An Integrated Genomic Approach to the Assessment and Treatment of Acute Myeloid Leukemia

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Traditionally, new scientific advances have been applied quickly to the leukemias based on the ease with which relatively pure samples of malignant cells can be obtained. Currently, our arsenal of approaches used to characterize an individual's acute myeloid leukemia (AML) combines hematopathologic evaluation, flow cytometry, cytogenetic analysis, and molecular studies focused on a few key genes. The advent of high-throughput methods capable of full-genome evaluation presents new options for a revolutionary change in the way we diagnose, characterize, and treat AML. Next-generation DNA sequencing techniques allow full sequencing of a cancer genome or transcriptome, with the hope that this will be affordable for routine clinical care within the decade. Microarray-based testing will define gene and miRNA expression, DNA methylation patterns, chromosomal imbalances, and predisposition to disease and chemosensitivity. The vision for the future entails an integrated and automated approach to these analyses, bringing the possibility of formulating an individualized treatment plan within days of a patient's initial presentation. With these expectations comes the hope that such an approach will lead to decreased toxicities and prolonged survival for patients.
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The current evaluation of patients with acute leukemia involves careful hematopathologic review of leukemic cells. Leukemic cells are most often present in the bone marrow and peripheral blood but are occasionally found in other sites, such as in the cerebrospinal fluid and/or in granulocytic sarcomas, which can occur almost anywhere in the body. Complete assessment of leukemia consists of hematopathologic assessment of involved tissue, most typically the bone marrow core and aspirate samples, as well as peripheral blood, and complementary studies, such as flow cytometry and cytogenetic and molecular analyses, including reverse transcription–polymerase chain reaction (PCR) and/or sequencing.[1,2] A full description of the current evaluation for patients with acute myeloid leukemia (AML) can be found in the accompanying article by Odenike et al in this issue of *Seminars in Oncology*.

Given the rapid progress in genomic analysis and sequencing of the human genome, one can envision a new approach to patients with acute leukemia that relies more heavily on genomic-based analyses.[3-10] This article will focus on that vision—a vision for how patients may be assessed clinically in the future, a promising one that is approaching quickly. This assessment will be based on molecular profiling of the leukemic cells, as well as host factors that influence the development and treatment of the disease (Figure 1). Some of the techniques are described more fully in the article by Seiwert et al in this issue of *Seminars*. Note

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Initial leukemia analysis: current versus potential future approaches		
Profiling the Leukemia		
Goal	**Current Technique**	**Genomic Approach**
Determine the leukemia subtype	Hematopathologic analysis, including morphology, flow cytometry, cytogenetic and molecular analyses	Hematopathologic analysis, including morphology and gene/miRNA expression profiling
Determine the mutations present	DNA sequencing of genomic or cDNA for particular genes of interest (eg. *FLT3, KIT, NPM1*) and/or immunohistochemical evaluation of subcellular localization (eg. NPM1)	Whole genome and/orTranscriptome sequencing
Determine the cytogenetic abnormalities present	Cytogenetic analysis	Transcriptome sequencing and/or Single Nucleotide Polymorphism arrays
Determine chemosensitivity	Empiric administration of chemotherapy combinations	*In vitro* drug sensitivity testing
Profiling the Host		
Goal	**Current Technique**	**Genomic Approach**
Determine predisposition to disease	Identify a clinical/familial syndrome	Genome-wide association study

Figure 1. Initial leukemia analysis: current versus potential future approaches.

that although this review will focus on AML, the principles are applicable to all forms of hematopoietic malignancies.

PROFILING THE LEUKEMIC CELLS

Whole Genome Sequencing

The development of next-generation, high-throughput DNA sequencing offers the possibility to sequence a genome completely, providing entire genomic sequence information on which to characterize a patient sample. For patients with AML, this approach is eminently feasible, given the relative ease with which leukemic cells can be collected from the peripheral blood or bone marrow, or by leukapheresis. In addition, populations of immature cells (hematopoietic stem and progenitor cells) that are enriched for the leukemic population, or mature cells can be isolated using flow cytometry. This approach was first used to characterize a genome from a patient with a normal karyotype, given the assumption that cytogenetically normal AML is driven by mutations not observable by traditional cytogenetic techniques. From this first sequenced AML, 10 mutations in individual genes were discovered, two in pathways already known to be involved in AML (*FLT3* and *NPM1*), and eight in genes not previously implicated in AML: nonsense mutations in *CDH24* and *SLC15A1;* and missense mutations in *KNDC1*, *PTPRT*, *GRINL1B*, *GPR123*, *EBI2*, and *PCLKC*.[11] Ley and Mardis and their colleagues went on to sequence a second AML genome from another cytogenetically normal case and found somatic mutations in 12 genes: missense mutations in *CDC42*, *NRAS*, *IDH1*, *IMPG2*, *ANKRD26*, *LTA4H*, *FREM2;* a splice site mutation in *C19orf62;* silent mutations in *SRRM1* and *PCDHA6;* and in-frame insertions in *CEP170* and *NPM1*.[12] One hope of using this technology is that by studying individual AML genomes, we will identify genes commonly mutated in AML and other cancers but heretofore unrecognized.[13]. Indeed, to date, *IDH1* mutations have been confirmed as recurrent mutations in 14% of cytogenetically normal AMLs.[14] In addition, the *IDH2* gene is mutated in 19% of AMLs with a normal karyotype, with mutation of either *IDH* gene conferring a relatively poor prognosis.[14] Thus, from these two AML genomes, it appears that AMLs with a normal karyotype have about 10 to 15 somatic mutations, a number that is remarkably similar to that seen in solid tumors.[15] In the future, the identification of genes commonly mutated in AML will reveal novel pathways that can be targeted therapeutically to improve patient survival.

Gene Expression Profiling

Currently, the major ways in which we distinguish AML subtypes involve hematopathologic diagnosis with flow cytometry and cytogenetic/molecular analyses. Inherent in the distinction among the subclasses of AML is the notion that different subtypes of AML express different proteins, either at the cell surface, defining AML types distinguishable based on flow cytometry, or as a result of chromosomal rearrangements or gene mutations. Therefore, it is logical that a technique that can define transcriptional gene expression globally should be able to distinguish among AML subtypes.[9] (For the purposes of this article, the term "transcriptional gene expression" will be shortened to "gene expression.") That prediction was first proven when AML samples were shown to have different gene expression profiles compared to ALL cases.[16] Additional studies have followed and, at present, gene expression profiling is able to predict accurately some of the major cytogenetic subgroups of AML, including those with *MLL* rearrangements, t(8;21), inv(16)/t(16;16), and t(15;17), but it is less accurate with other types of

AML.[17] Furthermore, a gene expression signature for activation of FLT3 was better able to predict prognosis for AML cases with a normal karyotype than the detection of a *FLT3* internal tandem duplication mutation by molecular testing.[18]

The hope for this technology is that it will be able to refine our subclassification scheme for AML and define new subtypes of AML that will be more predictive of the likelihood of remission and survival than conventional prognostic factors.[19,20] A second application of the technique is that it could be used at diagnosis to predict the chemosensitivity of a patient's AML cells and to help guide the choice of an induction chemotherapy regimen. The vision for future diagnosis would be that gene expression profiling could be used as a central diagnostic modality to define AML subtypes. International cooperative studies such as the Microarray Innovations in Leukemia (MILE) study will help to standardize gene expression profiling and usher it in as a standard clinical test.[21]

EPIGENETIC PROFILING

Profiling Based on DNA Methylation Patterns

In addition to expression profiling, DNA methylation patterns can also be used to distinguish cell types. The ability to distinguish the major cytogenetic AML subgroups based on DNA methylation distribution was demonstrated by Figueroa et al, who performed a microarray-based *Hpa*II tiny fragment enrichment by ligation-mediated PCR (HELP) assay to classify 344 cases of AML.[22] HELP was able to distinguish 16 AML subgroups each with distinctive DNA methylation patterns, including some subgroups recognized by their cytogenetic abnormalities, those with t(8;21), inv(16), and t(15;17). Notably, the fusion proteins produced by these chromosomal rearrangements are known to interact with DNA methyltransferase and histone deacetylase enzymes, and therefore, it is reassuring that an assay that detects epigenetic changes can distinguish these subgroups. Importantly, the HELP assay was able to identify five new AML subgroups, as well as those with *CEBPA* alterations. Again, as seen with the other emerging genomic technologies, the power of these high-throughput techniques is that by measuring a novel feature of AML cells, new biological insights will be gained.

Profiling Based on MicroRNA Expression Patterns

Similar to the justification for gene expression profiling, microRNA (miRNA) profiling is based on the hypothesis that expression of these small, non-coding RNAs varies widely among cells, and this variability in expression can be used as a basis for cell classification.[23-26] Indeed, miRNA expression patterns, like gene expression profiling, can distinguish some of the major subclasses of AML, including those with particular cytogenetic or molecular features, such as *MLL* rearrangements, trisomy 8, internal tandem duplications of *FLT3*, core-binding factor rearrangements, t(15;17), and mutations of *NPM1* or *CEBPA*.[27-29]

The true power of genome-wide miRNA profiling techniques, however, lies in their ability to reveal insights into cellular biology not already appreciated by current methods. Toward that end, Marcucci and colleagues characterized miRNA expression in patients with normal karyotype AML and found that increased expression of the miR-181 family was associated with expression of genes of innate immunity, such as those encoding interleukin-1β and the toll-like receptors TLR2, TLR4, and TLR8, and a decreased risk of failure to achieve complete remission, relapse, or death.[30] Furthermore, downregulation of miR124, miR128–1, miR194, miR219–5p, miR220a, and miR320 was linked to an aggressive clinical course.[30] In addition, Garzon et al found that patients with high expression of miR-191 and miR-199a had worse outcomes than those with lower expression levels.[27]

Single-Nucleotide Polymorphism Arrays

The identification of recurrent chromosomal rearrangements in a particular patient's AML, typically performed by G-banding of chromosomes and karyotype analysis, is one of the most critical prognostic indicators and drives recommendations regarding the mode of consolidation chemotherapy. Genome-based techniques seek to define chromosomal changes that are less than 5 Mb in size and beyond the level of detection by traditional methods.[3,7] Single-nucleotide polymorphism (SNP) arrays are microarray-based platforms that allow the detection of single-nucleotide variations at up to 900,000 separate genomic loci, at an average intermarker distance of less than 700 bp. This approach allows the analysis of DNA copy number variations (CNVs) that may not be detectable by standard karyotype analysis, such as amplifications and deletions, while also yielding information about smaller regions of DNA loss, including both loss of heterozygosity (LOH) and the presence of copy-neutral LOH (sometimes referred to as uniparental disomy [UPD]).

The technique has been applied to patients with normal karyotype AML to determine whether cytogenetic abnormalities were present that were undetectable by standard methods. Akagi et al were able to identify cryptic chromosomal abnormalities in 24% of AMLs with a normal karyotype, with chromosomal losses encompassing numerous tumor-suppressor genes, including *NF1*, *ETV6 (TEL)*, and *CDKN2A/2B*.[31] In addition, this approach has been used to identify cryptic copy number losses of chromosome arm 20q in

AML patients.[32] Serrano et al identified loss of heterozygosity without an associated CNV, indicating acquired copy-neutral LOH in 23% of 22 AML cases with a normal karyotype.[33] Walter et al used similar methodology on a larger cohort of 86 AML cases, of which 34 had a normal karyotype, and found six cases of copy-neutral LOH within those samples with a normal karyotype (18%).[34] Cryptic CNVs were identified in 40% of the samples overall. The largest published series of normal karyotype AML cases involved the analysis of 157 patients, and copy-neutral LOH was identified in 12% of cases, with 6p, 11p, and 13q being the genetic loci most commonly affected.[35] Copy-neutral LOH was commonly associated with cases that had mutations in *NPM1* or *CEBPA*, a finding also seen by the Serrano group, albeit with fewer cases.

The method holds great promise due to its ability to detect these abnormalities at high resolution. However, because of their exquisite sensitivity, it is crucial that the technique is performed on leukemia (tumor), as well as matched normal DNA, in order to distinguish cancer-specific lesions from constitutional changes that may be otherwise undetectable.[36] Moreover, balanced rearrangements, such as recurring translocations, are not detected typically by this approach. Whether the changes identified provide prognostic information beyond that conferred by conventional cytogenetic and molecular testing remains to be determined with larger prospective analyses. To date, Parkin et al have studied the largest series of AML patients using this technique, demonstrating that among 114 AML patients, the presence of more than two genomic lesions detected by SNP arrays doubled the risk of a patient's death when controlling for age and conventional cytogenetic karyotype, with increased risk conferred by additional chromosomal changes.[37] In addition, patients with a *TP53* mutation or a *TP53* mutation combined with LOH for chromosome arm 17p were at least twice as likely to die than patients without these changes.

Because reciprocal chromosomal translocations do not involve a net loss of genetic material, but instead have a rearrangement of the chromosomes, SNP arrays will not detect most recurring reciprocal chromosomal translocations unless they contain large deletions at the breakpoints. Thus, in the future, SNP arrays will likely be used in conjunction with cytogenetic/fluorescence in situ hybridization (FISH) analysis and/or high-throughput sequencing to characterize the full complement of genetic abnormalities present in a leukemia sample. Whole genome/transcriptome sequencing (see below) can identify cryptic translocations and juxtapositions of DNA sequences that are not detectable by cytogenetic analysis, and therefore may serve as an important adjunct to traditional methods, with increasing utility in the future.

Transcriptome/Exon Capture/ Array-Based Genomic Resequencing

The introduction of high-throughput DNA sequencing technologies in recent years has made full-scale exon sequencing feasible.[38] Two general approaches can be envisioned. If genes of a certain class are the focus, resequencing can be directed at those genes in particular. Otherwise, a genome-wide approach can be taken, without bias to the gene class of interest. Both schemes have been applied to AML, and several groups have performed large-scale resequencing of AML genomes, allowing the identification of somatic mutations.

Because of the relatively high frequency of mutation of genes encoding tyrosine kinases, several groups have led resequencing efforts focused on genes encoding these receptors. Ley and colleagues performed resequencing of 26 tyrosine kinase genes in AML patients, identifying novel mutations in *JAK1*, *DDR1*, and *NTRK1*, each seen at a frequency of 2%.[39,40] Jiang et al focused on the *FLT3* gene and identified novel point mutations at amino acid N841, at least one of which resulted in constitutive activation of the encoded receptor.[41]

By taking an unbiased approach, Yamashita et al have identified 11 somatic mutations in 19 cases of AML and one case of a myeloproliferative disorder, including *JAK3* mutations present in 3% of AML cases and an R882H mutation found in DNMT3A, one of the de novo DNA methyltransferases.[42]

The spectrum of mutated genes in different AML cytogenetic groups remains unknown. Similarly, we do not yet know how many of these mutated genes are shared across samples or are unique to each individual case. Large numbers of patients may be required to recognize the true incidence of these alterations. Furthermore, we do not yet know whether whole genome/transcriptome sequencing will be effective in defining multiple subclones within a single patient. The hope is that the identification of somatic mutations in leukemias will yield new insights into disease pathogenesis and will highlight new pathways and/or implicate pathways characterized in other systems that can become therapeutic targets.

Assessing Chemosensitivity

One of the roadblocks to successful treatment of AML is the inherent drug resistance of the tumor cells. Despite decades of research into the disease, standard induction chemotherapy regimens have remained largely unchanged, and consist of combinations of cytarabine and an anthracycline or anthracenedione. Clinically, drug sensitivity is measured empirically by the degree of clearance of leukemic blasts in the peripheral blood and bone marrow at designated time points after treatment.

New technologies may be able to measure inherent chemosensitivity rapidly at diagnosis to facilitate the choice of drug combinations and predict clinical response. For example, a chemosensitivity index, C_i, has been developed that allows a predictive accuracy of 98% for treatment response and is a strong prognostic factor in overall survival.[43] Briefly, a patient's leukemic cells are incubated in vitro with a chemotherapy drug of interest, and the C_i is calculated from the area under the curve as an exact measure for the total dose response relation. In the future, assays such as this may be combined with information about a patient's genetic composition (see below) to predict response to particular agents.

PROFILING THE HOST

Candidate Gene Approach

Among the different subtypes of AML, therapy-related myeloid neoplasms (t-MNs), those that arise after treatment with chemotherapy and/or radiation for another condition, may have the most influence from inherited host factors.[44,45] For this reason, much effort has gone into identifying the germline loci and pathways that increase a patient's risk for developing this complication of prior treatment. Most studies in the past have focused on determining the frequency of a few key polymorphisms in genes that comprise pathways that are critical for hematopoiesis or drug metabolism (reviewed by Seedhouse and Russell[46]). For example, several groups have reported an increased frequency of an inactivating polymorphism (*187Ser*) in the gene encoding NAD(P)H:quinone oxidoreductase, *NQO1*.[47–49] The NQO1 enzyme converts quinones derived from benzene into hydroquinones, which are less toxic. Therefore, people expressing an inactive form of NQO1 would be expected to be more susceptible to the carcinogenic effects of benzene and possibly other chemicals. Homozygous variant carriers may be particularly vulnerable to leukemogenic changes induced by carcinogens, and heterozygotes are at risk for treatment-induced mutation or loss of the remaining wild-type allele in their hematopoietic stem cells. A large Japanese study of patients with AML de novo and t-AML found that the *NQO1* polymorphism was more strongly associated with t-AML than polymorphisms in *GSTM1*, *GSTT1,* and *CYP3A4*.[50] Furthermore, patients carrying the *NQO1–187Ser* allele who had been exposed to chemotherapy had significantly shorter telomeres in their neutrophils and lymphocytes and were more likely to develop clonal hematopoiesis than patients with wild-type *NQO1* alleles.[51] These findings provide a molecular link between *NQO1* genotype and an increased risk of developing t-AML.

Guillem et al identified a haplotype in *MTHFR*, the gene encoding methylene tetrahydrofolate reductase, which conferred an increased risk in particular patient populations.[52] This enzyme regulates cellular folate metabolism, and therefore, is critical to the action of methotrexate and other chemotherapy drugs that are dependent on folate pools. Two SNPs were included in the haplotype: 677C/T and 1298A/C. An increased risk of developing t-AML was associated with the 677T/1298A haplotype in breast cancer patients and the 677C/1298C haplotype in patients with a primary hematopoietic malignancy.

Several groups have examined the genes encoding components of DNA repair pathways, like *hMSH2* and *hMLH1*. Patients with t-AML who have been previously treated with O(6)-guanine alkylating agents, such as cyclophosphamide and procarbazine, have an increased frequency of a variant C SNP that occurs within an intron splice acceptor of the *hMSH2* gene.[53] A variant SNP at position –93 of the *hMLH1* promoter was found in 75% of patients with t-AML who had received methylating chemotherapy as part of prior therapy for Hodgkin disease.[54] In contrast, this variant SNP was found in only 30% of patients with t-AML without prior exposure to methylating agents. In patients who had been treated with a methylating agent, the presence of the variant –93 SNP conferred a significantly increased risk of developing t-AML, with an odds ratio of 5.3.

Because de novo AML is so heterogeneous, a systematic review of these predisposing factors is usually not undertaken. However, certain inherited susceptibilities predispose to AML, and a careful family history may suggest one of these in rare cases.[55] Inherited *RUNX1* mutations, found in familial platelet disorder (OMIM 601399), may be discovered through the identification of unaffected family members with mildly lowered platelet counts.[56,57] Children with neurofibromatosis type 1 who have germline *NF1* mutations that result in altered RAS signaling are at an increased risk of developing a myeloproliferative neoplasm or t-MN.[58] In addition, patients with Fanconi anemia, who have mutations in one of 11 genes that encode proteins involved in DNA damage and repair, are at increased risk of developing t-MN.[59] Li-Fraumeni syndrome involves inherited *TP53* mutations and predisposes to breast cancers, brain tumors, osteosarcomas, and leukemias, including an increased risk for developing t-AML.[60] Furthermore, constitutional genetic variations in the p53 pathway affect t-AML risk.[61] The *MDM2* SNP309 polymorphism is located within a binding site for the SP1 transcription factor in the *MDM2* core promoter, and SP1 binds more effectively to the G allele compared to the T allele,[62–64] resulting in more efficient transcription of *MDM2* and consequently lower basal levels of *TP53*. The *TP53* Arg72Pro polymorphism alters the ability of the TP53 protein to induce apoptosis versus cell-cycle arrest.[65–67] Although neither polymorphism alone influenced risk of t-AML, an interactive effect was detected such that *MDM2* TT *TP53* Arg/Arg

double homozygote variants, and individuals carrying both a *MDM2* G allele and a *TP53* Pro allele are at increased risk of t-AML (*P* for interaction = .009). In addition, the risk of developing t-AML was 2.7-fold higher in *TP53* Pro/Pro homozygotes who received radiotherapy compared to *TP53* Arg/Arg homozygotes (*P* = .04). These data indicate that the *MDM2* and *TP53* variants interact to modulate responses to genotoxic therapy and are determinants of risk in t-AML.

Genome-Wide Association Studies

Genome-wide association studies (GWAS) are currently being used to assess several aspects of host biology.[68] For example, GWAS have been performed to identify inherited sequence variants that predispose to disease. Again, these approaches have been applied early on to the t-MNs, since these patients have already demonstrated a predilection for cancer development.

In the first such GWAS to identify inherited sequence variants that predispose to the development of t-MNs, Knight et al used multiple patient cohorts to identify multiple genetic loci that are linked with disease.[69] In the future, patients may be screened for the presence of these alleles and counseled about their risk of developing t-MN prior to receiving chemotherapy for their first diagnosis of cancer. Further, physicians may, in the future, avoid the use of particular chemotherapy agents or regimens depending on the genotype of the patient and if suitable alternative therapies are available.

GENETIC VARIANTS AND INDIVIDUAL DRUG SENSITIVITY

Both candidate gene approaches and GWAS have been used to determine genetic variants in particular genes and/or genetic loci that may be related to the variability seen in drug sensitivity among individuals. As an example, we will review the studies relating to cytarabine and daunorubicin, the drugs typically used for AML induction chemotherapy.

The Dolan group has harnessed the power of genetics by studying cytotoxicity within lymphoblastoid cell lines derived from members of family pedigrees to determine that 29% of the variation in daunorubicin sensitivity in vitro is due to genetic factors.[70,71] Furthermore, expression of *CYP1B1*, specifically, and the presence of particular SNPs correlated with cellular sensitivity to daunorubici.[71] Naoe and colleagues have shown that polymorphisms within *GSTT1* correlate with progression-free survival and overall survival for AML patients treated with daunorubicin-containing chemotherapy regimens.[72]

Hartford et al have used a GWAS to identify population-specific genetic variants that correlate with in vitro sensitivity to cytarabine.[73] In this work, multiple SNPs were identified that correlated with altered gene expression of potential target genes. Among the identified SNPs, four of them accounted for about half of the variability seen in susceptibility to cytarabine. Interestingly, the most "usual" suspects, ie, SNPs that are likely to regulate genes in the cytarabine metabolic pathway (eg, *DCK, CDA, NT5C2, DCTD, RRM1*, and *RRM2*), were not significantly associated with cytarabine sensitivity at the genome-wide level. However, SNPs that affect *DCK* expression and activity were identified to be associated with cellular sensitivity to cytarabine at a less stringent *P* value.[74]

THE NEED FOR ANALYSIS OF CONSTITUTIONAL SAMPLES

As we embark on more genomic analysis of patient samples, the need for analysis of constitutional DNA becomes critical to distinguish somatic from inherited mutations. Techniques such as transcriptome sequencing, exon capture approaches, and array-based resequencing schemes will all identify single–base pair variants and CNVs, some not detectable by other methods. Because de novo AML and t-MN can involve somatic mutations of the same genes that cause inherited leukemia predisposition (eg, *TP53*, *RUNX1*, and *CEBPA*), sequencing of constitutional DNA becomes essential to be able to distinguish inherited from somatic mutations.

A VISION OF THE FUTURE FOR LEUKEMIA ASSESSMENT

One can envision a new approach to the characterization of an individual's AML in which initial classification is performed using a combination of traditional methods, like hematopathologic review and cytogenetic and molecular methods, complemented by next-generation DNA sequencing techniques and genome-wide microarray-based testing (Figure 2). Disease classification, in the future, may depend on a full characterization of gene and miRNA expression, DNA methylation patterns, chromosomal rearrangements, and predisposition to disease and chemosensitivity. The diagnosis, characterization of the initial disease, development of a treatment plan, and subsequent patient follow-up are likely to involve an iterative process, one in which genetic and genomic analyses are used at each stage of treatment, with the resulting information informing the next stage of therapy.[75] Multiple assays will probably be used simultaneously along with oncoinformatic analyses to give an integrated description of the genetic and epigenetic alterations that are present at initial presentation and subsequent stages. It is unlikely that whole genome/transcriptome sequencing will be used routinely after therapy or when patients are in remission, since the burden of disease will be lowest

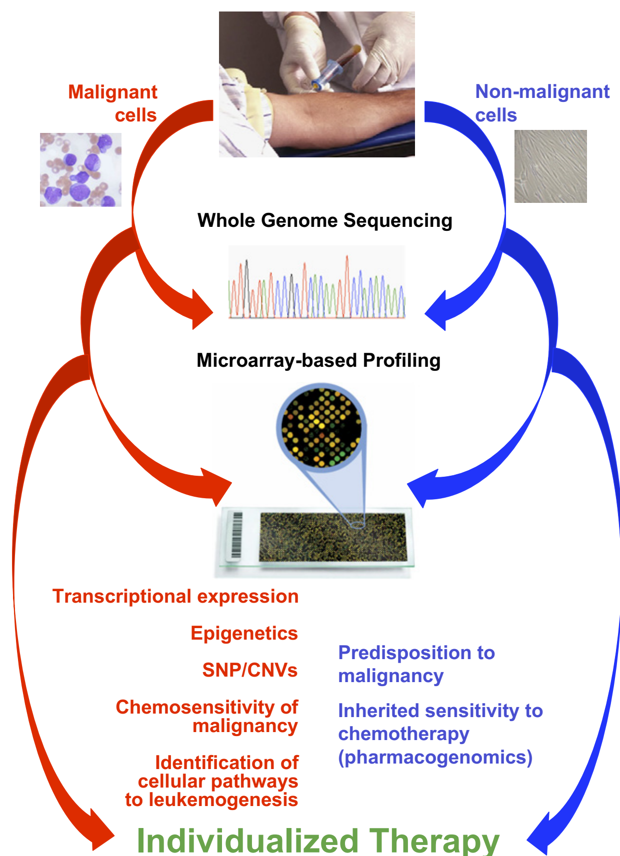

Figure 2. A vision of potential future automated leukemia analysis. Initial samples of malignant leukemia cells will be collected by venipuncture, bone marrow biopsy, or leukapheresis. Nonmalignant cells will be collected from oral washes or biopsies of the skin or bone marrow. DNA and RNA will be isolated from these cells and will be subjected to an array of approaches to characterize the leukemia, as well as to define the constitutional make-up of the patient. Whole genome or transcriptome sequencing will define the genetic subtype of leukemia and the presence of chromosomal or genetic changes. Microarray profiling will enable characterization based on transcriptional expression, distribution of epigenetic modifications, copy number variations, and an initial assessment of chemosensitivity. These analyses also may identify pathways that contribute to leukemogenesis. Analysis of nonmalignant cells will determine genetic predisposition to disease and chemosensitivity. Combined, these approaches will lead to optimized, individualized treatment plans for patients, which will result in decreased toxicity and increased survival.

then, and as performed today, this technique is best for defining the majority cell population. Molecular assays to detect specific mutations and/or FISH analysis will likely be used to detect specific mutations identified in the original leukemic clone. In the future, these analyses may be automated so that these data are available within days/weeks of identifying a new AML patient, conferring the ability to customize therapy based on individual molecular parameters.

Some of the techniques described above provide overlapping information. For example, information about gene expression is obtained both from microarray-based gene expression profiling, as well as from transcriptome sequencing. Whether both techniques will be used in the future, or whether one approach will predominate in the clinical setting may be influenced by the bioinformatic tools and specialized equipment and training needed to perform the analyses. The widespread adaptation of these methods will also require that companies produce and market machines and software tools needed for the assays. At present, neither gene expression profiling nor transcriptome sequencing is a routine analytical diagnostic tool. Whether this reflects the fact that we have not yet developed the informatics tools to classify patients completely, or whether this is related to practical aspects of availability, cost, and the expertise needed to perform the assays, is not clear. Physicians also may not be motivated yet to incorporate the vast amount of genetic data into their treatment programs until the field at large demonstrates that the information gained provides prognostic/treatment data beyond that currently obtained by cytogenetic and/or molecular analysis.

The actual adaptation of moving from applying genomic-based methods to achieve more sophisticated characterization of leukemic cells to using such analyses in clinical decision-making is challenging. These genomic approaches will have to be combined with advances in drug development,[75] so that when a profile is obtained that suggests a poor prognosis and relative resistance to cytarabine, physicians have effective alternative chemotherapy regimens to offer a patient. The mere characterization of disease will not improve patient survival unless we develop an array of efficacious treatment plans, ones aimed at killing cells with various growth characteristics.

One promise of the use of these high-throughput approaches and sophisticated informatics platforms is that they can be used, not just for clinical assessment of individuals, but also to define new cellular and molecular pathways that lead to leukemogenesis, identify new therapeutic targets, and discover new biomarkers that can be used to monitor treatment response.[75] Personalized medicine based on a global assessment of the diseased cells and the patient's genetic make-up is feasible and will hopefully bring with it advances in patient survival as treatments are geared to individuals.

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