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Advances in the Treatment of Acute Myeloid Leukemia: From Chromosomal Aberrations to Biologically Targeted Therapy

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Abstract We describe several recent advances in our understanding and treatment of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) including the use of cytogenetics to classify these diseases and to identify therapies that are specific for the abnormalities. Cell lines have provided readily available and very relevant models to understand these diseases. The two clear successes include the use of retinoic acid for acute promyelocytic leukemia and tyrosine kinase inhibitors (e.g., imatinib) for chronic myelogenous leukemia. Very recent results suggest a particular activity of lenalidomide, an analogue of thalidomide, in MDS patients with deletions of the long arm of chromosome 5 (so-called 5q minus syndrome), and notable activity of azanucleoside DNA demethylating agents in MDS with loss of chromosome 7. However, for the vast majority of cytogenetic abnormalities found in AML/MDS, no specific therapies have been identified. The use of a variety of molecular biology techniques have identified a large number of genomic abnormalities; the challenge of the next several decades is to identify specific therapies for these molecular defects. J. Cell. Biochem. 104: 2059–2070, 2008.

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The first 20 years of the last century, the diagnosis and prognosis of acute myeloid leukemia (AML) and Myelodysplastic syndrome (MDS) were predominantly determined by morphology. The physician looked through the microscope to determine which cell types were abnormal in the peripheral blood and bone marrow. In the 1960s, cytogenetics allowed us to identify specific chromosomal changes in AML and MDS. We became aware that one of the more frequent types of alterations in leukemia where chromosomal translocations fusing one chromosome with another resulting either in a fusion product of two genes [e.g., BCR-ABL in chronic myelogenous leukemia (CML)] or placement of a gene under the influence of the strong promoter of the other gene (i.e., c-myc translocated to near the immunoglobulin gene in Burkitt's lymphoma). Cytogenetics have, therefore, allowed us to classify AML and MDS. For example, those individuals whose AML cells have a normal karyotype or a translocation between chromosome 8 and 21 [t(8;21)] or between chromosomes 15 and 17 [t(15;17)] have a favorable prognosis. In contrast, those individuals that have loss of one chromosome 7, or 3 or more chromosomal changes have a very poor prognosis. Those elderly (often females) with MDS that are missing a small region on the long arm chromosome 5 (q31) have a disease known as 5q- syndrome and have a favorable

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prognosis. Cytogenetics are now standard of care for leukemia and MDS patients. Their importance for diagnosis is clearly reflected in the implementation of cytogenetic subgroups in the WHO classification of AML in 1999, as well as their use to help determine prognosis in MDS as outlined in the International Prognostic Scoring System (IPSS). Most of the fusion genes associated with these translocation have been identified, and several have become targets for various therapies such as imatinib for CML or all-trans-retinoic acid (ATRA) for APL.

An additional breakthrough was the development of polymerase chain reaction (PCR) which allows amplification of DNA or complementary DNA (cDNA). This technique has been used to help in the diagnosis of various translocations such as BCR-ABL (CML) and PML-RARα (APL). In addition, PCR has been used to monitor minimal residual disease (MRD). After therapy, patients may morphologically appear to be in remission, but residual leukemic cells may be identified by PCR. Therefore, physicians can gauge the effectiveness of their therapy by monitoring MRD. In addition, relapse can be identified at an earlier time point, facilitating the decision to re-institute therapy.

Several other techniques are extensively used in research and will, with time, work their way into the clinic. For example, microarray expression analysis allows investigators to look at expression of genes in the entire genome. Certain expression signatures have been associated with good and bad prognosis as well as suggesting specific therapies. The most common clinical application for this technique at this time is breast cancer. Its use in AML and MDS as a way of predicting classification and therapy has not reached the same level of refinement. The assay, however, has been a valuable tool for studying the cellular and molecular biology of AML and MDS.

Two other techniques to look at the entire genome are comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) chips. We have used the latter and have noted that approximately 50% of AML patients with normal karyotype have prominent genomic abnormalities not seen by cytogenetics. Likewise, approximately 50% of APL samples have additional genomic changes besides the t(15;17). The technique is rapid, robust, and provides data that are not readily available by cytogenetics. This technique likely will become a standard over the next decade and will provide a genomic signature that will allow subclassification of AML and MDS. Furthermore, SNP chip analysis will become a powerful tool to identify new genomic abnormalities that can serve as therapeutic targets.

Also, establishment of myeloid cell lines derived from patients with either AML or blast crisis of CML has provided important tools. The initial human myeloid cell lines were: K562 (erythroleukemia with the capacity to differentiate towards red cells and megakaryocytes); HL-60 cell line, a myeloblast cell line that has the capacity to differentiate to granulocytes or monocytes; and KG1, CD34+ very early myeloid cells. These and additional cell lines have been useful models to study the cellular and molecular biology of AML and MDS. As one of us [Koeffler, 1983] outlined 25 years ago, these cell lines have also served as very important tools for the investigation of novel therapeutic approaches in AML, CML and preleukemia, by virtue of their ability to respond with partial or even full morphological differentiation, to numerous physiologic and non-physiologic ompounds. Specifically, the development of retinoids as differentiation-inducing agents for acute promyelocytic leukemia (APL, i.e., FAB subtype M3) would have been hindered without the availability of such cell lines as HL60, KG1, U937, and, eventually, the NB-4 APL line bearing the translocation (15;17) [Lübbert et al., 1991]. Similarly, the K562 model of bcr-abl-fusion has been an invaluable tool in the development of the inhibitors of tyrosine kinases exemplified by imatinib [Mahon et al., 2000]. A third model of myeloid leukemia is represented by the Kasumi-1 cell line with a balanced translocation (8;21) expressing the AML1/ETO fusion gene. These cells have been extensively studied in their response to biological agents aimed at differentiation, cell cycle arrest and/or apoptosis.

TARGETED THERAPIES FOR DISTINCT CYTOGENETIC AND MOLECULAR SUBGROUPS

Acute Promyelocytic Leukemia (APL)

Abnormal promyelocytes are a defining morphological feature in APL also known as FAB-M3. Morphologically, some patients show a variant form of APL which is classified as FAB-M3v. The pathogenetic features and

treatment options are similar to classical APL. APL was considered one of the most fatal and difficult-to-treat entities in AML (particularly due to the initial bleeding diathesis) until the advent of ATRA treatment at the beginning of the 1990s. Clinically, the disease is characterized by frequent coagulopathy associated with bleeding. Cytogenetically, a recurrent translocation t(15;17)(q22;q21) occurs in more than 95% of the patients. This translocation leads to a chimeric fusion of the PML gene and the retinoic acid receptor alpha (RARα) gene resulting in PML-RARα. Rare patients carry alternative translocations involving the RARα gene, for example, PLZF-RARα. These fusion genes invariably induce an APL-like disease in murine models [Minucci et al., 2002; Westervelt et al., 2003] indicating the causal relationship between the translocation and leukemia development on the one hand; and on the other had, highlighting the specific association of these translocations with APL features.

The RARα receptor as a nuclear hormone receptor is involved in cellular differentiation. Since its functions partially overlap, for example, with RARβ, it is not essential for hematopoiesis and myeloid differentiation. As a transcription factor, RARα binds to retinoic acid response elements (RARE). Similiar to other nuclear hormone receptors, RARα acts as a transcriptional repressor in the absence of its ligand. Retinoic acid as a ligand for RARα induces a conformational change that leads to induction of RARα target genes. PML, the most frequent fusion partner of RARα, is a nuclear protein that is localized in nuclear bodies and interacts with a multitude of other proteins including the p53 tumor suppressor and transcriptional co-repressors. In the t(15;17), the chimeric PML-RARα fusion protein has been shown to recruit several transcriptional co-repressors such as DAXX, HDAC (histone deacetylase) and the SUV39H1 histone methyltransferase to promoter regions of genes with retinoic acid response binding sites. RARβ2 is the most extensively studied target gene [Grignani et al., 1998; Di Croce et al., 2002; Zhu et al., 2005; Carbone et al., 2006; Villa et al., 2006] PML-RARα also interacts with HDAC1 [Khan et al., 2001]. It has also been suggested that PML-RARα recruits DNA methyltransferase to its target gene promoters, but only the promoter of the RARß2 gene has so far been described to

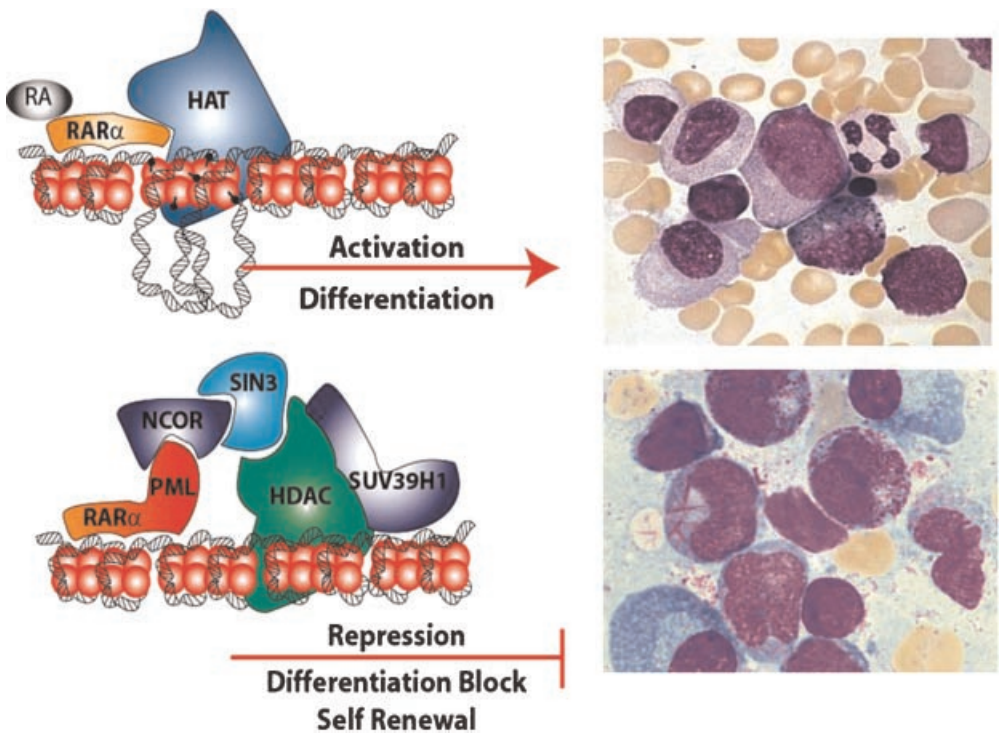

Fig. 1. The differentiation block of acute promyelocytic leukemia mediated by the PML/RARAα fusion protein is relieved by all-trans retinoic acid (ATRA).

be methylated due to direct DNA binding of PML-RARα. PML-RARα homodimerization has been shown to relax the relatively stringent RARα DNA binding specificity [Kamashev et al., 2004; Zhou et al., 2006]. This gain of function supposedly leads to many additional genomic binding sites that are not well defined.

On a phenotypic level, the PML-RARα fusion protein blocks differentiation and apoptosis and enhances self-renewal [Grignani et al., 1993; Tussie-Luna et al., 2006]. Microarray analyses elucidated several leukemogenic mechanisms and pathways [Alcalay et al., 2003; Park et al., 2003; Müller-Tidow et al., 2004; Walter et al., 2004; Meani et al., 2005]. For example, PML-RARα, similar to PLZF-RARα and AML1/ETO, induces activation of the Wnt signaling pathway, which might be associated with self-renewal properties [Müller-Tidow et al., 2004]. Also, PML-RARα alters the apoptotic response and expression of differentiation-associated genes.

In contrast to RARα, the PML-RARα fusion gene is unresponsive to physiological concentrations of retinoic acid and acts as an universal repressor at all of its target genes. At pharmacologically relevant concentrations, retinoic acid can overcome the transcriptional repressor functions of PML-RARα (Fig. 1). The treatment of APL with ATRA has dramatically improved the outcome of this disease. Nowadays, APL has by far the best prognosis of all AML subtypes, with cure rates approaching 90%. Ironically, this molecular targeted therapy was discovered before the molecular mechanisms of APL (PML-RARα) leukemia was known or how ATRA reversed the transcriptional repression of the fusion protein. An initial scientific report from Shanghai [Huang et al., 1988] in 1990 described the successful therapy of APL with ATRA. French, Italian and North American Study Groups followed their lead and designed clinical trials that rapidly showed the benefit of ATRA.

Upon ATRA exposure, primary APL cells as well as cell line models undergo terminal differentiation. While treatment with ATRA alone can induce complete remissions of the disease, relapse occurs in almost all patients. Therefore, APL is currently treated with a combination of chemotherapy (usually consisting of an anthracycline either with or without cytarabine) and ATRA which is given for at least 3–4 weeks. Although remission induction with this treatment regimen is a bit slower than with classical chemotherapy in AML, most patients achieve complete remission, and the vast majority are cured after consolidation chemotherapy. Several studies are further aiming to improve the outcome by different chemotherapy regimens. According to established risk factors, for example, high leukocyte count, the intensity of the chemotherapy can be tailored to the patient́s relapse risk. For induction therapy in low risk patients even non-cytotoxic combinations of arsenic trioxide (ATO, see below) and ATRA has been successfully used. However, even upon non-cytotoxic induction therapy, consolidation therapy includes classical chemotherapy. While further improvements in therapy regimens, for example, by adjusting the doses of cytotoxic drugs might be achievable, the main obstacle to further improvements in clinical practice are the early deaths. Up to 10% of APL patients die due to coagulopathy, mostly severe bleedings, for example, intracranial hemorrhage despite rapid initiation of therapy. Another problem specifically associated with ATRA, is the so-called ATRA syndrome that is associated with fluid retention and capillary leakage.

In addition to ATRA, APL cells are also sensitive to ATO. Several mechanisms including the rapid degradation of the PML-RARα fusion protein have been postulated but the exact molecular mechanism remains incompletely understood. ATO can be used as induction therapy (in combination with ATRA) but is more frequently used as relapse therapy. Here, as a single agent it can induce complete hematological and molecular remission in the majority of patients. However, without further therapy, preferably allogeneic stem cell transplantation, most patients relapse. Treatment with ATO is also used in APL patients with significant levels of minimal residual disease (MRD). MRD can be readily analyzed in APL patients by quantifying PML-RARα transcripts in bone marrow or blood. A rise or insufficient decrease in PML-RARa transcripts should lead to relapse treatment even in the absence of overt leukemia. Thus, most patients with APL are nowadays routinely monitored by real-time RT-PCR. In conclusion, the success of ATRA-based therapy in APL has set the stage for similar improvements in other AML entities. It also highlights the importance to define molecular subtypes of AML.

Acute Myeloblastic Leukemia With Maturation and Translocation (8;21)

The chromosomal translocation t(8;21)(q22; q22) represents one of the most frequent cytogenetic events in de novo AML. This AML subtype usually is morphologically associated with the FAB AML M2 subtype; indeed about 30–40% of AML M2 samples bear the t(8;21) chromosomal change [Downing, 1999]. The t(8;21)(q22;q22) generates the AML1/ETO fusion gene by juxtaposing coding sequences of the AML1 gene on chromosome 21 with sequences of the ETO gene on chromosome 8 [Miyoshi et al., 1991; Erickson et al., 1992] AML1 (RUNX1, PEBP2αB, or CBFA2), is a member of the RUNX family of transcription factors, characterized by a *Runt homology domain* (RHD) at their amino terminus. The RHD is required for heterodimerization with CBF*β* and for binding of DNA. AML1 activates transcription from enhancer core motifs (TGT/cGGT), which are present in a number of genes with functional roles in hematopoietic development [Meyers et al., 1993]. Furthermore, AML1 contains a transactivation domain, nuclear matrix attachment signal and two inhibitory domains [reviewed by Peterson and Zhang, 2004]. ETO (for eight twenty-one; also called MTG8 and CDR) encodes a zinc finger-containing protein belonging to a protein family characterized by four evolutionary conserved Nervy homology regions (NHR).

The chimeric protein consists of the NH2-terminal portion of the wild-type AML1 protein fused in-frame to the nearly full-length ETO protein. While the RHD at the amino terminus of AML1 is included in the fusion protein, the transactivation domain at the COOH terminus (which interacts with the transcriptional coactivators p300 and CBP), is replaced by ETO [Miyoshi et al., 1991]. The AML1/ETO fusion protein exerts a dominant-negative effect on AML1-dependent transcriptional activation, through interaction of the ETO moiety with the nuclear receptor corepressor N-CoR-mSin3-HDAC1 complex. This interaction results in a lower level of histone acetylation, inactive chromatin, and thus repression of the transactivation activity of wildtype AML1 [Lutterbach et al., 1998; Wang et al., 1998]. However, AML1/ETO also regulates target genes that are not regulated by AML1 [Shimada et al., 2000].

Since very few in vivo models of human AML1/ETO function are available to investigate the target genes of this chimeric transcription factor, we engineered a U937 cell line to express AML1/ETO under the control of an ecdysone-inducible promoter construct. This tool was then employed to identify target genes regulated by conditional expression of AML1/ETO. By combination of different techniques, LAT2/NTAL/LAB was identified as a strongly repressed gene in this model [Fliegauf et al., 2004]. Repression of LAT2 occurred within 4 h of conditional AML1/ETO expression. This (direct or indirect) repression by the fusion protein was validated in the Kasumi-1 and SKNO-1 cell lines constitutively expressing AML1/ETO, as well as in primary AML patients. The human lysozyme gene was also repressed three- to fourfold after conditional AML1/ETO-expression. This repression was sensitive to treatment with TSA, suggesting that repression was mediated by recruitment of HDAC-activity to the lysozyme promoter. Also, this repression was validated in cell lines [Claus et al., 2006]. A third gene studied was the human neutrophil elastase (ELA2) gene: direct binding of conditionally expressed AML1/ETO to this promoter could be demonstrated by ChIP in the absence of the gene being transcriptionally regulated [Lausen et al., 2006].

The ability of AML1/ETO to act as a modifier of sensitivity to the growth inhibitory and proapoptotic effects of a demethylating agent, Decitabine (DAC), was examined both in Kasumi-1 cells and U937-AML1/ETO cells treated with increasing concentrations of DAC. These experiments demonstrated that AML1/ETO enhanced the sensitivity of myeloid cells to the cellular effects of the drug. We asked whether AML1/ETO may mediate a DNA methylator phenotype. Conditional expression of AML1/ETO for up to 120 h did, however, not result in detectable methylation of the almost completely unmethylated p15 promoter in this U937 cell line model. This is in contrast to the heavily methylated p15 promoter in Kasumi-1 cells, which constitutively express AML1/ETO [Berg et al., 2007; Berg et al., Personal Communication, September 2007].

Others have also addressed a possible role of AML1/ETO as an epigenetic modifier of gene expression. The group of Hiebert had first shown that AML1/ETO recruits HDACs 1–3 to target genes normally regulated by

AML1 [Lutterbach et al., 1998]. Marcucci and colleagues were the first to show that AML1/ETO silenced the interleukin-3 promoter in human cells [Klisovic et al., 2003]. This repression was not only sensitive to treatment with the HDAC inhibitor depsipeptide, but also to DAC. This group showed that at the interleukin-3 promoter, AML1/ETO exists in a repressive complex with HDAC1 and DNMT1. This complex can be disrupted by treatment with the HDAC-inhibitor valproic acid, but not with the DNMT1 inhibitor DAC. Thus, evidence is accumulating that implicates AML1/ETO as an important epigenetic modifier, similar to the data obtained with PML-RARA.

The transcriptional repressor functions of AML1/ETO have also been utilized as a novel therapeutic approach by several groups. "Knock-down" of the mRNA by siRNA results in growth arrest, granulocytic differentiation and induction of numerous transcripts related to cell-cycle control and differentiation [Heidenreich et al., 2003; Dunne et al., 2006]. One of us performed investigations where AML1/ETO was re-directed by a therapeutic fusion protein that contained the AML1/ETO binding domain of MEF and the DNA-binding domain of MYB. As a consequence, AML1/ETO was redirected to myb target genes which are essential for hematopoietic survival and proliferation. Redirection of AML1/ETO specifically induced apoptosis in AML1/ETO positive cells but not in healthy progenitor cells. The advantage of this approach is that the oncogene is not inhibited but directly used to initiate cell death. The resistance mechanisms of simple inhibitors are unlikely to develop given that AML1/ETO function is instrumental in initiation and maintenance of leukemia. Despite the conceptual advance, no clinically applicable drugs have been developed. Signature-based drug screening [Stegmaier et al., 2004] offers an interesting and rational basis to identify drugs that induce effects that are similar to siRNA-mediated knock-down of AML1/ETO.

The Myelodysplastic Entity of 5q- Syndrome

Deletions on the long arm of chromosome 5 are the most frequent genetic abnormalities in MDS. They can either occur as the sole aberration or combined with one or more additional defects. The major commonly deleted region (CDR) has been delineated at band q31.1. This CDR is associated with exposure to toxic chemicals such as benzene or alkylating chemotherapy agents. These patients have a poor response to chemotherapy with complete remission rates of under 30%. The important genes in the CDR in these disorders have yet to be clearly identified. We examined a series of genes within the CDR and noted that α-catenin transcript levels were markedly diminished compared to other patients with AML or normal CD34 stem cells [Desmond et al., 2007]. In further studies, we showed that an inhibitor of histone deacetylase (HDAC) enhanced expression of α-catenin. Furthermore, genes in the immediate vicinity of alpha catenin did not differ significantly between samples with and without the 5q- abnormality. We have examined a number of AML and MDS samples and did not find methylation of the promoter region of α-catenin. This is in contrast to studies done by Liu et al. [2007] who did find increased methylation of CpG islands in the region of α-catenin. Alpha-catenin binds to and links β-catenin to cellular cytoskeleton. Loss of α-catenin may cause destabilization of β-catenin at the cell surface causing its nuclear translocation and subsequent activation of oncogenic β-catenin target genes. However in our experiments, we could not find a change in localization of β-catenin or an increase in β-catenin mediated transcription of target genes [Desmond et al., 2007]. Clearly, further experiments to determine the importance of α-catenin silencing in AML with 5q- deletions are required.

Another gene of interest is the early growth response gene 1 (Egr-1), a member of the WT 1 transcription factor family located in the CDR on chromosome 5. It is involved in cellular response to growth factors, mitogens and stress stimuli. Beside animal models, evidence exists that low ERG1 expression is involved in the pathogenesis of different cancer types in humans partially via regulation of p53 [Ferraro et al., 2005; Krones-Herzig et al., 2005; Ronski et al., 2005]. Joslin et al. [2007] found that $ERG^{-/-}$ and $ERG^{+/-}$ mice yielded a higher susceptibility to develop myeloid malignancy and suggested haploinsufficieny of ERG1 as an initiating event in the development in MDS and AML.

In contrast to therapy-related AML/MDS with 5q-, a sole deletion on the long arm of chromosome 5 between bands 31 and 33 is associated with the 5q- syndrome. This is a

disease most frequently observed in elderly females who develop macrocytic anemia, normal or elevated peripheral platelets and less than 5% blasts in the bone marrow. These individuals have a favorable prognosis with only 25% evolving to AML after 15 years. Moreover, patients with 5q- syndrome show excellent response rates when treated with the immunomodulatory drug lenalidomide [List et al., 2006]. However, the exact mechanism and genes affected by the drug are still under investigation.

We examined the mRNA levels of 33 genes within the common deleted region of the 5q-syndrome which spans approximately 1.5 megabases in 12 patients with 5q- syndrome [Lehmann et al., 2007]. The expression levels were compared to those of mononuclear bone marrow cells from patients with MDS having a normal karyotype. Eight genes had markedly decreased expression in 5q- syndrome samples: SLC36A1, G3BP, CSFR1, ATOX1, RPS14, PDGFRβ, TNIP and SPARC. The SPARC gene is a matricellular protein which has the capacity of regulating extracellular matrix formation, cellular proliferation and cell adhesive properties. In addition, it is known to be methylated in lung and pancreatic cancer cells consistent with its role as a tumor suppressor gene. We examined hematopoiesis in SPARC-null mice and found that they had significantly lower platelet counts compared to wild type animals [Lehmann et al., 2007]. Red cell counts also were slightly lower than in the wild-type mice associated with decrease numbers of erythroid burst forming units. Recently, Pellagatti et al. [2007] demonstrated that lenalidomide led to upregulation and increased protein expression of the SPARC gene in erythroblasts in vitro, which also highlights the potential importance of the gene in the 5q- syndrome.

EPIGENETIC THERAPY: A NOVEL TREATMENT OPTION FOR OLDER MDS AND AML PATIENTS WITH POOR-RISK CYTOGENETICS

The median age for patients newly diagnosed with AML or MDS is above the age of 65 years [Deschler and Lübbert, 2006]. These patients are notoriously under-represented in clinical trials which investigate various induction chemotherapies [Deschler et al., 2006]. Even with intensive treatment, the median survival of these patients is probably around 6 months and long-term survival rarely exceeds 10%. This unsatisfactory situation drives the quest for novel treatment approaches. Several are listed below.

DNA Demethylating Agents

The demethylating agents 5-azacytidine and Decitabine are remarkably active, even at low doses with mild hematologic toxicity, in patients with high-risk MDS. This disease shares many poor prognostic features with AML of the elderly. 5-azacytidine was the first drug approved by the FDA (2004) for the treatment of myelodysplastic syndromes (all subtypes). Phase II studies of 5-azacytidine in MDS had been initiated by Silverman and the CALGB [7 daily administrations of 75 mg/m^2, total dose 525 mg/m^2, repeated every four weeks, Silverman et al., 1993, 1994]. An overall response rate of 49% was obtained, with 12% CRs and a median response duration of 14.7 months. A pivotal phase III study of the CALGB compared subcutaneous 5-azacytidine randomized against best supportive care (BSC), with the possibility of "cross-over" from BSC in case of progressive disease. An overall response rate of 60%, with 7% CRs and 16% PRs and a median response duration of 15 months was achieved in the experimental arm. Quality of life was also significantly improved in 5-azacytidine treated patients. Side effects included mainly myelosuppression and associated effects, particularly during the first cycles. Non-hematological toxicities, e.g. nausea and vomiting, were rare, but skin reactions occurred more frequently.

In a large confirmatory trial [Fenaux et al., 2007], 5-azacytidine was compared to conventional treatment as determined prior to randomization by the treating physician (either BSC, low-dose ara-C, or induction chemotherapy). Of 358 patients included, 179 were randomized to 5-azacytidine, 179 to conventional care (105 to BSC, 49 to low-dose ara-C, 25 to standard induction chemotherapy). Study drug was administered for a median of 9 cycles. 28.5% of patients in the experimental arm achieved CR or PR. Median survival was 24.4 months in the 5-azacytidine group compared to 15 months in the conventional care group ($P = 0.0001$), with a doubling of the 2-year survival (50.8 vs. 26%, $P < 0.0001$).

Whereas in the 1970s and 1980s, more than 100 trials of high dose 5-azacytidine were

performed in AML (mostly in combination with other chemotherapies a very limited number of trials using low-dose Decitabine or 5-azacytidine in AML have been published. The pioneering study by Pinto et al. [1989] described 27 patients (12 de novo AML, 9 MDS progressing to secondary AML), median age 74 years, who received low-dose Decitabine (15–90 mg/m^2 as a 4-h infusion 3 times daily for 3 days, repeated every 4–5 weeks). They achieved a remarkable 45% complete or partial remission rate; in another 30%, a definitive antileukemic effect was noted. The non-hematologic toxicity was acceptable, and this treatment approach was further developed for MDS.

A large series of high-risk MDS/early AML patients received 15 mg/m^2 in a total of nine doses over 72 h. Their overall response was 49% [Wijermans et al., 2005]. One of us did a phase II trial in untreated AML patients >60 years who were not eligible for induction chemotherapy [Lübbert et al. Blood (abstract) 300, 2007]. Median age of the patients was 72 years (range 56–85), with 33% >75 years. Complex karyotype and/or preceding MDS were present in 32% and 49%, respectively; both of these characteristics are associated with a bad prognosis. ATRA was given to the decitabine-responsive patients during their 2nd course of therapy. Complete responses (CR) and partial responses (PR) occurred in 23 patients (15%) and in 15 patients (10%), respectively. Best responses occurred at a median of 13 weeks. Including all responses, a total of 54% individuals responded. 15 (10%) had progressive disease [Lübbert et al., Blood ASH Annual Meeting Abstracts, November 2007; 110: 300]. Preliminary results of another low-dose decitabine trial in older patients with AML were reported. Cashen et al. (Blood, ASH Annual Meeting Abstracts, November 2006; 108: 1984) gave the drug over 1 h on 5 consecutive days (20 mg/m^2 per day), repeated every 28 days. CR was 26%, and 36% had stable disease with >25% reduction in bone marrow blasts.

Valproic Acid, an Inhibitor of Histone Deacetylases (HDACs)

Valproic acid (VPA) is an inhibitor of class I HDACs. Over the last 5 years, the drug has been studied as either a single agent or in combination with various drugs including ATRA. VPA has provided a 50% overall response rate in low-risk MDS [Kuendgen et al., 2004, 2005] and a lower rate of response in high-risk MDS [Kuendgen et al., 2005] and AML [Bug et al., 2005b; Cimino et al., 2006]. The contribution of ATRA probably was modest. Thus, the role of single-agent VPA may be rather limited in AML. Nevertheless, the drug in combination with an active drug such as decitabine, may have enhanced activity, as demonstrated in multiple in vitro studies [e.g., Yang et al., 2006; reviewed in Lübbert, 2005]. Surprisingly, murine [Milhem et al., 2004; Araki et al., 2006] and ex vivo [Bug et al., 2005a,b] data showed that the combination of DAC and an HDAC inhibitor may provide beneficial effects on normal hematopoiesis (with possible expansion of the early progenitor pool), as well as active suppression of the abnormal clone. A large phase II study of AML and MDS performed at the MD Anderson Cancer Center demonstrated the feasibility of DAC combined with 10 days of intravenous VPA [Garcia-Manero et al., 2006].

Sensitizing Non-M3 AML to Retinoic Acid With Epigenetic Drugs

ATRA as single agent, when given at the doses established for APL, has no role in the treatment of non-M3 AML. However, it has been shown that in combination with drugs that reactivate the RA signaling pathway, ATRA sensitivity may be restored also in vivo [Trus et al., 2005]. Several large clinical studies have had mixed results when examining the role of ATRA in combination with induction chemotherapy. One studied found that addition of ATRA to induction chemotherapy in AML patients >60 years resulted in a survival benefit [Schlenk et al., 2004]. In contrast, several other large studies showed that adding ATRA to the chemotherapy was no better than chemotherapy alone [Estey et al., 1999; Burnett et al., 2007]. Also, the combination of a demethylating agent with a second epigenetic drug and an inducer of differentiation were used in a trial of 53 patients with AML (49) or MDS (4); the overall response was 42% [Soriano et al., 2007].

CONCLUSION

Since the discovery of the Philadelphia chromosome t(9;22) as a recurrent genetic abnormality in CML in 1961, dramatic strides have been made towards the development of specific therapies which may serve not only as adjuncts to conventional chemotherapy but may in the

TABLE I. Chromosomal Abnormalities Provide Surrogate Markers for Treatment Targets in Myeloid Leukemia and Preleukemia (MDS)

Drug	Disease entity	Karyotype	Gene	Reference
All-trans retinoic acid	Acute promyelocytic leukemia	t(15;17)	PML-RARA	Huang et al., 1988
Arsenic trioxide	Acute promyelocytic leukemia	t(15;17)	PML-RARA	Shen et al., 1997
Imatinib	Ph1-positive leukemia	t(9;22)	bcr-abl	Mahon et al., 2000
Lenalidomide	5q-syndrome (low-risk MDS)	del(5)	?	List et al., 2006
5-azacytidine, decitabine	High-risk MDS	−7	?	Lübbert et al., 2001; Raj et al., 2007; Rüter et al., 2007

future replace standard treatment. One of the first was the treatment of CML with a tyrosine kinase inhibitor. Even earlier was the treatment of APL with ATRA. Other examples are also given in Table I. Sadly, the vast majority of recurrent genomic abnormalities described in AML do not have a targeted therapy. This is the frontier for the next 50 years.

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