Published in final edited form as:
Curr Hematol Malig Rep. 2013 June ; 8(2): 149–155. doi:10.1007/s11899-013-0161-6.

Are Results of Targeted Gene Sequencing Ready to Be Used for Clinical Decision Making for Patients with Acute Myelogenous Leukemia?

Arati V. Rao, M.D[1] and **B. Douglas Smith, M.D.**[2]

[1]Duke University Medical Center Division of Hematologic Malignancies and Cell Therapy 2400 Pratt Street, Suite 9010 Durham NC 27710 Tel: 919-684-8964 Fax: 919-668-1091 arati.rao@dm.duke.edu

[2]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins 1650 Orleans Street, CRB1 – Room 246Baltimore, MD 21287 Tel: 410-614-5068 Fax: 410-614-7437 bdsmith@jhmi.edu

Abstract

Acute Myeloid Leukemia (AML) is the most common acute leukemia in the US, which despite recent advances, continues to have a high mortality rate. It is a biologically active disease characterized by numerous cytogenetic abnormalities and multiple genetic mutations. Next-generation sequencing will perhaps not reveal all the factors that make AML a complex disease, but does have the potential to impact the diagnosis and risk-stratification of AML patients and allow for more personalized therapy. AML cells are easy to access from the patient and samples are only minimally contaminated with normal cells, which makes it an attractive cancer to study. Several studies have now demonstrated that the majority of AML patients are cytogenetically normal and the genome of these patients may contain fewer mutations than cancer genomes that are highly aneuploidy, suggesting that mutations in diploid genomes are more likely to be pathogenetically relevant. Whole-genome, exome, transcriptome, and targeted gene sequencing studies have been conducted successfully in AML and have provided us with valuable information. The challenges for the future include: reducing the cost of sequencing, understanding epigenetic changes, managing data across various platforms, separating the driver mutations from the sea of passenger mutations and finally, educating future generations to allow a better understanding and easy availability of these complex methodologies.

Keywords

AML; exome; transcriptome; targeted gene sequencing

Introduction

Acute Myeloid Leukemia (AML) is the most common acute leukemia in the US with an estimated 13,780 cases diagnosed in the US in 2012. [1] Unfortunately, the majority of these patients ~10,200 succumbed to this deadly disease. Despite extensive research and the continued search for targeted agents, AML patients continue to have poor response rates and overall survival. In addition to the karyotype, there are now several genetic mutations and gene overexpressions that have been identified over the years of intensive research that all

Corresponding author: Tel: 919-684-8964 Fax: 919-668-1091 arati.rao@dm.duke.edu.

Disclosure A. Rao: none; B. Smith: none.

play a role in the diagnosis and prognosis of AML. [2] Understanding the genetic lesions associated with AML is important in order to design and tailor-make therapies for these complex patients. The best example for this comes from Acute Promyelocytic Leukemia (APL), characterized by t (15;17) i.e. the reciprocal translocation of the retinoic acid receptor-alpha (*RARa*) gene on chromosome 17 with the promyelocytic leukemia gene (*PML*) on chromosome 15. This led to the discovery that APL patients treated with a differentiation-inducing agent ATRA (All-trans-retinoic-acid) and chemotherapy had high remission rates of 75-85%. [3]

The size (3 billion base pairs) and complexity of the human genome has been a challenge and made sequencing of cancer genomes impractical, cumbersome and expensive. [4] However, newer techniques like whole-genome sequencing (WGS), exome sequencing and transcriptome sequencing have made it easier to study and interrogate cancer genomes. More recently, there have been several WGS and exome sequencing studies in AML that have provided us with valuable prognostic information. The aim of this review is to provide an overview of the knowledge acquired from various sequencing methodologies and studies conducted thus far in AML patients. This will provide information on the feasibility and clinical applicability of genome sequencing in AML.

Whole Genome Sequencing in AML

There are several sequencing modalities available and each of these molecular techniques has it's own advantages and disadvantages as described in Table 1. One of the first studies that demonstrated the presence of somatic mutations in cancer using NGS technologies was actually in an AML patient (M1 subtype) with normal karyotype. [5] The authors performed a 32.7-fold 'haploid' coverage (98 billion bases) for the tumor genome, and 13.9-fold coverage (41.8 billion bases) for the normal sample. Of the numerous well-supported Single Nucleotide Variants (SNVs) found in the tumor genome, 97.7% also were detected in the patient's skin genome, and after downstream analysis they found only eight heterozygous, non-synonymous somatic SNVs in the entire genome. All were novel, including mutations in protocadherin/cadherin family members (CDH24 and PCLKC), G-protein coupled receptors (GPR123 and EBI2), a protein phosphatase (PTPRT), a potential guanine nucleotide exchange factor (KNDC1), a peptide/drug transporter (SLC15A1), and a glutamate receptor gene (GRINL1B). They also detected previously described, recurrent somatic insertions in the *FLT3* and *NPM1* genes. The patient under question in this study relapsed at 11 months and expired at 24 months from her disease. The authors were able to demonstrate that the same mutations were detected in the tumor cells in the relapse sample at approximately the same frequencies as in the primary sample. All of these mutations were thus likely present in the resistant tumor cells that contributed to the patient's relapse, further suggesting that a single clone contains all 10 mutations.

The authors of this study followed this with paired-end WGS in another patient with normal karyotype normal AML (M1 subtype).[6] Here, they identified 12 acquired (somatic) mutations within the coding sequences of genes and 52 somatic point mutations in conserved or regulatory portions of the genome. All mutations appeared to be heterozygous and present in nearly all cells in the tumor sample. They then tested these mutations in 188 AML samples and found that four of the 64 mutations occurred in at least one additional AML sample that was tested. Previously identified mutations in *NRAS* and *NPM1* were again identified, but two other new mutations (*IDH*1 and a tier 2 mutation in chromosome 10) were also identified. Notably, the mutations, in the *IDH1* gene, was present in 15 of 187 additional AML genomes tested and was strongly associated with normal cytogenetic status; it was present in 13 of 80 cytogenetically normal samples (16%) thus suggesting that these mutations are not random and are likely important in the pathogenesis of AML. Over the

next two years with improvements in sequencing techniques, Ley, et. al., decided to reevaluate the first case with deeper sequence coverage, during which they discovered a frameshift mutation in the DNA methyltransferase gene *DNMT3A*. [7] Further, they sequenced the exons of *DNMT3A* in 281 additional patients with de novo AML to define recurring mutations. A total of 62 of 281 patients (22.1%) had mutations in *DNMT3A* of which there were 18 different missense mutations, the most common of which was predicted to affect amino acid R882 (in 37 patients). They also identified six frameshift, six nonsense, and three splice-site mutations and a 1.5-Mbp deletion encompassing *DNMT3A*. These mutations were highly enriched in patients with an intermediate-risk cytogenetic profile (56 of 166 patients, or 33.7%) but were absent in all 79 patients with a favorable-risk cytogenetic profile. The median overall survival among patients with *DNMT3A* mutations was significantly shorter than that among patients without such mutations (12.3 months vs. 41.1 months, $P<0.001$). The authors concluded that *DNMT3A* mutations were associated with adverse outcomes among patients with an intermediate-risk cytogenetic profile or *FLT3* mutations, regardless of age, and were independently associated with a poor outcome.

In many cases of suspected cancer susceptibility, the family history is unclear and genetic testing of common cancer susceptibility genes is unrevealing. Link et al. identified a novel cancer susceptibility gene by sequencing leukemic bone marrow and normal skin samples from a patient with therapy-related AML and multiple early onset primary tumors but without any significant family history of cancer. [8] They detected a germline deletion variant that had caused the elimination of exons7–9 of the *TP53* gene. The authors discovered 16 non-synonymous SNVs, 2 variants in splice sites, 2 indels in coding regions, 8 SVs, and 12 somatic copy number alterations.

Finally, Welch et al. have also utilized WGS to identify cryptic, actionable mutations in a clinically relevant time frame with regards to therapeutic decision-making.[9] This became relevant in an interesting case of a young female patient who presented with morphologic and clinical features of M3-AML. Chromosomes 15 and 17 appeared to be normal by metaphase cytogenetics, and FISH was not diagnostic for PML-RARA. However, multiple chromosomal alterations were identified, including unusual translocations and the loss of whole chromosomes, consistent with high-risk complex cytogenetics. The patient was able to achieve a complete remission after induction therapy with cytarabine and idarubicin, and her sister was found to be an HLA match. Thus, the oncologist was presented with a diagnostic conundrum with divergent treatment options: is this APL(which would be treated with ATRA-Arsenic consolidation) or is this AML with complex cytogenetics (which would be treated with allogeneic stem cell transplantation (SCT) in first remission)? The authors applied WGS to the patients leukemia cells and this led to the identification of the insertion of a segment of chromosome 15 (containing the *LOXL1* and *PML* genes) into the second intron of *RARA* on chromosome 17 generating the *PML–RARA* fusion gene and two other fusion genes: *LOXL1–PML* and *RARA– LOXL1*. Ultimately, the patient was correctly diagnosed with APL and was able to avoid the high transplant related mortality from allogeneic SCT.

Exome Sequencing in AML

Exome sequencing (also known as targeted exome capture) is an efficient strategy to selectively sequence the coding regions of the genome. [10] It is a cheaper but still effective alternative to WGS. Exons are short, functionally important sequences of DNA, which represent the regions in genes that are translated into protein and the untranslated region (UTR) flanking them. UTRs are usually not included in exome studies. In the human genome there are about 180,000 exons and these constitute about 1% of the human genome, which translates to about 30 megabases (Mb) in length. It is estimated that the protein

coding regions of the human genome constitute about 85% of the disease-causing mutations. In addition to solid tumor malignancies and AML, exome sequencing has been utilized in identifying functional variations in Alzheimer's disease and some other genetic disorders.

One study by Grossman et al [11] sought to use whole exome sequencing (WES) to compare the sequences of leukemic cells at diagnosis and normal hematopoietic cells at the time of complete remission from a patient with CN-AML and no mutations of *NPM1*, *CEBPA*, or *FLT3* nor presence of *MLL*-PTD. The genes found mutated by WES of the index patient were further screened in 2 independent cohorts of 200 and 353 adult AML patients at diagnosis, respectively. The mutated genes identified in the index patient included the previously reported *DNMT3A*, and 3 additional genes, *BCOR*, *YY2*, and *SSRP1*, which the authors selected for further mutational screening because of their biologic functions and/or putative implication in AML pathogenesis. Analysis in the 553 AML patients showed that *BCOR* mutations occurred in 3.8% of unselected CN-AML patients and represented a substantial fraction (17.1%) of CN-AML patients showing the same genotype as the AML index patient *BCOR* somatic mutations were: a) disruptive events similar to the germline *BCOR* mutations causing the oculo-facio-cardiodental genetic syndrome; b) associated with decreased *BCOR* mRNA levels, absence of full-length BCOR, and absent or low expression of a truncated BCOR protein; c) virtually mutually exclusive with *NPM1* mutations; and d) frequently associated with *DNMT3A* mutations, suggesting cooperating genetic alterations. More importantly, patients with *BCOR* mutations (like *DNMT3A* mutations) had an inferior outcome in a cohort of 422 CN-AML patients (25.6% vs. 56.7% overall survival at 2 years; $P < .032$).

Yan and colleagues also studied paired samples from nine patients with AML-M5 by exome sequencing. [12] They identifying 66 somatic mutations in 63 genes and these included previously known variants (*NRAS, FLT3*) as well as a mutation in the *MLL– MLLT4* fusion gene. They further performed WES in five AML-M5 cases without matched normal samples focusing on the 63 genes found to have somatic mutations in their initial sequencing set. Then, all the sequence changes detected in these 63 genes were genotyped in bone marrow DNA samples from 98 additional cases of newly diagnosed (94 cases) or relapsed (4 cases) AML-M5 (M5 validation set) by Sequenom analysis. The authors thus found somatic mutations (confirmed by sequencing of paired peripheral blood control samples at complete remission in some cases in the M5 validation set) or very probable somatic mutations (defined as those not identified in 509 samples in the control validation set) in 14 genes, each detected in at least two cases, among a total of 112 AML-M5 cases. Finally, six genes (*DNMT3A*, *NSD1*, *GATA2*, *CCND3*, *ATP2A2* and *C10orf2*) were selected for sequencing of their whole coding regions in the M5 validation set. The frequency of *DNMT3A* mutations in this entire AML-M5 series was 23 out of 112 (20.5%). In an effort to explore rare SNPs in *DNMT3A*, particularly the non-synonymous ones they also sequenced the whole coding regions of 31 samples from healthy individuals but detected no such variations. In addition, they found sequence variations in *NSD1*, *GATA2*, *CCND3*, *ATP2A2* and *C10orf2*. To clarify molecular abnormalities in AML-M5, they also checked for known gene mutations present at relatively high frequency and identified *MLL* abnormalities and mutations in *NPM1, NRAS, FLT3* in 19.6%, 25.9%, 10.7% and 18.8% respectively in the 112 cases. Clinically, patients with *MLL* abnormalities had ≥80% monoblasts in the bone marrow, whereas only 22% of cases with *DNMT3A* mutations showed a high percentage of monoblasts, and the bone marrow of most *DNMT3A* mutation cases was infiltrated with promonocytes and monocytes. Moreover, the mean age of disease onset was 54.9 years in patients with *DNMT3A* mutations, but was 37.0 years in the group with *MLL* abnormalities and this may partly explain the differences in survival: no difference in OS between M5-AML patients with and without *MLL* mutations, however much worse survival in M5-AML patients with *DNMT3A* mutations compared to those without.

Finally, Greif, et. al. performed exome sequencing in three patients with APL at diagnosis and at remission.[13] No patient had a *FLT3*-ITD or an *NPM1* mutation and all were treated with arsenic trioxide. The authors confirmed mutations in gene including a single base pair insertion in *WT1* (Wilms Tumor 1 gene) as well as a *KRAS* missense mutation. *WT1* mutations are seen in ~ 10% patients with CN-AML.*KRAS* mutations are found in about 10% of APL patients and has been previously shown to work in conjunction with *PML/RARA* to induce leukemia in a murine model. In addition, the authors found mutations in genes that may play a role in the pathogenesis of APL and these include missense mutations in the highly divergent homeobox gene *HDX*, as well as missense mutations in the tyrosine kinase gene *LYN*. There was also a missense mutation in the *APOC2* (apolipoprotein C-II) gene, which is expressed during myelomonocytic differentiation.

Transcriptome sequencing (RNA-seq) in AML

The size of a diploid human genome is about 6 Gbp, the transcriptome, is only 0.6 Gbp in size. The transcriptome is the complete set of transcripts in a cell, including their quantity, for a specific developmental stage or physiological condition. Studying the transcriptome is requisite for understanding disease development, interpreting the functional elements of the genome, and integrating the molecular constituents of cells. The transcriptome constitutes mRNAs, non-coding RNAs and small RNAs. Transcriptomics helps us determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other post-transcriptional modifications; and it aids in quantifying the changing expression levels of each transcript during development and under different conditions.[14]

RNA-seq is a method of studying the transcriptome by NGS. RNA-Seq can reveal the precise location of transcription boundaries, to a single-base resolution. Short reads of 30-bp from RNA-Seq can provide information about how two exons are connected, whereas longer reads or pair-end short reads can reveal connectivity between multiple exons. RNA-Seq has the advantage of being highly reproducible and is helpful in revealing sequence variations (e.g., SNPs) in the transcribed regions. Greif, et. al., have utilized transcriptome sequencing in a patient with M1 AML with normal karyotype at diagnosis and in remission.[15] They identified 11 candidate mutations from a total of almost 10 Gbp of primary transcriptome sequences. Five of these mutations were confirmed by sequencing of genomic DNA from both the tumor and remission samples and the authors noted five SNVs, which affected the genes *RUNX1, TLE4, SHKBP1, XPO7* and *RRP8.* Three of these mutations (*RUNX1, TLE4, SHKBP1)* affect genes in pathways involved in AML pathogenesis.

In AML, miRNA signatures can distinguish between cytogenetic and molecular subtypes, predict response to hypomethylating agents, and provide prognostic information. Ramsingh, et. al., utilized NGS technology to be the first to characterize a microRNAome in primary human cancer.[16] RNA sequencing of leukemic myeloblasts from a patient with M1 AML and from CD34 + cells pooled from healthy donors showed that 472 miRNAs were expressed, including 7 novel miRNAs, some of which displayed differential expression. Sequencing of all known miRNA genes revealed several novel germline polymorphisms but no acquired mutations in the leukemia genome. Analysis of the sequence of the 3′-untranslated regions (UTRs) of all coding genes identified a single somatic mutation in the 3′-UTR of *TNFAIP2*, which is highly expressed in hematopoietic cells, and is a direct target for transcriptional repression by the *PML-RARA* and *PZLF-RARA* oncogenes.

Targeted gene sequencing in AML

Over the past few years, the acquisition of NGS data has been quite straightforward; however, its analysis is extremely complicated and time consuming. This is largely due to the large volume of data generated (>100GB/run), the computational difficulty in aligning

short reads, and of course the heterogeneity of AML. As an example, there are now several easily identified mutations (i.e., NPM1, CEBPA, KIT, DNMT3A, FLT3, IDH1, IDH2, WT1, RUNX1, ASXL1, MLL, and *NRAS*) that have been shown to occur with varying effects on outcome. Targeted gene sequencing provides a comprehensive, unbiased mutational profiling of many genes simultaneously using a single method and may be a more ideal tool for understanding the overall impact of numerous mutations within an individual AML. It is able to identify the full spectrum of prognostically significant gene mutations including translocations, SNVs, and insertions/deletions (indels). This technique also requires less technician labor and is less expensive than WGS or exome sequencing. One proof of concept study performed hybrid capture using a panel of 20 genes implicated in leukemia prognosis from five leukemic cell lines and one patient with AML.[17] This analysis and found all published gene mutations including *NPM1, FLT3*, and *KIT* in these cell lines. The same authors went on to sequence *FLT3* and 26 other cancer associated genes from 24 AML patients, 25 lung cancer patients, and two cell lines.[18] They also tested several bioinformatics tools and demonstrated that Pindel was the only tool with 100% sensitivity and specificity in detecting insertions. This method may be poised to replace a variety of more labor-intensive methods with the ability to analyze sequencing data using freely available software tools.

Clinical applications and future directions

AML remains a lethal cancer with most patients eventually dying of their disease, despite the ability to achieve remission in most patients. It is a disease fraught with karyotypic abnormalities, genetic mutations, and gene overexpressions and leukemia researchers are continuing to study the immediate impact of key mutations on how we treat these patients. NGS technologies are powerful in the discovery of AML-associated mutations and studies thus far have led to the identification of 281 mutated genes in AML of which only 16 (~6%) of the mutated genes were previously known. NGS studies also have lead us a to better understanding subtypes of AML.[19,20] For example, in AML with primary translocations like APL with t (15;17), NGS has helped identify mutations (albeit occurring at low frequency of ≤ 5%) that cooperate with fusions proteins and impact disease progression. This may be most import for CN-AML, which is the largest cytogenetic subgroup of AML and often without other clear prognostic markers. Most of the NGS studies thus far have been in patients with CN-AML where in ~25% patients have mutations of the *NPM1* gene and these patients in addition were associated with 34 novel non-recurrent mutated genes which may indeed be cooperating mutations. NGS studies have also led to the discovery of more frequently occurring mutations like *DNMT3A* (seen at a frequency of ~20-25%), which we now know never occurs in AML with primary translocations, is often associated with mutations in *NPM1, IDH1*, and carries poor a prognosis for patients treated with cytotoxic chemotherapy.[7] Mutations discovered in AML genomes may also be relevant in other cancers (e.g., *RAS* in lung cancer, *IDH1* in gliomas). Moreover, NGS studies can be utilized to study cancer susceptibility genes and can aid in relevant clinical decision-making (e.g., chemotherapy vs. SCT during consolidation).[9] There is a growing need to develop and validate clinical assays that can probe multiple mutations simultaneously. Newer NGS modalities: exome, transcriptome, and targeted-gene sequencing have decreased the cost of sequencing and the quantity of sample required per patient.

Yet, there are several challenges that researchers continue to face, the greatest being the ability to distinguish the benign pre-existing passenger mutations from those that are relevant in the pathogenesis of AML (initiation and progression), so-called driver mutations. Secondly, it remains unclear how many of the newly discovered mutations interact with epigenetic changes and other non-cell intrinsic factors like angiogenesis, stroma, cytokines, and chemokines.[23] Some of these questions may be answered by the genome sequencing

of 500 *de novo* AML cases by the The Cancer Genome Atlas project (http://tcga-data.nci.nih.gov/tcga) which is an NIH consortium aiming to contribute to the understanding of the molecular basis of cancer through the gathering and analysis of different high throughput data like DNA-sequencing, methylation, gene expression and miRNA expression data. Finally, even with the decreasing costs for NGS studies, these tests remain expensive and require expertise to accurately analyze and decipher the complex data. This requires a team dedicated to understanding and treating patients with AML including: molecular and computational biologists, geneticists, hematopathologists, IT consultants, systems support specialists, and physicians and research nurses who have in-depth knowledge of the disease and can begin to apply the data generated in therapeutic decision-making. The clinical team must also develop a strategy to carefully present this data to the patients and families facing the diagnosis of AML.

Conclusion

In the future, our ability to study diseases at the molecular level will require that we wrestle with fundamental questions on how to protect as individual patient's genetic data, how to pay for these tests, and how to best interpret multiple prognostic markers simultaneously. Ultimately, it will be up to the treating oncologist to learn how to integrate results from these tests into their patient care planning. With NIH funding at an all-time low, major academic institutions are working to invest funds to develop centers for genomic-guided medicine hoping that with more awareness, understanding and a larger number of Cancer Centers performing NGS studies, we will ultimately provide equitable access for better diagnosis and treatment decision-making with the aims of improving remission rates and decreasing mortality for AML patients.

References

1. Surveillance, Epidemiology, and End Results (SEER) Program Populations (1969-2009). National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch; www.seer.cancer.gov/popdata [released January 2011]
2. Marcucci G, Haferlach T, Döhner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clinc Oncol. 2011; 29(5):475–86.
3. Tallman MS, Andersen JW, Schiffer CA, Appelbaum FR, Feusner JH, Ogden A, et al. All-trans-retinoic acid in acute promyelocytic leukemia. N Engl J Med. 1997; 337(15):1021–8. [PubMed: 9321529]
4. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature. 2001; 409(6822):860–921. [PubMed: 11237011]
5. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature. 2008; 456(7218):66–72. [PubMed: 18987736]
6. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009; 361(11):1058–66. [PubMed: 19657110]
7. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010; 363(25):2424–2433. [PubMed: 21067377]
8. Link DC, Schuettpelz LG, Shen D, Wang J, Walter MJ, Kulkarni S, et al. Identification of a novel TP53 cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML. JAMA. 2011; 305(15):1568–1576. [PubMed: 21505135]
9. Welch JS, Westervelt P, Ding L, Larson DE, Klco JM, Kulkarni S, et al. Use of whole-genome sequencing to diagnose a cryptic fusion oncogene. JAMA. 2011; 305(15):1577–1584. [PubMed: 21505136]
10. Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. Hum Mol Genet. 2010; 19(R2):R145–51. [PubMed: 20705737]

11. Grossmann V, Tiacci E, Holmes AB, Kohlmann A, Martelli MP, Kern W, et al. Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. Blood. 2011; 118:6153–6163. [PubMed: 22012066]
12. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011; 43(4):309–315. [PubMed: 21399634]
13. Greif PA, Yaghmaie M, Konstandin NP, Ksienzyk B, Alimoghaddam K, Ghavamzadeh A, et al. Somatic mutations in acute promyelocytic leukemia (APL) identified by exome sequencing. Leukemia. 2011; 25:1519–1522. [PubMed: 21606962]
14. Feng H, Qin Z, Zhang X. Opportunities and methods for studying alternative splicing in cancer with RNA-Seq. Cancer Lett. 2012; (12):S0304–3835. 00657-X.
15. Greif PA, Eck SH, Konstandin NP, Benet-Pagès A, Ksienzyk B, Dufour A, et al. Identification of recurring tumor-specific somatic mutations in acute myeloid leukemia by transcriptome sequencing. Leukemia. 2011; 25:821–827. [PubMed: 21339757]
16. Ramsingh G, Koboldt DC, Trissal M, Chiappinelli KB, Wylie T, Koul S, et al. Complete characterization of the microRNAome in a patient with acute myeloid leukemia. Blood. 2010; 116:5316–5326. [PubMed: 20876853]
17. Duncavage EJ, Abel HJ, Szankasi P, Kelley TW, Pfeifer JD. Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia. Mod Pathol. 2012; 25(6): 795–804. [PubMed: 22425908]
18. Spencer DH, Abel HJ, Lockwood CM, Payton JE, Szankasi P, Kelley TW, Kulkarni S, 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. [PubMed: 23159595]
19. Walter MJ, Graubert TA, Dipersio JF, Mardis ER, Wilson RK, Ley TJ. Next-generation sequencing of cancer genomes: back to the future. Per Med. Nov 1.2009 6(6):653. [PubMed: 20161678]
20. Riva L, Luzi L, Pelicci PG. Genomics of acute myeloid leukemia: the next generation. Front Oncol. 2012; 2:40. [PubMed: 22666660]
21. Welch JS, Link DC. Genomics of AML: clinical applications of next-generation sequencing. Hematology Am Soc Hematol Educ Program. 2011; 2011:30–5. [PubMed: 22160009]
22. Mardis ER, Wilson RK. Cancer genome sequencing: a review. Hum Mol Genet. 2009; 18(R2):R163–8. [PubMed: 19808792]
23. Mardis ER. A decade's perspective on DNA sequencing technology. Nature. 2011; 470(7333): 198–203. [PubMed: 21307932]

TABLE 1

Comparison of the different NGS techniques [19-22]

Sequencing Target	Advantages	Disadvantages	Starting material and Cost
Whole-genome	- Comprehensive (whole genome including non-coding genes sequenced) - All structural variants: deletions, amplifications, chromosomal translocations, and uniparenteral disomy identified	- Expensive - Generates large amounts of complex data for analysis	- 1 μg of DNA - $5000-$20,000/sample
Exome	- Selectively sequences 1%-2% of the genome containing coding genes and microRNAs - Lower cost yet still relatively deep sequence coverage	- Misses mutations in non-exome regions (>98% of the genome) - Misses most structural variants, i.e., chromosomal translocations with intronic break points - Hard to identify copy # alterations	- 3 μg of DNA - $1000-3000/sample
Transcriptome	- Sequences all transcribed genes, both coding + noncoding RNAs - Quantitative information about gene-expression levels available - Posttranscriptional changes in gene expression, i.e., alternative splicing can be detected - Fusion transcripts produced by chromosomal rearrangements can be detected - Cheapest method available	- Only identifies mutations in expressed genes - Low or no coverage of genes expressed at lower levels - Cannot detect mutations that cause the loss of one or both copies of a gene or mutations that accelerate RNA turnover (e.g., frameshift or nonsense mutations that cause “nonsense mediated decay”) - Hard to identify corresponding normal samples - Errors in reverse transcriptase and RNA editing can make data interpretation difficult	- 0.1-4 μg of DNA - $300-500/sample
Targeted-gene	- Any region in the genome can be targeted for enrichment: 1) exons from a list of genes known to be associated with diseases (e.g. FLT3) 2) specific functionally important biological pathways 3) disease-associated chromosome segments (including both exons + introns) suggested by linkage analysis - Detects spectrum of gene mutations - Regions of interest (promoters or highly conserved sequences in	- Analysis software tools still be developed -Pindel has been used to detect FLT3-ITD insertions in AML patients but the sensitivity and specificity needs to be tested beyond this locus	- 0.2–4.8 μg of DNA

Sequencing Target	Advantages	Disadvantages	Starting material and Cost
	intron regions) can be targeted for capture - Significant cost saving - Shorter turnaround time - Provides a more feasible dataset for analysis outcome that is functionally interpretable		