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Leukemia and Lymphoma: An update of acute myeloid leukemia after Whole genome sequencing (WG)

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Abstract

Leukemia, known for several centuries, is a cancer of the blood and bone marrow. It starts when the DNA of a single cell in the bone marrow mutates and develops abnormal growth, which spill into blood stream. Based on the types, leukemia is classified in several groups for diagnosis and treatment. With the discovery of whole genome sequences (WGS), scientists are attempting to find out accurate diagnosis and treatment. Primary Acute myeloid leukemia (AML) samples are feasible and can detect novel, clinically relevant mutations including the clonal heterogeneity of this disease and clonal evolution that occurs over time. Some of the novel mutations are highly recurrent (>20% of patients), but there appears to be a continuum of mutation frequency down to rare (<5%) or even singleton mutations that may be relevant for the biology of this disease. Large cohorts of well-annotated samples are needed to establish mutation frequencies, implicate biological pathways, and demonstrate genotype: phenotype correlations. Recent advances in genomic techniques have unraveled the molecular complexity of AML leukemogenesis, which in turn have led to refinement of risk stratification and personalized therapeutic strategies for patients with AML

Keywords: Genetic mutation in AML; Recent advances in leukemia; WGS as a clinical tool; Management of patients with AML

1. Introduction

Leukemias are clonal disorders of hematopoietic stem cells or immature progenitors. Several subtypes of leukemia are associated with disease-specific karyotype anomalies in the malignant blasts.as described previously (1). Most cases of acute promyelocytic leukemia, a subtype of acute myeloid leukemia (AML), are associated with a t(15;17) chromosomal rearrangement that results in the production of the ***(*** *Progressive multifocal leukoencephalopathy)* PML-RARA(Retinoic Acid Receptor alpha) fusion-type oncoprotein (1-3). Similarly, another subtype of AML is associated with a t(8;21) rearrangement, resulting in the production of the oncogenic (runt-related transcription factor 1) RUNX1-CBFA2T (Core Binding Factor Subunit Alpha 2) protein (4).

The karyotype of leukemic blasts is an important determinant of the long-term prognosis of affected individuals. AML with t(15;17), t(8;21) or inv(16) rearrangements thus constitutes a subgroup of leukemias with a 'favorable' karyotype, with a 5-year survival rate of >60%, whereas AML with an 'adverse' karyotype (monosomy 7, monosomy 5 or complex anomalies) has a 5-year survival rate of only <15%) (5,6). The prognosis of AML with a normal karyotype (constituting ~50% of all AML cases) is substantially worse than that with a favorable karyotype, with a 5-year survival rate of 24% (7), indicational blasts with a normal karyotype may contain transforming genes generated as a result of (a) sequence alterations, (b) epigenetic abnormalities or (c) small chromosomal rearrangements not detectable by the G-banding technique. Indeed, several genes, including *NPM1* and *KIT*, have been found to be mutated and activated in AML blasts with a normal karyotype (7).

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The identification of transforming genes in AML will require large-scale resequencing of the blast genome. Although a new generation of sequencing technologies is now available, whole-genome resequencing of many samples remains a demanding task (8,9). Although DNA microarray-based sequencing is suitable for analysis of multiple samples, currently available platforms are limited in the number of nucleotides that each array can probe. To overcome such limitations, two-step analysis of human leukemia specimens (n=20) has identified a novel transforming mutation in the gene for Janus kinase 3 (JAK3) and a hylomorphic mutation in that for DNA methyltransferase 3A (DNMT3A) (10,11).

On the other hand, Acute lymphoblastic leukemia (ALL) is the most common childhood leukemia (10). Although the cure rate for ALL is very high but remains a leading cause of childhood morbidity and mortality. As age increases, the frequency of genetic alterations associated with favorable outcome declines. Poor outcome due to alterations such as *BCR-ABL1* are more common. Current therapies do not target specific genetic alterations and are associated with substantial short- and long-term toxicities that limit further dose escalation except for tyrosine kinase inhibitors (TKIs) such as imatinib in the treatment of *BCR-ABL1*+ leukemia. So, the physicians have great interest in the use of high-resolution genomic profiling to characterize the genetic basis of leukemogenesis. This may help to understand and predict treatment failure, and to provide novel markers that may be integrated into diagnostic testing and be targeted with novel therapies. These studies also provide critical insights into the nature of clonal heterogeneity and tumor evolution. Ongoing next generation sequencing of ALL has already provided important insights in acute leukemia not evident genetic profiling approaches (10). Recent insights obtained from genomic profiling of ALL, with an emphasis on high-risk B-progenitor ALL, the following rearrangements are made although approximately 75% of childhood ALL cases bear a recurring chromosomal alteration detectable by karyotyping, FISH (fluorescence ion situ hybridization), or molecular techniques) :

- In B-progenitor ALL, these include hyper diploidy with greater than 50 chromosomes, hypodiploidy with less than 44 chromosomes, and chromosomal rearrangements including t(12;21) *ETV6-RUNX1* (TEL-AML1), t(1;19) *TCF3-PBX1* (E2A-PBX1), t(9;22) *BCR-ABL1*, and rearrangement of *MLL* at 11q23 to a diverse range of fusion partners.
- T-lineage ALL is characterized by activating mutations of *NOTCH1* and rearrangements of transcription factors *TLX1 (HOX11)*, *TLX3 (HOX11L2)*, *LYL1*, *TAL1*, and *MLL*.2
- High hyper diploidy, *ETV6-RUNX1* (both associated with favorable outcome), and *TCF3-PBX1* are less common in adult ALL.

These rearrangements are widely used in diagnosis and risk assessment algorithms to understand leukemogenesis but are insufficient to fully explain the process (leukemogenesis). Rearrangements such as *ETV6-RUNX1* are present years before the development of leukemia, and many do not alone result in the development of leukemia in experimental models. It is now known that most of ALL cases are characterized by structural and genetic alterations and sequence mutations.

Table 1 Frequency of cytogenetic subtypes of pediatric ALL

B-ALL		**T-ALL**	
Subtypes	**Rearrangement (%)**	**Subtypes**	**Rearrangement (%)**
ETV6-RUNX1	22	**TLX1**	**0.3**
Hyperdiploid	20	**LYL1**	1.4
MLL	6	**ETP**	2.0
TCF3-PBX1	4	**TLX3**	2.3
CRLF2	3	**TAL1**	7
ERG	3		
Dicentric	3		
BCR-ABL1	2		
Hypodiploid	3		
Others (B-ALL)	10	BCR-ABL1-like	9

ALL genomes typically contain fewer structural alterations than many solid tumors, but more than 50 recurring deletions or amplifications have been identified, many of which involve a single gene or a few gene. reviewed in Millighan and Downing (10) (Table 1). Many of the genes involved encode proteins with key roles in

- Lymphoid development (e.g., pax5, izkf1, ebf1, and lmo2),
- Cell-cycle regulation and tumor suppression (cdkn2a/cdkn2b, pten, and rb1),
- Lymphoid signaling (btla, cd200, tox, and the glucocorticoid receptor nr3c1), and
- Transcriptional regulation and coactivation (tbl1xr1, etv6, and erg).

Several genes are involved in multiple types of genetic alteration, including copy number alteration translocation and sequence mutation, such as *PAX5*, *WT1*, and *PTEN*, indicating that microarray profiling is alone incapable of detecting all genetic alterations in ALL (10).Recently identified mutations in B-progenitor ALL are discussed in Table 2.

Table 2 Rearrangement and mutations in B-progenitor ALL

Gene	Frequency of rearrangement and mutations
PAX5	1/3 of B-progenitors of ALL cases
IKZF1	15% of all pediatric B-All cases>70% of BCR ABL1 lymphoid leukemia, and 1/3 rd of BCR-ABL1~B-ALL
JAK ½	18-35% DS-ALL and 10% high risk BCR-ABL 1-ALL. Jak-1 mutations also identified in T-ALL
CRLF2	5-16% pediatric and adult B-ALL and > 50%DS-ALL
IL:7R	Up to 7% of B and T-ALL
CREBBP	19% of relapsed ALL; commonly acquired at relapse
TP53	12% of B- ALL; commonly acquired at relapse
Kinase	Present in half of BCR-ABL1-like ALL cases

The above genes are altered, and the frequency of such alterations is reported (10). Such alterations may impact the various aspects on clinical treatments. So the clinical consequences are described in Table 3.

Table 3 Clinical consequence of gene alteration

Gene-altered	Impact of gene alteration on clinicians
PAX5	Transcription factor for B-lymphoid development. Mutations impair DNA binding and transcriptional activation. Cooperates in leukemogenesis, but no association with outcome
IKZF1	Transcription factor for B-lymphoid development. Deletions and mutations result in loss of function or dominant-negative isoforms Cooperated in pathogenesis of BCR-ABL1+ ALL. and BCR-ABL1- B-ALL associated with risk of ALL
JAK ½	Results pf JAK-STAT activation in model cell lines and primary leukaemia cells, may be responsive to JAK inhibitors
CRLF2	Associated with mutant JAK in up to 50% of cases ; Associated with IKZF1 alteration and poor outcome, particularly in non-DS-ALL
IL:7R	Results in receptor dimerization and constitutive IL7R signalling and JAK-STAT activation; JAK inhibitors may also be useful.
CREBBP	Mutations result in impaired histone acylation and transcriptional regulation associated with glucocorticoid resistance
TP53	Loss of function or dominant negative; associated with poor outcome
Kinase	Result in kinase signalling activation that attenuated with TKIs

2. Leukaemia and Genetic mutation in AML

2.1. Identification of the JAK3(M511I) mutation

Screening of the leukemic blasts of the 20 individuals for point mutations in phase I yielded 9148 nonsynonymous changes among 3403 independent genes, a frequency like that observed in other large-scale resequencing studies performed with capillary sequencers (12,13). However, analysis of $CD4^+$ fractions showed that most of these sequence changes were also present in the paired control genome, leaving only 11 nonsynonymous somatic mutations in 11 genes . Such a small number of somatic mutations is in good agreement with the eight somatic mutations found in AML through WGS (14). All 11 somatic changes were confirmed by analysis of both genomic DNA and cDNA of the corresponding specimens with a capillary sequencer. These data thus support the necessity of examining paired noncancerous specimens to pinpoint somatic changes in the cancer genome (14-18).

One of the gene mutations found in $CD34^+$ fractions in Met-to-Ile change at amino-acid position 511 of JAK3. A heterozygous *JAK3* mutation responsible for the amino-acid change was confirmed in both genomic DNA and cDNA from the $CD34^+$ fraction, but not in those from the corresponding $CD4^+$ fraction of patient ID JM07, who had *de novo* AML (M1 subtype) and a normal karyotype (18).

In contrast to JAK2, activating mutations are preferentially associated with myeloproliferative disorder. Several gain-of-function mutations (such as I87T, P132T, Q501H, A572V, R657Q and V722I) of JAK3 have recently been associated with acute megakaryoblastic leukemia of children (15,16). Other JAK3 mutations (such as A573V and A593T) were also identified in the same disorder, and an M576L substitution was detected in adult with acute megakaryocytic leukemia (AML, M7 subtype) (18), although the transforming potential of these changes remains unknown.

Fig. 1 shows M511 residue. Such a residue is in the linker region located between the Src homology 2 (SH2) domain and the pseudo kinase domain of JAK3 (18). The transforming mutation Q501H that is associated with juvenile acute megakaryoblastic leukemia is also located in this region. Since JAK3 is abundant and has an essential role in the development of lymphocytes (17), the expression level of *JAK3* in AML blasts was examined. The gene was expressed at a high level in most AML specimens (n=52), with its expression level being greater than that expression level of *JAK2* in all cases (18). Fig 3. C57BL/6 mice were irradiated and then injected intravenously with syngeneic $CD34^-$KSL hematopoietic stem cells infected with a retrovirus encoding JAK3(M511I) or the corresponding empty virus (control).

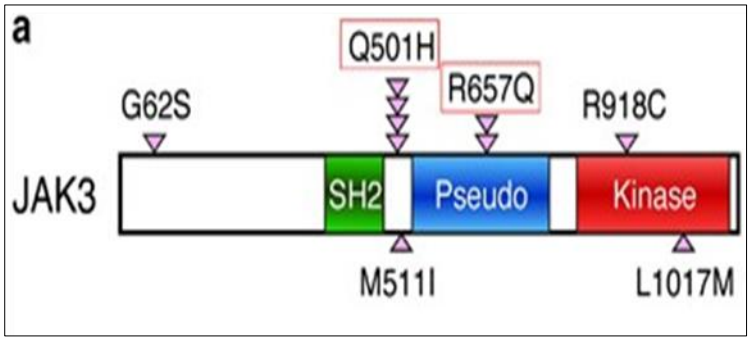

Figure 1 Identification of JAK3 mutants in leukemia. (a) Amino-acid substitutions detected in this study are shown relative to the domain organization of JAK3. The mutations M511I (one case) and Q501H (four cases) are located in the linker region between the SH2 and pseudokinase domains of JAK3, whereas G62S (one case), R657Q (two cases) and R918C (one case) are located in the amino-terminal region, the pseudokinase domain and the kinase domain, respectively. The KCL22 cell line also harbors an L1017M mutation within the kinase domain of JAK3. Activating mutations of JAK3 (Q501H and R657Q) are indicated by red rectangles which is known previously.

Greenberger et al., introduced the mutant or wild-type protein into the interleukin-3 (IL-3)-dependent mouse cell line 32D to examine the transforming potential of JAK3(M511I (19). 32D cells underwent rapid apoptosis after withdrawal of IL-3, however those expressing JAK3(M511I) continued to grow even in the absence of IL-3, although at a reduced rate compared with that of cells expressing artificially generated, highly transforming mutant JAK3(V674A) as discussed by Greenberger et al (19)). 32D cells differentiate into terminal granulocytes in the presence of granulocyte colony-stimulating factor. How ever, cells expressing the M511I or V674A mutant of JAK3 maintained an exponential rate of growth, without any sign of differentiation, in the presence of granulocyte colony-stimulating factor, suggesting the presence of transforming potential of M511I mutant (19).

A recombinant retrovirus encoding this mutant was prepared to examine for the leukemogenic activity of JAK3 (M511I) and used it to infect murine hematopoietic stem cells. Reconstitution of the bone marrow of lethally irradiated mice with such infected cells resulted in marked lymphocytosis in peripheral blood and enlargement of the spleen in the recipient animals (Figure 2).

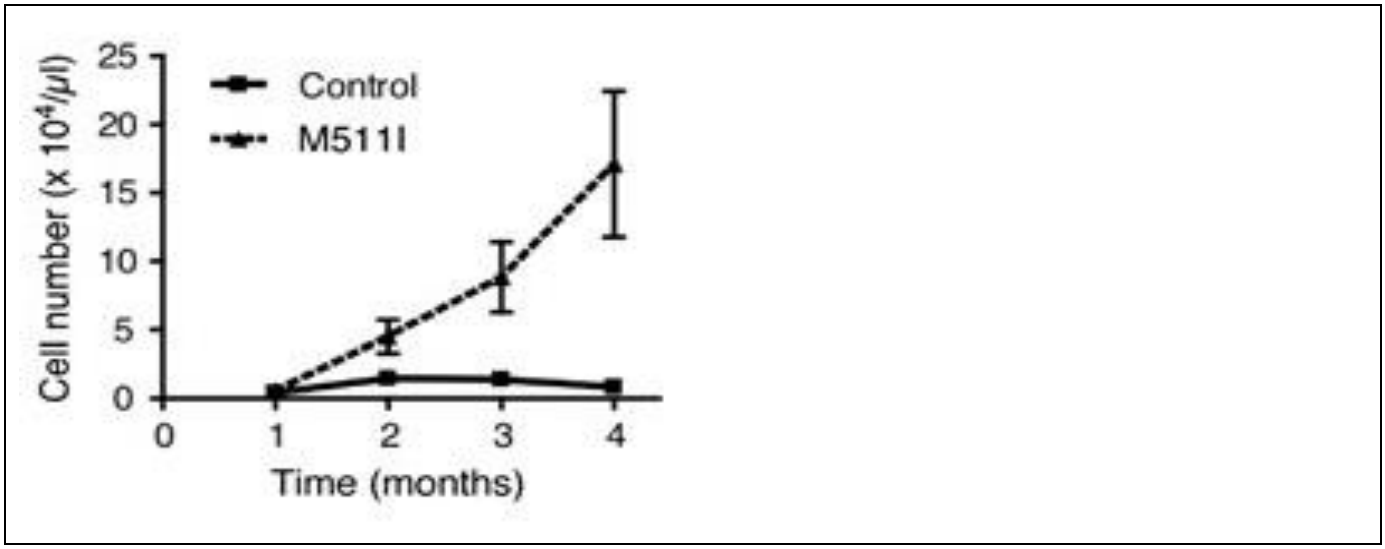

Figure 2 C57BL/6 mice were irradiated and then injected intravenously with syngeneic $CD34^{-}KSL$ hematopoietic stem cells infected with a retrovirus encoding JAK3(M511I) or the corresponding empty virus (control).

The cells in the peripheral blood, spleen and bone marrow of the recipients responsible for these phenotypes manifested a medium-sized, blastic morphology, and flow cytometric analysis revealed them to be $CD8^{+}$ T cells . The clonal nature of these proliferating T cells was further confirmed by Southern blot analysis (18), indicative of the development of T-cell acute lymphoblastic leukemia in the recipient mice.

The nucleotide sequence of the entire coding region of *JAK3* cDNA to assess the prevalence of *JAK3* mutations in adult leukemia was further examined in an additional 266 specimens of leukemic blasts. The coding region of *JAK3* cDNA was successfully amplified by PCR from 83 specimens. Four distinct *JAK3* sequence changes could further be identified in 8 of these 83 samples: One case with G62S, 4 cases with Q501H, 2 cases with R657Q and 1 case with R918C. Within 20 cases evaluated in the phase I analysis, a total of 9 cases was identified with a mutant form of JAK3 (3.1%) among 286 cases of leukemia. The identification of known transforming JAK3 mutants (Q501H and R657Q) originally associated with acute megakaryoblastic leukemia prompted us to determine the prevalence of these two changes in another cohort of AML (n=148), revealing two cases with JAK3(Q501H) and one case with JAK3(R657Q). In addition, analysis of a hematopoietic cell line (KCL22) (20) established from a patient with chronic myeloid leukemia in BC revealed yet another mutation (L1017M) of JAK3 .

The transforming potential of various JAK3 mutants were compared by introducing each protein into the IL-3-dependent mouse B-cell line BA/F3 and examined the growth properties of the resulting transfectants. Cells expressing the JAK3 mutants proliferated in a similar manner in the presence of IL-3 whereas culture without IL-3 revealed marked differences in the transforming potential among the mutants. JAK3(M511I) was the most efficient oncokinase, with a transforming activity similar to that of JAK3(V674A). The frequent mutants JAK3(Q501H) and JAK3(R657Q) exhibited weaker but still pronounced transforming potential, whereas the remaining mutants (G62S, R918C and L1017M) showed an even lower potential .

2.2. Somatic mutations of DNMT3A

Somatic mutation data set, obtained in phase II, was a heterozygous change in *DNMT3A* that happens in an R882H substitution in the encoded protein (Figure 3a,). DNMT3A, together with DNMT3B, has an essential role in *de novo* methylation of the human genome (21), and an aberrant methylation profile (hypermethylation of CpG islands and hypomethylation of other regions) is an indication of cancer cells (22). Despite a direct linkage between such methylation changes and silencing of tumor-suppressor genes in cancer, the molecular mechanism responsible for such abnormal methylation remains unknown. The data thus provide somatic mutation of a DNA methyltransferase gene in cancer cells. Mutations in the catalytic domain of DNMT3B have been shown to be responsible for a hereditary syndrome

characterized by ICF (immunodeficiency, instability of the centromeric region of chromosomes and facial anomalies) in humans (23). One of the mutation sites of DNMT3B (R823) associated with the ICF syndrome corresponds to the residue of DNMT3A (R882) shown to be mutated in this study (Figure 3).

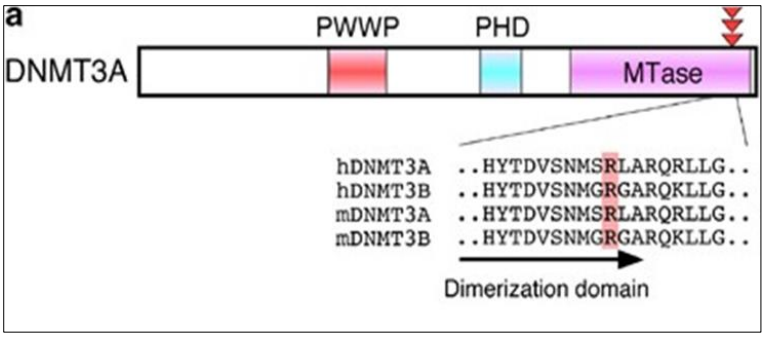

Figure 3 Identification of a DNMT3A mutant in leukaemia. (a) Domain organization of human DNMT3A showing that the R882 residue found to be mutated in leukaemia is conserved among human (h) and mouse (m) members of the DNMT3 family. DNMT3A contains a tetrapeptide PWWP domain, polybromo homology domain (PHD) and methyltransferase (MTase) domain. The R882 residue is in the homodimerization region present within the MTase domain.

The R882 residue of DNMT3A is considered to participate in the homodimerization and activation of the protein (17) To determine whether the R882H mutation affects the catalytic activity of DNMT3A, mutant and wild-type proteins were expressed separately in insect cells, purified them to near homogeneity and subjected them to an *in vitro* assay of methyltransferase activity with a synthetic substrate (24). .The catalytic activity of DNMT3A(R882H) was <50% of that of the wild-type protein . DNMT3L acts as a coactivator for the methyltransferase activity of DNMT3A or DNMT3B through its association with the latter proteins (25). The R882H mutation did not affect the interaction of DNMT3A with DNMT3L in transfected mammalian cells or its sensitivity to DNMT3L as examined by *in vitro* assay of methyltransferase activity indicating R882H mutation directly inhibits the enzymatic activity of DNMT3A.

Screening of another cohort of leukemia cases (*n*=54) for mutant forms of *DNMT3A* revealed another two patients with a mutation of the same amino acid (R882H in one patient and R882C in the other) . Therefore, a total of 3 cases with an R882 mutation (4.1%) were identified among 74 cases of leukemia. Screening for mutations of *DNMT3B* failed to detect any somatic changes in the same individuals (data not shown), suggesting that *DNMT3A* is a preferential target in leukemia.

3. Recent advances in leukaemia after genomic sequencing

3.1. AML and Genome

The higher resolution genome scans utilizing microarray-based comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) array karyotyping led to the identification of additional recurring and singleton copy number alterations in patients with AML, many of which are below the size threshold for detection by routine cytogenetics (29,30). With these approaches, a structural chromosomal lesion can be detected in up to 65% of AML patients.(29-35) This line of investigation has been productive, but arguably less informative in adult AML, compared to other acute leukemias (32-36).

Recurrent mutations in a growing number of genes, in addition to large structural variants, have been detected in AML samples. The best characterized mutants are the *fms*-like tyrosine kinase 3 gene (*FLT3*) which accommodates activating mutations in the juxta membrane region in 20–27% of AML patients or the kinase domain in 5–7% (35-40) The juxta membrane lesions (internal tandem duplications, or ITDs) have negative prognostic significance (36,37)(Both classes of *FLT3* activating mutations provide targets for inhibition by small molecules. In-frame insertions in the *NPM1* gene, found in ~30% of AML patients, an aberrant protein is produced that is mis localized from the nucleolus to the cytoplasm (NPMc) (41) These mutations are associated with a relatively favorable prognosis in patients that lack *FLT3* ITD (38).

Even the above advancement, most patients of AML are still fall in the intermediate risk category, without a known cytogenetic or molecular driver. Furthermore, with rare exceptions (39-42), single mutations (point mutations or

translocations) are not sufficient to cause AML in genetically engineered mouse models. Taken together, these results suggest that our understanding of AML genomics is still incomplete. “As the extent of genetic heterogeneity in AML became more evident, there was waning confidence that candidate gene studies would reveal the genetic rules of AML” in a timely and cost-efficient manner. A strategy to perform unbiased surveys of entire AML genomes was needed to reach a comprehensive understanding of AML genomics and other human cancers “ (43,44) .

3.2. Whole genome sequencing in AML

Next generation sequencing (NGS), also known as “massively parallel” sequencing is an enabling technology that has transformed cancer biology over the past decade. The genome is resequenced at a high level of redundancy (generally, 30–40x the size of the 3 billion base pairs haploid human genome, or ~100–120 billion base pairs) to ensure that all regions of the genome are adequately sampled. Sequencing both ends of each DNA fragment (“paired end” sequencing) improves the accuracy of realigning short reads to their site of origin in the genome and facilitates detection of structural variants (e.g., translocations, inversions, and copy number alterations). Robust algorithms have been developed to manage all phases of processing these data, from genome alignment to mutation detection (45)

The first cancer genome sequence was reported in 2008 (46). The subject was a young (age<60) female patient with intermediate risk AML, characterized by a normal karyotype. She has no molecular abnormalities detected by standard cytogenetics, molecular diagnostics, or array-based comparative genomic hybridization; and typical FAB M1 morphology, immunophenotype, and gene expression profile. Ten mutations with predicted translational consequences were identified in coding genes, including classic NPMc and *FLT3* ITD abnormalities. The eight remaining genes had not been previously implicated in AML and no recurrent mutations in the same exons were detected in 187 other patients with AML. This suggests that these were either rare pathogenic alleles, or (more likely) non-pathogenic somatic mutations acquired in a normal self-renewing hematopoietic cell prior to transformation. This experiment extended for WGS using small amounts of primary clinical samples and motivated many other groups to apply similar approaches in other cancers. The results refuted prior predictions that cancer genomes would be highly unstable, resulting in a landscape of point mutations and structural variants that would be difficult to resolve. The findings did suggest that achieving the goal of identifying all biologically important genetic changes in AML genomes would be challenging.

Two other AML genomes were analyzed by scientists both yielded novel genetic factors with prognostic significance. A very similar pattern of somatic mutations from another young patient with typical M1 AML was detected: mutations with translational consequences in ten genes, including two known factors (NPMc and $NRAS^{G12D}$) (47) . Six of the eight novel genes were not recurrently mutated in 187 other AML patients. Recurrent mutations were detected in two genes, including the mitochondrial gene, *ND4* (2/93 AML samples) and codon R132 of *IDH1* (17/182; 9.3% of AML patients). *IDH1* encodes cytoplasmic isocitrate dehydrogenase. *IDH1* mutations are common (>70%) in malignant glioma,

R132H allele predominates in glioma (88% of cases), whereas the R132C is more common in AML (~50% of cases).(48,49). These findings have been replicated by several other groups and extended to include mutations in *IDH2*, the mitochondrial homolog of cytoplasmic IDH1. Together, *IDH1/2* mutations are detectable in 12–17% of AML patients and enriched in patients with normal karyotype (22–33%).(50-53). Mutated *IDH1* is associated with adverse outcomes in patients with the NPMc/$FLT3^{wt}$ genotype.(50-53). The pathophysiologic consequences of mutated IDH enzymes appear to include production of the “oncometabolite” (54). 2-hydroxyglutarate that impairs TET2-mediated hydroxylation of methyl cytosine residues.(55).

Recurrent mutation was detected when the first AML genome using optimized NGS approaches was resequenced. that led to detection of a frameshift mutation in the *DNMT3A* gene, which encodes a methyltransferase that catalyzes *de novo* methylation of cytosine residues.

DNMT3A mutations with predicted translational consequences (including missense, nonsense, frameshift, splice site alterations, and deletions)was detected in 62/281 (22.1%) of *de novo* AML patients. Such mutations were mutually exclusive with favorable risk karyotypes (0/79 patients), enriched in patients with intermediate risk cytogenetics (33.7% of cases),.Moreover, they were associated with higher white blood cell count and lower survival, but not related to age or *FLT3* genotype in patient cohort (56). The biological consequences of mutated DNMT3A are not yet known. Clustering of mutations at codon R882 suggests that they may confer gain-of-function properties. In contrast, other *DNMT3A* mutations (e.g., deletions, truncations) almost certainly result in loss of function. Neither class of mutations has yet been associated with a consistent pattern of altered DNA methylation or gene expression in primary AML samples.(56-59)

3.3. WGS as a clinical tool in the management of patients with AML

As mentioned above, AML and other cancers can be identified by WGS technology. The question arises whether this technique can be used in the clinics or be confined in research laboratories ? Let us discuss the pros and cons of the technology utilized in clinical settings. The cost of sequencing continues to fall, and the results are more accurate with the improvement of analytical technology. The fact that all classes of genetic variants can be detected on one platform (including point mutations, insertion/deletions, copy number alterations, and chromosomal rearrangements), makes WGS a particularly attractive alternative to the existing diagnostic workup that employs multiple, expensive platforms (including morphology, flow cytometry, cytogenetics, FISH, single gene mutational profiling, RT-PCR). WGS cannot replace all these tools, but it is rapidly moving into position as a cost-effective alternative to several of them.(60)

Clinicians (61) used WGS to resolve an ambiguous case of AML. "A 39-year-old woman was admitted with features typical of acute promyelocytic leukemia but lacked the characteristic t(15;17) that is present in nearly all cases. In fact, she had a complex karyotype that, in the absence of the t(15;17), is associated with poor risk AML. In view of these findings, she was referred to the Cancer center for allogeneic stem cell transplantation (appropriate therapy for poor risk AML, but patients with more favorable risk acute promyelocytic leukemia would instead be treated with targeted chemotherapy that is associated with a relatively good outcome without the risks of a stem cell transplant). To resolve this clinical dilemma, her genome was sequenced, all somatic mutations were confirmed by resequencing on a second platform, and a clinical report was generated in less than 50 days. WGS revealed an insertion of the *PML* gene in the *RARA* locus, an event that recapitulated the molecular consequences of the t(15;17) but was cryptic using conventional cytogenetics and FISH analysis. Optimized molecular assays were developed to confirm this finding (and show that two other cases with similar features also had cryptic *PML/RARA* rearrangements), and the patient went on to receive appropriately targeted chemotherapy and remains in remission without undergoing stem cell transplantation"(61).

There are many other challenges that must be overcome before WGS of AML in clinics. These include :

- Scaling up sequencing production/analysis to produce "clinical grade" data in real-time,
- Establishing protocols and procedures to perform this work in a cap/cilia environment,
- Generating reports that can be interpreted by clinicians who may lack formal training in clinical genetics,
- And returning results to patients that may include findings of direct relevance to their cancer care but will likely also include incidentally detected findings that could be medically important for them or their offspring.

It should be noted that most of the somatic mutations and nearly all the germline variants detected by WGS today are of uncertain clinical and biological significance. Therefore, mechanisms must be in place to allow the data to be reinterpreted and reported back to patients and clinicians in the future as knowledge increases (62).

Cancer genome sequencing is still largely restricted to large academic sequencing centers. Although this technology has wide applicability. These tools will require to be refined and deployed in formats that can be used outside these specialized centers or clinics. Physicians, in particular, pathologists, will need to be retrained to address the interpretation of analyzed genomic data from sequencing-based assays (63). Regardless, the potential power of these techniques to transform the clinical approach to AML is considerable, and they will likely become routine practice within a few years.

Abbreviations

- ALL : Acute lymphoblastic leukemia
- AML : Acute myeloid leukemia
- BCR: B-cell receptor signaling
- BMSCs: Bone marrow stromal cells
- CARs: Chimeric antigen receptors
- CRISPR: Short palindromic repeats interspersed with regular intervals
- DSBs: Double-strand breaks
- FISH: fluorescence ion situ hybridization
- HiPSC: Human induced pluripotent stem cell
- HR: Homologous recombination
- HSPCs: Hematopoietic stem and progenitor cells
- IAPs: Inhibitors of apoptosis proteins
- IR: Ionizing radiation

- MMEJ: Micro-homology-mediated end-joining
- NGS: Next generation sequencing
- NHEJ: Non-homologous end-joining
- PBMC: Peripheral blood mononuclear cell
- Sias: Sialic acids
- SMACs: Second mitochondrial-derived caspase-activators
- TALENs: Transcription-activating type nucleases
- TCR: Transcription-activating type nucleases
- THC: Tetrahydrocannabinol
- ZFNs: Zinc finger nucleases

4. Conclusion

WGS in AML and other cancers may now be an established technology with proven capacity to identify novel, clinically relevant genetic findings. The fact that all classes of genetic variants can be detected on one platform (including point mutations, insertion/deletions, copy number alterations, and chromosomal rearrangements), makes WGS a particularly attractive alternative to the existing diagnostic workup Many mutations that contribute to the pathogenesis of acute myeloid leukemia (AML) are undefined. The relationships between patterns of mutations and epigenetic phenotypes are not yet clear. AML genomes have fewer mutations than most other adult cancers, with an average of only 13 mutations found in genes. Of these, an average of 5 are in genes that are recurrently mutated in AML. A total of 23 genes were significantly mutated, and another 237 were mutated in two or more samples. Nearly all samples had at least 1 nonsynonymous mutation in one of nine categories of genes that are almost certainly relevant for pathogenesis.

There are many remaining challenges that must be overcome before WGS of AML could become a widely utilized clinical test. These include scaling up sequencing production/analysis to produce “clinical grade” data in real-time, establishing protocols and procedures to perform this work in a cap/cilia environment, generating reports that can be interpreted by clinicians who may lack formal training in clinical genetics, and returning results to patients that may include findings of direct relevance to their cancer care, but will likely include incidentally detected findings that could be medically important for them or their offspring. It should be noted that most of the somatic mutations and nearly all the germline variants detected by WGS today are of uncertain clinical and biological significance. Therefore, mechanisms must be in place to allow the data to be reinterpreted and reported back to patients and clinicians with furtherance of knowledge.

Compliance with ethical standards

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