiating normal and malignant cells.[14,17] By identifying gene mutations through NGS and mapping the frequency of mutations to specific cancer subtypes, drug developments and therapeutic clinical trials could benefit patients.[18] Individuals who have histologically-confirmed metastatic cancer are treated with broad chemotherapy regimens and generally have poor quality of life and prognoses.[18] NGS studies allow for deeper genomic investigation for diagnoses. Site-specific and targeted therapies based on tumor molecular profiles may be identified, providing patients with precision medicine when standard treatments are not beneficial.[18]

Next-Generation Sequencing in Standard Testing

While traditional hematopathology-based methods are the gold standard for identifying and diagnosing AML, NGS-based approaches may support and improve these testing techniques. NGS could become a high-quality diagnostic tool for accurately determining the genetic basis of AML on a patient-specific level. Implementing NGS-based approaches may enhance the results obtained with hematopathology-based and molecular diagnostic methods to better understand genetic mutations in AML.

Standard Diagnostic Techniques

Hematopathology- and molecular-based diagnostic tests are used to identify and diagnose AML in patient specimens. By taking a multidisciplinary approach to characterize the severity of a patient's disease through cytomorphology, immunohistochemistry, and molecular methods, patient care teams can provide a definitive diagnosis and an optimal treatment plan for disease management.[19] Cellular abnormalities are identified by hematopathology, and clinically relevant gene mutations associated with AML have been historically identified through molecular biology techniques.[2]

Benefits of Hematopathology Techniques

One major advantage of the traditional routine diagnostic workup for AML is the fast turnaround time. Results from bone marrow aspirates and blood smears can be obtained from the laboratory within hours of receiving

specimens.[20] The speed of results from these methods is important for the initial disease diagnosis. While stable patients can wait longer for more detailed genetic testing results, a short duration from sample collection to disease determination is crucial for clinically unstable patients to begin chemotherapy or other interventional therapies. Physicians recommend starting treatment within 48 hours of diagnosis in severe circumstances for optimal clinical outcomes.[4,20]

To perform AML diagnostic techniques in the clinical laboratory, laboratories do not require additional equipment to perform testing. This should be considered a significant benefit in favor of hematopathology-based methods. Most laboratories perform automated differential blood counts to measure the percentage of each cell type in specimens, and the equipment in a clinical diagnostic laboratory can be used for more applications than AML testing alone. Protocols and reagents can be also used for evaluating bone marrow and blood samples for other diseases, improving the utility of the reagents in the laboratory. While these testing methods are beneficial for diagnosing AML, there are drawbacks to be considered.

Limitations of Hematopathology Methods

Even though hematopathology approaches are widely used for elucidating the recurrent phenotypic abnormalities in AML testing, these methods have weaknesses. AML is characterized by the presence of greater than or equal to 20% leukemic myeloblasts in the bone marrow.[1,5,23] Normal myeloblast counts are usually less than 5% in bone marrow, and no myeloblasts should be visible in the blood.[4,15] Considering the WHO diagnostic criteria of defining AML with defining genetic abnormalities or lacking genetic abnormalities, hematopathology approaches do not provide information to characterize AML entities into classifying groups. While hematopathology provides fundamental information for diagnosing AML, results should be supported by a multidisciplinary testing approach including molecular-based testing methods.[4]

While the utility of hematopathology in a clinical laboratory is valuable for AML testing, not all clinical laboratories are equipped to analyze specimens using the complex hemopathology methods required for a comprehensive AML diagnosis. Bone marrow and blood samples identified as abnormal by automated testing may need to be sent to larger institutions for testing. By sending out plausible AML specimens to other laboratories the time from sample intake to result reporting increases, delaying diagnoses.

Another disadvantage of hematopathology is that the technique does not provide patient-specific genetic information. Without knowledge of a patient's present genetic mutation or pattern, inadequate treatment decisions may be made to combat a patient's specific AML.[23] The limitations of hematopathology-based methods can be overcome by incorporating molecular methods, like sequencing and FISH, to better understand the diagnosis.

Benefits of Molecular Diagnostic Techniques

Molecular testing methods provide information about genetic abnormalities that cannot be determined through hematopathology approaches. These methods generate comprehensive genetic data, allowing for better understanding of a patient's diagnosis. Cytogenetic and molecular methods include karyotype testing to indicate chromosomal changes, FISH for visualizing genetic mutations, PCR-based assays for amplifying genetic regions to identify abnormalities, and Sanger sequencing to determine the full sequence of targeted genomic regions.

Chromosome evaluation through cytogenetic testing is beneficial for identifying abnormalities in the chromosomes of leukemic cells by microscopy. Conventional cytogenetic analysis by karyotyping is recommended for all AML cases.[5,16] This method is useful for determining if AML leukemic cells have complex genetic changes at the chromosomal level including

translocations, inversions, deletions, additions, and duplications.[15] Determining anomalous chromosomes can help classify the severity of AML, providing valuable diagnostic and prognostic information. Cytogenetic testing results can be used to influence treatment decisions, assess disease response to treatments, and aid in monitoring disease progression and kinetics.[19]

FISH is useful for identifying changes in cellular DNA, similarly to cytogenetic karyotype testing, but is better for visualizing genetic mutations. FISH involves small fragments of DNA conjugated to fluorophores, termed probes, designed to bind to short genomic DNA sequences.[16] When probes bind to targeted genomic DNA in patient specimens the fluorophores can be visualized by fluorescence microscopy. Since FISH is a targeted analysis approach, the method can be advantageous to cytogenetic testing by identifying genetic changes with higher sensitivity and specificity.[15,16] FISH can detect smaller chromosomal abnormalities with higher analytical resolution. Cytogenetic testing can resolve structural abnormalities to 2000 kilobases and resolution of FISH is achieved down to 100 kilobases.[16]

PCR-based assays involve amplification of targeted DNA to generate more copies. Like the binding of FISH probes, short oligonucleotide fragments called primers bind to complementary sequences in the genomic DNA. These primers are significantly shorter than FISH probes, so specific DNA changes can be identified with even higher resolution.[16] The resolution threshold of PCR assays is significantly lower than both cytogenetic testing and FISH at 75-100 base pairs.[16] Most PCR-based assays are single-gene assays, where one specific gene in the patient genome is being evaluated for mutations. For AML, PCR assays focus on more commonly identified genes, such as *FLT3* alterations, as *FLT3* mutation is associated with severe prognostic implications but can be treated with targeted therapies.[13,16,24] This technique is helpful for finding genetic abnormalities in small numbers of cells, which is important for early detection and diagnosis of AML.

Sanger sequencing is widely considered traditional sequencing and can be used to assess the severity of AML diagnoses.[16] The method is similar to PCR-based testing methods, where DNA is amplified, and the nucleotide sequence is determined by tracking fluorophore emission. However, Sanger sequencing uses one primer to identify the full sequence of the target DNA in a linear fashion, where PCR uses a forward and reverse primer to create exponential copies of the DNA template.[16,19] Sanger sequencing is most frequently used in AML testing to detect *CEBPA* mutations, as they are difficult to detect through other molecular methods like FISH or karyotyping.[21]

Despite the benefits of incorporating molecular methods to understand genetic abnormalities in AML, these methods also have limitations. Molecular testing approaches have associated pitfalls, including limited results in cytogenetic testing, the requirement of locus-specific probe design for successful FISH assays, and false negatives in PCR- and Sanger sequencing methods.[16] Understanding the drawbacks of molecular methods is critical when determining which tests are best for diagnosing AML.

Limitations of Molecular Diagnostic Techniques

Cytogenetic testing and karyotyping rely on the generation of metaphase chromosomes for successful analysis. Cells must be grown in the laboratory for one to three weeks before microscopy to visualize the chromosomes.[15,21] If the cells in a patient specimen do not proliferate the chromosomes cannot be identified, which will not produce results.[16] Another caveat of this method is that abnormalities may involve small regions of the chromosome beyond the scope of visual recognition, which would require detection by alternative methods like FISH.[16]

For FISH assays, probes are designed to match specific regions in the patient DNA. The

design and optimization process for accurate FISH probes can be time-consuming and costly, and mutations will not be properly identified if the designed probes are not specific to the affected genes.[16] While probes can determine if specific mutations are present in a patient specimen, chromosomal abnormalities outside the genomic region targeted by the designed probe will not be identified.[21]

A limitation of PCR-based molecular testing is the identification of complex genetic variants. For example, translocations have multiple break points where chromosomes recombine.[16] Multiple primers spanning the break points must be designed to correctly amplify translocations, making PCR less practical for detecting these variants.[15,16] The phenomenon of allele drop-out is a common issue in PCR-based assays. Allele drop-out occurs when mutations are located within the primer binding site, leading to poor primer annealing and false negative results due to failed amplification.[25] Another common issue in PCR-based techniques is the potential for polymerase artifacts that result from the numerous amplification events for the method, which may also lead to false positive readouts.[25] The limitations of PCR-based testing assays can be resolved through careful primer design.

Sanger sequencing is limited by sequencing capacity. While Sanger sequencing analyzers may have multiple channels to sequence more than one sample at a time, this method is not capable of multiplexing, reducing the rate by which data is collected.[19] Sanger sequencing has the additional drawback of limited sensitivity. If the mutation of interest in a sample is found in less than 15% of the sample, the sequencing method will not be able to detect it which can be deciphered as a false-negative result.[16]

Improvements in testing efficiency for molecular methodologies are critical for combatting the shortcomings of each technique. Standard hematopathology and molecular testing methods limit the capacity and specificity of results necessary for diagnosing AML. Multidisciplinary approaches combining more than one method are important when diagnosing patients with AML. These challenges may be overcome with the consideration and addition of recent technological improvements and a, like NGS, to the AML diagnostic testing regimen.

NGS Diagnostic Utility

NGS platforms are becoming routinely used for identifying clinically critical malignancies for cancer diagnostics.[25,26] NGS is still considered a new technological advancement for molecular biology and diagnostics, as this method emerged within the last decade.[24] Despite the novelty of this method, major advantages can be identified when considering if or when to implement NGS into the standard diagnostic workflow for AML testing.

Advantages of Next-Generation Sequencing

Sanger sequencing and NGS are similar, as both methods amplify specific target fragments and determine the sequence of the DNA template strand.[19] Unlike Sanger sequencing, NGS has the capability for massive parallelization. Where Sanger sequencing is limited, NGS can sequence thousands of genetic regions simultaneously.[19] This allows for comprehensive genomic coverage and more data produced to establish diagnoses.

A considerable advantage for NGS is that the data generated by the method can be contributory for diagnosing clinically difficult neoplasms that emerge in AML. The classification and mutation profiling information provided by NGS may otherwise be missed when building a diagnosis.[26] NGS can simultaneously detect numerous mutations in multiple patient samples at once, which could improve the efficiency of AML screening if the method was integrated into routine clinical testing.

Genetic anomalies can appear anywhere in the genome of AML patients, leading to structurally diverse genomic abnormalities from patient to patient.[7,27] One of the biggest challenges identified when studying the genetic mutations of AML is the complexity and

specificity of mutations that can occur, including single nucleotide polymorphisms, insertions/deletions, copy number variants, chromosomal rearrangements, and fusion genes.[7,26] NGS can detect AML mutational discrepancies with 85-99.9% efficiency depending on which mutation combinations are present in patient specimens.[12,26,27] NGS-based testing is also significantly more specific than traditional Sanger sequencing. NGS reaches less than 5% specificity and Sanger cannot detect gene mutations with less than 15% specificity, which is important for accurate detection of mutant alleles in patient specimens.[16] This technological improvement can provide more detailed information to patients and physicians in less time.[25,26]

A revolutionary advantage of NGS in AML diagnostics is the potential to match patients with specific mutations to clinical trials that may provide significant clinical benefit.[2] With the advancement of molecular technology, novel molecular-targeted therapeutic strategies are being developed and entering clinical trial testing for AML and other clinically complex. NGS mapping has identified "drug-gable" genetic mutations that can be targeted using small molecule inhibitors, which provides options for patients who have previously undergone chemotherapy regimens with little to no clinical resolution or who have relapsed following remission after chemotherapy.[14,19] The information generated through NGS allows the patient care team to make informed decisions for a patient's medical treatment based specifically on the individual's mutational profile, ultimately improving the standard of care for the patient. As with the other testing methods discussed, NGS has limitations which must also be examined when determining the clinical utility of this approach.

Disadvantages of Next-Generation Sequencing

Although NGS is a promising resource for improving early detection and treatment for patients, more research needs to be performed before the method can be implemented in AML diagnostic testing.[25,27,28] One major downside to NGS for AML diagnostics is that there is no one consensus list of biomarkers for AML. The Association for Molecular Pathology leukemia focus group has published a definitive list of the minimum genes required for testing for chronic myeloid neoplasms, but no list currently guides which genes should be tested when diagnosing AML.[26] Fortunately, the understanding of AML, the genetic mutations involved, and novel therapeutic targets is also rapidly improving.

Another disadvantage of NGS compared to hematopathology and other molecular-based methods is while NGS is more specific and sensitive than traditional PCR approaches, NGS is still vulnerable to the shortcomings of PCR-based molecular methods previously addressed.[25] NGS assays are often multiplexed so that libraries can be generated and sequenced from more than one patient samples in a single run. Multiplexed sequencing reactions are susceptible to primer errors which lead to inaccurate results. The likelihood of errors increases when the number of primers and amplicons in a reaction are increased. Annealing temperature variation, the number of cycles needed for sufficient amplification, and extension times can vary.[25] These drawbacks can usually be mitigated through careful assay design.[25]

NGS is becoming a cost-effective method to obtain comprehensive genomic data for multiple patients in a single run. However, the upfront cost associated with NGS may not be feasible for laboratories operating on tight budgets or those that do not have a high enough sample volume to invest in the equipment and consumables needed. Studies have found that the advancement of the technology has improved the operational costs of NGS. The cost per sample tested by NGS is comparable to or lower than the expenses required for standard cytological and molecular analyses.[6,29] Even with the price reduction of NGS testing, the financial burden of purchasing the sequencing reagents,

training personnel to perform the complex sequencing protocols, the costs of performing the sequencing reactions, and deciphering the large datasets produced may be difficult for some clinical laboratories to justify.[13,29]

An additional caveat hindering the motivation to implement NGS in clinical laboratories is the time it takes to get results back to physicians. NGS runtimes vary based on the sequencing platform used and the workflow required for the system. Sequencing can take between three to five days to complete from library preparation to analysis.[6,13] This does not include the time to analyze the data produced and provide patients with a definitive genetic conclusion, which can take up to two weeks.[20] FISH, PCR-based testing, cytogenetic testing, and Sanger sequencing have turnaround times of roughly 72 hours or three days.[21]Although the genetic information obtained by NGS is highly specific, the prolonged timeframe is a limitation compared to Sanger sequencing and other conventional testing methods.

Considering the benefits and shortcomings of diagnostic approaches like NGS, the impact on early diagnoses and precision treatment options will continue to advance. These improvements will positively impact patient care and quality of life. Being aware of the potential pitfalls of this technology is critical for avoiding erroneous reporting in clinical diagnostics and practice.[25]

Discussion

A major strength of NGS is the sensitivity of the method, which allows for the detection of atypical variations that standard hemato-pathology and molecular methods are likely to miss in routine diagnostics. NGS has signify-cantly improved the understanding of AML pathogenesis and the genetic mutations which serve as prognostic indicators for patient survival and outcome.[30,31] Conventional AML testing only detects chromosomal abnor-malities in 50% of patients, so including NGS in the testing algorithm can dramatically improve genomic risk factor assessments and determine if targeted therapies will have a better clinical impact on patients compared to current chemotherapy regimens.[31] Considering that new therapies are being researched and tested in clinical trials, the breadth of knowledge for treating AML is rapidly expanding to positively impact patient care. With advancements in NGS technologies, NGS-based approaches for diagnosing AML may provide beneficial information on patient-specific basis.[30,31]

Despite the potential advantages of incorporating NGS-based approaches to the AML diagnostic methods, there are still shortcomings that need to be addressed before the technology can be implemented into clinical laboratory workflows. Some genes are difficult to identify with NGS-based methods, and current NGS technologies are still prone to the relatively high error rates that challenge traditional PCR-based methods.[30] Other pitfalls of the approach include long turnaround times from specimen collection to result reporting to physicians and the up-front costs of imple-menting NGS testing. The disadvantages must be considered and further optimized before NGS can be fully integrated into the daily clinical workflow for AML diagnostics.

While the argument can be made for and against the addition of NGS in the initial AML diagnostic workup, there are a significant number of cases in which patients do not respond to therapeutic interventions, or there are no effective drugs or applicable clinical trials available for patient-specific mutations. One main uncertainty of NGS implementation is the frequency by which patients should undergo genomic NGS testing to track genetic changes, proliferation, and MRD monitoring.[31] There are currently no standards in place for retesting patient specimens with NGS when patients fail to reach remission with traditional treatment methods. Important standards that must be defined before NGS can be used in daily clinical practice are the timepoints for NGS analysis during treatment and which genes should be included in NGS methods for clinical testing.[31] With further work, NGS-based

approaches could be combined with standard hematopathology to improve AML diagnostics.

The impacts of implementing NGS-based approaches concurrently with standard techniques for AML diagnostics include, but are not limited to, improving the depth of understanding of AML, providing qualitative results to physicians and patients, developing a less-invasive yet descriptive method for monitoring MRD, and bridging the gap in knowledge of treating remission and clinically complex cases through the advancement of precision medicine and patient-centric treatment plans. Precision oncology is a cancer treatment approach with the goal to identify and execute the exact therapeutic strategy for every patient at the right time to successfully reach and maintain remission.[32,33] Recent studies have shown that using NGS methods for genomic profiling-directed therapies correlate with increased survival rates and ultimately better outcomes.[34]

By elucidating the genetic underpinnings of patients' unique diagnoses, optimal therapies can be implemented to maximize survival outcomes and reduce the potential for relapsing post-remission, allowing patients to see beyond the cancer diagnosis. Fast-paced, groundbreaking research is being performed to discover druggable targets and novel compounds to challenge cancer progression and revolutionize the way cancers are diagnosed and treated.

Conclusion

The genetic variability of AML makes diagnosing and treating the heterogeneous disease incredibly difficult. It is of critical necessity to understand AML mutations on a case-by-case basis. Standardizing NGS approaches for classifying the genomic and molecular basis of disease on a patient-specific level will have diagnostic, prognostic, and predictive relevance for AML assessment.[31] Traditional hematopathology methods can be used to obtain rapid diagnostic results for urgent treatment interventions, while implementation of NGS methods provides qualitative results for clinically relevant genetic mutations improving overall patient care and outcomes. With further research, NGS has the potential to become a standard diagnostic technology for diagnosing AML.

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