



Acute Myeloid Leukemia: The Challenge of Capturing Disease Variety

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The difference between success and failure of treatment of acute myeloid leukemia (AML) is largely determined by genotypic leukemia-specific differences among patients. The diversity of AML genotypes result from somatic genetic alterations settling down in succession in an individual's leukemia clone during the development of the disease. Gene mutations, gene expression abnormalities and other molecular alterations (e.g., microRNA variations) affect critical functions in AML cells, and may exert profound effects on the therapeutic response and outcome of the disease. Prototypes of common

Fifty years ago when the American Society of Hematology was founded, the diagnosis of acute myeloid leukemia (AML) was based solely on microscopic evaluation. There was no generally accepted treatment. In those days three classes of experimental agents were being introduced into treatment according a new concept of combination chemotherapy: corticosteroids, folic acid antagonists (methotrexate) and purine antagonists (6-mercaptopurine). Patients showing a response to treatment had somewhat better survival than nonresponders but there were no long-term survivors.^{1,2} Since then more active antileukemic agents have been introduced into modern combination chemotherapy and brought cure to a significant fraction of patients. Initially AML was considered one monolithic disease for which “one size fits all” chemotherapy was applied. This is no longer true. AML is a genetically pleiomorphic disease. In 1958 the same cytotoxic treatment was applied to what was morphologically considered to be AML or acute lymphoblastic leukemia (ALL). In 2008 therapeutic and diagnostic developments have shifted the paradigm of AML therapeutics and created a perspective of personalized therapeutics. This review will highlight recent developments in AML that impact on diagnosis, prognosis assessment and treatment choice (Table 1).

Diagnosis and Prognosis of AML: Cytogenetic Analysis and Gene Mutations

Cytogenetic analysis has become part of the standard diagnostic approach of a patient suspected of AML. This allows the identification of (cyto)genetic entities that deserve targeted treatments (examples: acute promyelocytic leukemia and BCR-ABL–positive leukemia). It also allows the dis-

clinically significant gene aberrations involve transcription factors, signaling molecules and growth factor receptors. The expanding knowledge in this area allowing for risk stratified therapy decisions and the development of targeted drug therapy, is becoming an increasingly important part of the modern individualized clinical management of AML. This chapter highlights recent insights into the diagnostic, prognostic and therapeutic impact of chromosomal (e.g., the so-called monosomal karyotype) as well as particular genomic abnormalities, and presents examples of decision algorithms for individualized therapy.

inction of specific subtypes of disease with widely different prognosis (illustrating example: AML t(8;21)(q22;q22) with favorable risk versus AML abn3q26 with adverse risk) that deserve risk-guided treatment strategies. Numerous genetic abnormalities that escape cytogenetic detection (e.g., gene mutations, gene expression abnormalities) have more recently been discovered. These have allowed the further dissection of AML into molecular subtypes with distinctive prognosis (Table 2). Clinically favorable genotypes of AML, for example, involve mutations in the genes of the transcription factor *CEBPA* (CCAAT enhancer binding factor alpha)³⁻⁵ or *nucleophosmin-1* (*NPM1*),⁶⁻¹³ whereas unfavorable genotypes may include those with partial tandem duplications of the *MLL* gene (*MLL*-PTD),¹⁴⁻¹⁷ internal tandem duplications of the gene of *fms-like tyrosine kinase 3* (*FLT3*-ITDs)¹⁸⁻²⁴ and mutations in *Wilms' tumor 1* gene (*WT1*).^{25,26} These findings emerge from a field of intense scientific activity. There is little question that more genetic markers with clinical value will be discovered. These genetic events perturb diverse cellular pathways and functions, and they often confer a profound impact upon the clinical phenotype of the disease and treatment response.

Gene Expression Markers: EVI1-Positive Leukemia, One of the Most Aggressive Subtypes of AML

Besides gene mutations aberrant levels of expression of particular genes may also be informative as regards prognosis. Examples of aberrant gene expression with adverse prognostic significance include the overexpression of *Ecotropic Viral Integration-1* (*EVI-1*),^{27,28} *Brain and Leu-*

Table 1. Glossary of abbreviations.

BAALC – the *Brain and Leukemia Cytoplasmic Gene* that may be overexpressed in AML. Function of the gene is unknown. Overexpression of the transcript in leukemic cells has been shown to correlate with unfavorable prognosis.

CEBPA (CCAAT enhancer binding factor alpha) – the gene encodes for a transcription factor that is indispensable for granulopoiesis; gene mutations are seen in approximately 5% to 8% of AMLs and are associated with a relatively favorable prognosis.

CXCR4 – is a G-protein coupled receptor that is involved in chemotaxis, adhesion and migration of a variety of hematopoietic cell types, myeloid and lymphoid. The chemokine SDF1 is the ligand of the CXCR4 receptor.

ERG (Ets-related gene) – a member of the ETS family of transcription factors. Overexpression of the ERG transcript in AML blasts has been associated with an unfavorable prognosis.

EVI1 (ecotropic viral integration-1) – initially discovered as an oncogene causing myeloid leukemia in mice (following retroviral insertion mutagenesis). The gene is deregulated in clinical AML in cases of 3q26 chromosome abnormalities. The *EVI1* gene may also be upregulated in 5% to 10% of AMLs without 3q26 chromosomal translocations. There are 5 splice variants of EVI-1 transcripts, of which EVI1-D is most common. AML with cytogenetic 3q26 abnormalities and/or EVI1 overexpression have an unfavorable prognosis.

FLT3 (fms-like tyrosine kinase 3) – this gene encodes for a tyrosine kinase hematopoietic receptor. Two sorts of mutations have been described. Internal tandem duplications (**FLT3-ITDs**) and tyrosine kinase domain mutations (**FLT3-TKD**), both leading to constitutive activation of the receptor. *FLT3*-ITDs are common in AML (about 25% of cases) and predict a comparatively unfavorable outcome. *FLT3*-ITDs often are apparent in AMLs that also carry mutations of *NPM1*. The prognostic significance of *FLT3*-TKD (apparent in approximately 8% of AMLs) for the time being is less clear.

GEP (gene expression profiling) – a method that allows for the measurement of the expression levels of thousands of gene transcripts on a hybridization array that contains a great variety of cDNAs or oligonucleotide probes.

KIT – the tyrosine kinase hematopoietic receptor for stem cell factor (SCT or KIT ligand). Mutations in the gene are specially seen in core-binding factor AMLs, i.e., AMLs with translocations t(8;21) and inv(16)/t(16;16). AMLs with *KIT* gene mutations have been suggested to carry a somewhat inferior prognosis.

MDS/EVI1 – in addition to EVI1, AML cells may express MDS1/EVI1 (ME). MDS/EVI1 is an EVI1 fusion variant generated through intergenic splicing with MDS1, a gene with a currently unknown function that is located ~140 kb upstream of EVI1. A recent study has addressed the prevalence and the prognostic value of each of the splice variants of EVI1 as well as those of the intergenic splice form *MDS1/EVI1 (ME)*.

MicroRNA (miRNA) – small noncoding RNAs of approximately 19-25 nucleotides that play a role in transcriptional or translational regulation of genes involved in a variety of biological processes, including myelopoiesis and leukemogenesis.

MLL-PTD – partial tandem duplications of the *Mixed-Lineage Leukemia* gene (*MLL*-PTD) involve duplications of a genomic region of the gene and are seen in 5% to 10% of normal karyotype AML. *MLL*-PTDs are associated with unfavorable prognosis. *MLL* is also involved in a variety of gene fusions/chromosomal translocations.

MN1 (meningioma-1) – a gene originally reported as a candidate gene for sporadic meningioma encodes for a transcriptional coactivator. *MN1* is involved in the translocation (12;22)(p13;q11) that creates a fusion between the *MN1* and *TEL* (or *ETV6*) genes. Overexpression of the *MN1* transcript in AML blasts has been suggested to predict unfavorable prognosis.

NOTCH1 – a gene encoding a single-pass, heterodimeric receptor that is essential in T-cell development. *NOTCH1* mutations are commonly seen in T-ALLs; they activate signal transduction.

NPM1 (Nucleophosmin-1) – a gene that encodes for a nuclear protein with multiple functions. Mutations in *NPM1* are seen in approximately one-third of AMLs and are associated with a comparatively favorable prognosis. *NPM1* mutations are often seen along with *FLT3*-mutations in the same AMLs.

SDF1 α (stroma-derived factor 1 α) – a chemokine produced by marrow stroma. It is the stimulating ligand for the CXCR4 receptor and is involved in diverse cellular functions including cell trafficking and homing.

VEGF (vascular endothelial growth factor) – one of the endogenous regulatory peptides involved in stimulation of angiogenesis. AML cells may produce VEGF and may carry receptors for the cognate receptors (VEGF-R), thus establishing an autocrine loop of stimulation.

WT1 (Wilms' Tumor 1) – this gene was originally reported as a suppressor gene responsible for Wilms tumor, a childhood kidney neoplasm. *WT1* mutations have recently been reported in AML and have been suggested to predict unfavorable outcome.

Table 2. Examples of molecular markers additional to cytogenetics with independent prognostic significance as regards remission duration or survival in acute myeloid leukemia (AML) of adults.

	Gene
Gene mutations with favorable impact	<i>CEBPA</i> ^{mut} <i>NMP1</i> ^{mut} but <i>FLT3-ITD</i> ^{neg}
Gene mutations with unfavorable impact	<i>KIT</i> [*] <i>FLT3-ITD</i> but no <i>NMP1</i> ^{mut} <i>MLL-PTD</i> ^{**} <i>WT-1</i> ^{**}
mRNA overexpression with unfavorable prognostic impact	<i>BAALC</i> ^{**} <i>ERG</i> ^{**} <i>MN1</i> ^{**} <i>EVI-1</i>

* among core-binding factor AMLs

** among AML with normal cytogenetics

FLT3 internal tandem duplications (*FLT3-ITD*) and mutations in *nucleophosmin 1* (*NMP1*^{mut}) are often seen in combination in the same leukemia.

kemia Cytoplasmic Gene (BAALC),^{29,30} *meningioma-1* gene (*MN1*)³¹ and *Ets-related gene (ERG)*^{32,33} (Table 2).

EVI-1-positive AML represents a genotype with a particularly adverse prognosis as has been demonstrated by Delwel and coworkers.^{27,28} For quite some time the AML subtype with 3q26 chromosomal lesions has been recognized as belonging to the most aggressive forms of human AML.^{27,34} The oncogene ecotropic viral integration site-1 (*EVI-1*) is involved in the cytogenetic translocations of 3q26.^{27,35} AML with *abn3q26* represent only about 1% of AML.³⁴ However, there are another 5% to 10% of AML that overexpress the oncogene *EVI-1* (*EVI-1* positive) in the absence of the *abn3q26* anomaly.²⁷ In the *EVI-1*-positive subset CR rates are low, relapse rates are high and survival is low. Besides the common *EVI1-1D* splice form, four other splice variants of *EVI1* were recently identified: *EVI1-1A*, *-1B*, *-1C*, and *-3L5*, which mainly differ in their 5' untranslated regions. In addition, AML cells may also express *MDS1/EVI1* (*ME*), an *EVI1* fusion variant generated through intergenic splicing with *MDS1*, a gene with a currently unknown function that is located ~140 kb upstream of *EVI1*.²⁸ A recent study has addressed the prevalence and the prognostic value of each of the splice variants of *EVI1* as well as those of the intergenic splice form *MDS1/EVI1* (*ME*) by quantitative RT-PCR.²⁸ Among 534 AML patients 41 *EVI1*⁺ cases were identified. High *EVI1* expression levels predicted a significantly reduced event-free survival (EFS) (3% vs 29%) and overall survival (OS) (13% vs 39% at 5 years) ($P < .001$). Multivariate analysis revealed that high *EVI1* levels are an independent predictor of inferior EFS (HR = 1.9, $P = .002$) and DFS (HR = 2.1, $P = .006$). Seventeen of the *EVI1*⁺ leukemias were *ME*-negative, i.e., *EVI-1*^{pos}/*ME*^{neg}. Several of the latter *EVI-1*^{pos}/*ME*^{neg} AMLs

with a dissociated high *EVI1* expression but low *MDS1/EVI1* expression had cryptic cytogenetic 3q26 aberrations.²⁸ These *abn3q26* abnormalities apparently may quite easily escape cytogenetic identification but they can be properly documented by FISH. The AML with the *EVI-1*^{pos}/*ME*^{neg} genotype represent one of the most unfavorable forms of AML. For the time being it remains elusive why AML with high *EVI-1* expression represent such a bad prognostic subtype of AML that does not respond to currently available treatments. Exploring the mechanisms of transformation and treatment resistance by the *EVI1* protein remains a major challenge of current research. *EVI1* encodes a nuclear protein, which interacts with several proteins important in transcriptional control, e.g., *CtBp1*, *HDAC*, *SMAD3*, *P/CAF* and *GATA1*. Investigations into how these interactions take place may provide the necessary insights for developing tools to therapeutically target *EVI1*⁺ leukemia cells.

Monosomal Karyotype: Better Predictor of Adverse Prognosis than Complex Karyotype

Particular cytogenetic abnormalities have been implicated to confer a poor prognosis in AML. These include -5, -7, del(5q), del(7q), *abn3q*, *abn11q23*, *abn17p*, t(6;9) t(9;22). However these abnormalities not infrequently are seen together or in association with other abnormalities. The negative prognostic value of coexisting multiple clonal cytogenetic abnormalities, often referred to as "complex karyotypes," is widely accepted. Commonly applied definitions of AML with complex cytogenetics are based on the summing up score of any type of abnormality. In a recent study in adults up to 60 years of age,³⁶ the value of each of the different sorts of cytogenetic abnormalities as determinants of poor prognosis has been assessed, such as loss of one or more chromosomes, extra copy of one or more chromosomes and structural cytogenetic abnormalities. The presence of autosomal chromosomal monosomies strongly predicted for an adverse prognosis. While the negative prognostic impact of autosomal monosomies in AML had been described for monosomies of chromosomes 5 and 7, it was demonstrated in multiple comparisons that there is nothing special about loss of chromosomes -5 or -7 as regards prognostic significance. Any type of autosomal monosomy in AML is associated, in fact, with a dismal outcome.³⁶ On the other hand, in direct comparisons extra copies of 1 or more chromosomes did not appear to exert an additive negative effect on prognosis in AML. Trisomies, tetrasomies or the presence of ring or marker chromosomes and structural chromosomal aberrations appeared to contribute minimal prognostic impact when they were considered in relationship to monosomal abnormalities. The negative prognostic influence of multiple, i.e., 2 or more, autosomal monosomies or 1 autosomal monosomy in combination with at least 1 structural chromosomal abnormality in AML was profound (4-year OS: 4%). These findings have led to the

proposal of the novel monosomal karyotype index for prognostically highly unfavorable AML with multiple cytogenetic abnormalities.³⁶ The “monosomal karyotype” index defines AML with a highly adverse prognosis as a karyotype with at least two autosomal monosomies or one single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities. In a head-to-head comparison with the traditional complex karyotype, the simplified “monosomal karyotype” index is significantly superior. This is true in two ways: On one hand, AMLs with a complex karyotype but not fulfilling the criteria of monosomal karyotype have a better prognosis that is similar to that of cytogenetically abnormal AML in general. On the other hand, AMLs without a complex karyotype but with a monosomal karyotype have an extremely poor outcome. The better predictability of very unfavorable risk AML by the monosomal karyotype in comparison to “complex karyotypes” holds up regardless whether complexity is defined by ≥ 3 or ≥ 5 clonal cytogenetic abnormalities.³⁶

In patients 60 years old and younger the monosomal karyotype enables (in addition to normal cytogenetics and core binding factor leukemias) the prognostic classification of two new aggregates of cytogenetically abnormal AML: abnormal cytogenetics (4-year OS: 26% \pm 2) and the highly adverse risk monosomal karyotype categories (4-year OS: 4% \pm 1).³⁶

Genome-wide Methods, New Genotypes, New Markers

The great variation of genomic aberrations in AML have opened a search for genome wide analysis that provides a snap shot of the complex genetic signature of a particular leukemia. Gene expression profiling (GEP), microRNA analysis, methylation profiling and whole genome sequence analysis offer powerful technologies that create opportunities for characterizing an individual’s leukemia in a genome-wide manner in diverse terms (gene expression, microRNA expression, gene mutations and epigenetics).

Gene expression profiling

GEP is a technology in which DNA microarrays containing cDNAs or oligonucleotide probes are used to simultaneously measure levels of thousands of mRNA transcripts. A straightforward way of using GEP is to compare expression profiles among cases of AML and to examine similarities and differences. Thus, particular cytogenetic AML subtypes—e.g., AMLs with t(8;21), t(15;17) and inv(16)—can each be recognized by highly distinctive GEP profiles among a mixed series of AML.³⁷⁻⁴³ Likewise, AML genotypes with mutations in *CCAAT/enhancer binding protein alpha* (*CEBPA*), and mutations in *nucleophosmin 1* (*NPM1*) show distinctive gene expression signatures.^{6,42} The apparent fact that the genetic abnormality places such a prominent stamp on the transcriptome of the leukemia has im-

portant implications. It implies that a particular expression profile that becomes apparent following gene expression profiling may reflect an underlying genetic abnormality for that particular subset of AML. Thus, a notable gene expression pattern may offer the key to the disclosure of new genotypes. Evidence indicating that a distinctive gene expression AML subtype may guide the way to the discovery of biologically and clinically relevant genotypes is highlighted by the example of the recent identification of a unique form of AML characterized by epigenetic *CEBPA* silencing and an immature myeloid/T-lymphoid biphenotype.⁴⁴ In fact, these acute leukemias with silenced *CEBPA* were found to carry expression profiles similar to AML cases with *CEBPA* mutations, but such mutations were not present. Subsequent experiments elucidated that in the novel subgroup *CEBPA* expression was silenced, often through promoter hypermethylation. Mouse modeling subsequently demonstrated that knock down of *CEBPA* expression evokes the expression of T-cell genes in immature hematopoietic progenitor cells, thus furnishing a mechanistic insight into why these leukemias carry both myeloid and T-lymphoid features. In this example, the analysis of human AML GEP data in combination with pathway analysis and the use of informative mouse models uncovered the pathobiology of a subgroup of leukemia that is defined by a myeloid/T-lymphoid phenotype, *CEBPA* silencing and, in fact, also frequent *NOTCH1* mutations.⁴⁴

Touw and colleagues have generated a series of myeloid leukemias in mice through retroviral insertion mutagenesis to search for critical suppressor genes involved in leukemogenesis. A direct comparison between the murine integration sites with a clinical GEP AML data set revealed that indeed mouse leukemia genes are frequently deregulated in human AML.⁴⁵ The comparisons of human AML GEP data with high-throughput murine data from dedicated experimental mouse models provide opportunities for identifying candidate suppressor genes (or oncogenes) of AML. Techniques such as chromatin immunoprecipitation on DNA microarray chips (ChIP-chip), ChIP-sequencing and RNA interference libraries may also be used in combination with the analysis of gene expression profiling and uncover target genes.

MicroRNA profiling

MicroRNAs are small non-coding RNAs of 19-25 nucleotides that play a role in transcriptional or post-transcriptional regulation of genes involved in numerous biological processes, including differentiation and proliferation. Three recent studies have found characteristic microRNA expression profiles in cytogenetically and molecularly defined subtypes of AML.⁴⁶⁻⁴⁸ It has been demonstrated that AML can be classified according their variable and distinctive microRNA expression profiles.⁴⁸ Analysis (significance analysis of microarrays [SAM]) revealed individual

differentially expressed microRNAs that characterize subtypes of AML, e.g., AML with t(8;21), inv(16), t(15;17) and AML with *NPM1* and *CEBPA* mutations. Subsequently these subtypes of AML can be defined and classified according limited sets of selected microRNAs.

For instance, in the AML genotype with *NPM1*-mutations (*NPM1*^{mut}) which is present in approximately 35% of patients with AML, the microRNAs -10a, -10b, -196a, -196b are highly expressed.⁴⁸ AML with *NPM1* mutations are not only characterized by typical microRNA patterns but also by specific mRNA gene expression signatures as had previously been shown.^{6,49} The mRNA signatures are characterized by overexpression of homeobox genes (*HOXA*, *HOXB* families and *TALE* genes). Interestingly, the microRNAs -10a, -10b, -196a, -196b that are upregulated in AML with mutant *NPM1*, are all located within the genomic cluster of *HOX* genes. Thus, these observations appear consistent with an aberrant regulatory network involving *NPM1*, *HOX* genes and microRNAs, which might be engaged in the arrest of cellular differentiation of hematopoietic progenitors and development of AML with mutant *NPM1*.

Marcucci et al⁵⁰ have reported data to suggest that microRNA profiling can have prognostic value in cytogenetically normal AML. The investigators derived a set of 12 microRNAs that can distinguish two subgroups of cytogenetically normal AML with EFS probabilities of 11% (bad risk) and 36% (intermediate risk). The profile was developed for the subset of AML with the genotypes *FLT3*-ITD^{pos} and *FLT3*-ITD^{neg}/*NPM1*^{wt}, which account for two-thirds of all cases of cytogenetically normal AML. The microRNA profile appeared independent of the prognostic effect of *FLT3*-ITD abnormalities. The survival estimates in this study, which were based on relatively small numbers of cases, require confirmation. These recent studies underscore the importance of microRNAs in the pathogenesis of AML and their potential usefulness in future diagnostics.

Single nucleotide polymorphism arrays and array-based comparative genomic hybridization

Changes in expression levels of critical genes may be due to small DNA amplifications or deletions undetected by conventional cytogenetics. Small chromosomal aberrations can be examined with single nucleotide polymorphism (SNP) arrays and array-based comparative genomic hybridization (CGH). A study of 60 patients with AML with complex karyotypes using array-CGH (bacterial artificial and P1-derived artificial chromosome clones) identified several recurrent lesions.⁵¹ It is not yet clear what the overall frequency and distribution of these genomic alterations in AML is. Copy-number neutral loss of heterozygosity through segmental uniparental disomy in AML is a relatively common and notable finding in AML.⁵²⁻⁵⁴ This phenomenon can point to the identification of homozygous mutations or deletions of leukemia-related genes, including *FLT3* and *CEBPA*.^{54,55}

Other global genomic techniques

Gene expression changes may be caused by aberrant methylation or mutations in yet-unknown genes. Whole genome sequencing is emerging as a method to address this issue on a global scale.⁵⁶ A challenge in this regard will be distinguishing functionally relevant mutations from the abundant so-called passenger mutations—unimportant genetic changes caused by genomic instability of cancer cells—that will be picked up at the same time. Studies on tyrosine kinase abnormalities have underscored the need for validation of the biological effects of novel mutations identified by high-throughput nucleotide sequencing.^{57,58}

Post Remission Therapy According to AML Genotype

After a remission has been attained the next therapeutic objective is to eradicate any residual leukemia (minimal residual disease) by applying additional chemotherapy, autologous stem cell transplantation or allogeneic stem cell transplantation. Allogeneic stem cell transplantation is the most effective antileukemic modality. *But an allogeneic stem cell transplantation for whom?* The reason to turn to allogeneic transplantation is that the immune-mediated graft-versus-leukemia effect of the transplanted cells reduces the risk of relapse considerably and improves relapse-free survival. The alloSCT advantage obviously has to be cautiously balanced against the increased risk of death and morbidity that is typically connected with alloSCT. Excess mortality (ranging between 10% and 40%) and morbidity due to transplant-related complications, such as infection and graft-versus-host disease, can abrogate all of the benefit of a reduced risk of relapse.⁵⁹ For this reason, allogeneic stem cell transplantation is usually avoided in a type of AML that has a pattern of cytogenetics with a relatively favorable prognosis, such as AML with the chromosomal translocations t(8;21) or inv(16)/t(16;16).^{59,60} In the latter subtypes the risk of relapse is in the order of 35% to 40% or less. In a recent study two low-risk genotypically defined subsets within the large category of cytogenetically normal AML were investigated,⁶⁰ each with a risk of relapse of about 35%. The first genotype was defined by the presence of ‘favorable’ mutations in *NPM1* and the absence of concurrent ‘unfavorable’ *FLT3*-internal tandem duplications (*NPM1*^{mut}/*FLT3*-ITD^{neg}).⁶¹ This genotype accounts for approximately 16% of all newly diagnosed patients younger than 60 years old. There was no demonstrable benefit from transplantation in patients with *NPM1*^{mut}/*FLT3*-ITD^{neg} AML. The second subset of AML with ‘favorable’ mutations in the transcription factor gene *CEBPA* (*CEBPA*^{mut}) could not be analyzed in this way because of a lack of statistical power due to a limited number of cases. The latter low-risk subtype *CEBPA*^{mut} accounts for 8% of all AML. Nevertheless, the available body of evidence suggests that these AMLs are unlikely to profit from

an alloSCT. Thus, by and large patients with AML with t(8;21), AML with inv(16)/t(16;16), AML with *NPM1*^{mut}/*FLT3*-ITD^{neg} and AML with *CEBPA*^{mut} are not considered for alloSCT.^{59,61} By contrast, a transplant would be more attractive in a patient whose leukemia cells bear a cytogenetic or molecular abnormality that predicts a high risk of relapse after chemotherapy.⁵⁹ Obviously, in the near future these results will also need to be considered more specifically in the light of the extended scale of allogeneic stem cell transplantation strategies with respect to reduced-intensity conditioning regimens and in relationship to different transplant sources and donor types (matched unrelated and haploidentical donors, umbilical stem cell grafts).⁶²

A Composite Prognostic Index for AML (HOVON/SAKK): Considering Genetic and Clinical Factors

As discussed above, cytogenetics as well as various genomic markers (gene mutations, gene overexpression) may provide input for algorithms for remission induction and post-remission treatment decisions. At the same time it remains appropriate to realize that prognostic factors in fact remain a moving target and they are only relevant to therapies available at a given time. Algorithms that provide a basis for risk-adapted therapeutic choices may include cytogenetic factors, molecular markers as well as clinical parameters (e.g., age, attainment of an early or late complete remission) and hematological determinants (e.g., secondary AML, white blood cell count at diagnosis). The Dutch-Belgian Leukemia Cooperative Group (HOVON) and the Swiss Leukemia Cooperative Group (SAKK) have recently introduced a prognostic stratification score for newly diagnosed AML in adults less than 60 years of age that distinguishes four prognostic categories with different outcomes. The risk classification has been derived from (a) an analysis of the data of 1975 patients from the previous HOVON/SAKK AML studies for patients up to 60 years of age, registered before January 1, 2004 and with successful cytogenetic analysis, and (b) an analysis of the data of a subset of 424 patients for whom marker information and microarray expression data were also available. The risk stratification system for prognosis is used as an algorithm for assigning patients in complete remission (CR) depending on their relapse risk to allogeneic stem cell transplantation (**Table 3**).

Specific Treatment per AML Genotype: An Individualized Approach?

As soon as the definitive diagnosis of AML has been established, the treatment begins with a remission induction phase aimed at establishing a CR. The anthracyclines (daunorubicin, idarubicin) and cytarabine have remained the cornerstones of modern remission induction therapy for adult AML since their introduction. They induce CR in an average of 70% to 80% of adults aged less than 60 years enrolled in clinical trials and on average in 50% of patients

older than 60. Continuous efforts are being made to improve the efficacy of remission induction treatment. Improved induction therapy should yield more and better quality CRs (i.e., CRs of longer duration). The responsiveness to therapy depends mainly on the genotype of the disease and age of the patient. Thus, for instance, leukemias with a monosomal karyotype or with *EVII* oncogene overexpression (see above) and patients of older age with AML respond very poorly to treatment. Is it possible to target particular genotypes of AML with specific drugs and advance the therapeutic possibilities for these subtypes?

Activating mutations in *KIT* (*KIT*^{mut}), the receptor of the hematopoietic growth factor SCF (stem cell factor) are predominantly seen in AML with core binding factor abnormalities (CBF) t8;21 and inv(16)/t(16;16). Some studies have suggested that AML with CBF and *KIT*^{mut} have a prognosis that is less than that of the other AMLs with CBF.⁶³⁻⁶⁵ The *KIT* gene encodes for a tyrosine kinase receptor that in case of the mutation is constitutively activated. Kinase inhibitors (e.g., dasatinib) that abrogate *KIT* signaling are currently in early clinical trial and await an evaluation as regards their possible therapeutic effect. Fms-like tyrosine kinase 3 or *FLT3* are membrane kinase receptors expressed on hematopoietic progenitor cells that are stimulated following ligand binding. Mutations may disrupt the function and lead to the constitutive activation of the receptor. The most common kinase receptor mutations in AML are internal tandem duplications in *fms-like tyrosine kinase 3* or *FLT3* (apparent in approximately 25% of AML), which express a negative effect on prognosis (**Table 2**). The other class of so-called *FLT3*-TKD mutations (i.e., point mutations in the tyrosine kinase domain of the receptor) seen in 8% of cases has controversial prognostic significance. Various small molecule *FLT3* kinase inhibitors or anti-*FLT3* blocking antibodies are in Phase I-III clinical trial. These include sorafenib, lestaurtinib and staurosporin and various others.⁶⁶⁻⁶⁹ These agents have produced responses in AML with *FLT3* gene mutations as well as in AML with wild-type *FLT3*. The *KIT* and *FLT3* mutations are generally held to have been acquired as late events during leukemogenesis. The question as to whether the *KIT* and *FLT3* inhibitors in combination with chemotherapy will add significant therapeutic benefit is currently addressed in various currently ongoing phase III trials. Although many of the new agents appear active in vitro and have generated much excitement, the clinical activity for the majority of them has as yet to be demonstrated.

Therapeutic Targeting of Chemoresistance

Mechanisms determining chemoresistance may not only reside in the leukemic cells themselves but may also depend on an interaction between the leukemia and the external environment. The microenvironment in which the AML cells seed and develop may influence the patho-

Table 3. HOVON/SAKK prognostic score for AML (less than 60 yrs of age) based on cytogenetics, molecular markers, clinical and hematological factors: relationship to treatment outcome. A summary of the CR rates, EFS and OS of the patients as assessed from diagnosis in HOVON/SAKK AML studies is shown for each of the risk (sub)groups. The table also gives the estimates for EFS and OS from consolidation which are relevant for post remission treatment decisions (EFS2, OS2). Those estimates give the values for 5-year OS and EFS measured from the start of consolidation treatment and which are restricted to patients who have reached a CR on protocol after cycle I or II.

Risk (% of cases*)	From diagnosis			From start of consolidation		
	CR1†, %	EFS§ at 5 y, %	OS‡, %	EFS2‡ at 5 y, %	OS2¶, %	
Good (30)						
	GR1	t(8;21), WBC ≤ 20	94 *	51	65	58
	GR2	inv(16)/t(16;16)	94	59	68	66
	GR3	no MK, <i>CEBPA</i> ^{mut}	93	44	68	50
	GR4	no MK, <i>FLT3ITD</i> ^{neg} / <i>NPM1</i> ^{mut} , CRe	84	48	61	59
			100 *	51	57	59
Intermediate (20)						
	IR1	t(8;21), WBC > 20	99 *	42	51	48
	IR2	CN -X -Y, WBC ≤ 100, CRe	87	32	46	35
			100 *	43	51	48
Poor (30)						
	PR1	CN -X -Y, WBC ≤ 100, not CRe	75 *	19	25	27
	PR2	CN -X -Y, WBC > 100	69 *	17	23	24
	PR3	CA, non CBF, no abn3q26, <i>EV11</i> -	74 *	23	27	32
			79	20	25	27
Very Poor (20)						
	VPR1	Monosomal karyotype (MK)	60	3	7	7
	VPR2	abn3q26	48	2	4	6
	VPR3	<i>EV11</i> ⁺	65	8	19	8
			79	10	17	10

*, % distribution of each risk subgroup of all patients at diagnosis

† % patients reaching a first CR after cycle I or cycle II

§ EFS, actuarial probability of event-free survival 5 years from diagnosis

‡ OS, actuarial probability of overall survival 5 years from diagnosis

‡EFS2, actuarial probability of event-free survival 5 years from start consolidation

¶ OS2, actuarial probability of overall survival 5 years from start consolidation

Abbreviations: CBF, the core-binding factor (CBF) leukemias involve AMLs with cytogenetic abnormality t(8;21)(q22;q22) or the *AML1-ETO* fusion gene and the cytogenetic abnormalities abnormality inv(16)(p13q22)t(16;16)(p13;q22) or the related fusion genes *CBFB-MYH11*; CN -X-Y, cytogenetically normal or only loss of X or Y chromosome as the sole cytogenetic abnormalities; CA, cytogenetically abnormal; CRe, attainment of early CR, i.e., after cycle I; *EV11*⁺, high *EV11* mRNA expression; *FLT3ITD*^{neg}/*NPM1*^{mut}, no *FLT3*-internal tandem duplication but *NPM1*-mutant positive; Fms-like tyrosine kinase receptor-3 internal tandem duplications (*FLT3*-ITD) and nucleophosmin-1 (*NPM1*) mutations often go together as dual genetic anomalies in the same AML

Note: in the available HOVON/SAKK analysis no significant prognostic effect was apparent among CBF leukemias as regards the *KIT*-mutated genotypes that are commonly seen in CBF AML.

biology and the treatment response of the disease. AML infiltrations are associated with increased angiogenesis, as is apparent from the increased microvessel density. Angiogenesis in AML may be promoted by various provascular factors, including vascular endothelial growth factor (VEGF), and basic fibroblast growth factor. VEGF may be produced by the AML blasts, and VEGF-receptors (VEGF-R) may be expressed by the AML cells themselves as well as by endothelial cells, thus enabling an autocrine and paracrine stimulatory circuit of vascular growth in leukemic areas (prosurvival effects). VEGF-C production by stromal cells, via interaction with VEGF receptor-3, has been shown to protect AML cells from chemotherapy-induced apoptosis.⁷⁰ Considering these data one might postulate that targeting VEGF or VEGF-R could offer a therapeutic target in AML.⁷¹ Interruption of these circuits, e.g.,

by antiVEGF-R antibodies or appropriate kinase blockers (peptides) is currently in study as a therapeutic strategy of overcoming leukemic chemoresistance.⁷² Chemotherapy resistance of AML may also be provoked by adherence of the leukemic cells to the stromal environment. Leukemic cells settling in the niches of the microenvironment are relatively protected against the cytotoxic effects of chemotherapy. Adhesion is accomplished through leukemic progenitor cell-stromal cell interactions via specific receptors and adhesion molecules. One such interaction is known as the CXCR-4-SDF1 α (stroma-derived factor 1 α) axis. SDF1 α is produced by marrow stroma and activates its cognate receptor CXCR4. CXCR4 has been implicated in chemotaxis, homing, survival/apoptosis of hematopoietic cells. AMLs with high CXCR-4 cell surface expression (e.g., on the AML progenitor population) and a presumed

high tendency of stromal protection have been shown to have a poor prognosis.^{73,74} CXCR-4 inhibitors that target the stromal interaction and release the leukemic cells from the microenvironment are currently in clinical trial. These inhibitors have both a mobilizing and cell-cycle activating effect upon the AML cells and may sensitize the AML for chemotherapeutic cell killing.^{75,76} The effects of CXCR-4 inhibition remarkably resemble AML cell stimulation by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF). Following in vivo stimulation with G-CSF or GM-CSF leukemia cells are also mobilized to the blood and also become activated. The administration of G-CSF or GM-CSF concurrently with chemotherapy, commonly referred to as CSF priming, has also been shown to sensitize for cell killing by chemotherapy in vitro. A clinical benefit of CSF priming has been demonstrated in two large randomized studies where a reduced relapse probability and improved disease-free survival have been reported.^{77,78} The positive effects on outcome were particularly apparent in intermediate-risk cytogenetic types of AML. Some other published studies, in particular in AML at older age, have failed to show enhanced therapeutic efficacy of CSF priming.⁷⁹ The reasons for these discrepant results for the time being has not been resolved but information of the genetic leukemia diversity has not been available in those studies. Interestingly, synergy between the activities of G-CSF stimulation and CXCR-4 inhibition has also been reported.⁸⁰ Future studies that make use of CXCR-4 inhibition and take the level of cellular CXCR-4 expression phenotype into account, and studies using the combination of G-CSF stimulation and CXCR-4 inhibition in combination with antileukemic chemotherapy appear warranted.

Concluding Remarks

In March, 1958 Frei et al submitted one of the first comparative studies in the chemotherapy of malignant disease.¹ The study dealt with patients with AML and ALL. They concluded from their studies that, “survival time from onset of treatment was greater in patients who attained remissions than in those who did not The combination of antimetabolites used in this study could exercise its optimum effect only on patients with leukemic cells sensitive to both drugs.” In 1960 F.G.J. Hayhoe wrote about acute leukemia in his renowned monograph “Leukemia, Research and Clinical Practice”: “While comparisons . . . suggest that an increase in longevity of some few months is the most that has been achieved by the advances in treatment of the past 10 years, they are in a sense misleading, and are not helpful in determining prognosis in individual patients”² These observations and statements half a century ago already pointed to the need of a better understanding of patient variability and disease heterogeneity for treatment development in AML and drew attention to the necessity

for the development of drugs that would overcome resistance to therapy. The occasion of the celebration of a half century of ASH may make us especially aware of the remarkable progress as regards the diagnosis, prognosis and treatment of AML that the last 50 years have witnessed. Nevertheless, there is no question that the challenges of therapeutics in AML today remain considerable. In 2008 most of our patients (primary refractory leukemias, recurrences after initial complete remission, leukemia at older age) continue to fail treatment. At the same time the intense scientific activity at multiple frontlines holds promise for a continuing positive trend in developmental therapeutics in AML.

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Conflict-of-interest disclosure: The author has equity ownership in Skyline.

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