

Letter From the Editors

Welcome to the second issue of **Frontiers in HematOncology**, a new international publication for close followers of cutting-edge clinical and scientific developments in hematologic oncology. It is our aim to present original articles on groundbreaking research and novel approaches to disease management, coupled with compact yet detailed news coverage of major conferences in the field. Additional features include challenging case studies by leading specialists and summaries of highlights from the recent literature.

In this issue, Stephen B. Baylin and James G. Herman report on their new understanding of epigenetic changes in carcinogenesis and the prospects for therapeutic reactivation of epigenetically silenced genes. Catherine M. Verfaillie and members of her stem cell research team describe their trailblazing work with multipotent adult progenitor cells. And from Salamanca, Spain, an unusual case report focuses on multiple allogeneic PBSC transplantation in an acute myeloid leukemia patient. There is also a report by Gareth Morgan on the Eighth International Conference on Malignant Lymphoma in Lugano, Switzerland, together with extensive news coverage from the recent meetings of the American Society of Clinical Oncology (ASCO) in Orlando, Florida, and the European Hematology Association (EHA) in Florence, Italy.

Your comments on this issue, as well as your recommendations and contributions for forthcoming issues, will be most welcome. Please write to the Editors at **Frontiers in HematOncology**, 1500 Broadway, New York, NY 10036, USA, or fax us at +212-704-0120. ■

Understanding the Role of Epigenetic Changes in the Initiation and Progression of Human Cancer

BY STEPHEN B. BAYLIN, MD,* AND JAMES G. HERMAN, MD†

*Ludwig Professor of Oncology, Associate Director for Research, and Director, Cancer Biology; †Associate Professor of Oncology; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Baltimore, Md

The importance of epigenetic changes in human cancer is only now being recognized by the scientific and medical communities. Of primary interest is aberrant promoter hypermethylation that leads to inappropriate gene silencing. Notably, such hypermethylation is at least as common as tumor-suppressor gene disruption through mutation, and it may even play as crucial a role.¹ These insights are leading to the exploration of newer therapeutic strategies aimed at blocking methylation.

A high degree of methylation takes place in the mammalian genome at dinucleotide cytosine guanosine sites.² Approximately 70% to 80% of the cytosine guanosine sites that

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Salamanca, Spain

GUEST CONTRIBUTORS

Stephen B. Baylin, MD

Ludwig Professor of Oncology
Johns Hopkins Medical Institutions
Baltimore, Md, USA

Catherine M. Verfaillie, MD

Director of the Stem Cell Institute
Professor of Medicine
University of Minnesota
Minneapolis, Minn, USA

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are candidates for methylation are methylated in the DNA. The exceptions are so-called CpG islands—CpG-rich areas very frequently found in the proximal promoter regions of almost half the genes in the genome.^{2,3} Most of the CpG islands associated with such promoters are protected from methylation in normal cells. In the case of both solid tumors and leukemias, however, hypermethylation has now been shown to occur in the promoter CpG islands of a growing list of genes. The de novo methylation of such CpG islands occurs early during the process of carcinogenesis and is associated with an unfavorable state for transcription or expression of the associated genes.^{4,5} This state, referred to as gene silencing, is now the best-characterized epigenetic change in tumors.¹ One can appreciate how such a state would have an adverse impact if a tumor-suppressor gene were among those genes silenced in this manner. Such a change would be tantamount to a mutation, since it would prevent the protein from being produced by the affected gene and would provide neoplastic cells with a selective advantage.

For each of the major types of cancer, there is a growing list of silenced genes in which the CpG regions have taken on heavy methylation around the transcription start site—for reasons that are not yet fully understood. Of the genes that cause cancer when mutated in families (Table), approximately half are methylated in various forms of cancers that are not inherited. In the case of acute myelogenous leukemia and acute lymphocytic leukemia, the most evident impact of hypermethylation is inactivation of the cell cycle regulating the *p15* gene.⁶ Inactivation of the *p15* gene also tends to occur in myelodysplastic syndromes at the

TABLE. METHYLATION STATUS OF FAMILIAL CANCER GENES

Methylated	Not Methylated
<i>Rb</i>	<i>NF1, NF2</i>
<i>P16^{INK4a}</i>	<i>MSH2</i>
<i>VHL</i>	<i>PTEN</i>
<i>NLH1</i>	<i>P53</i>
<i>E-cadherin</i>	<i>PTC</i>
<i>BRCA1</i>	<i>BRCA2</i>
<i>APC</i>	<i>TM</i>
<i>PJ (LKB1)</i>	

time this preneoplastic condition begins to transform toward leukemia.⁶

Role of Chromatin

A mounting appreciation of the role of chromatin in mediating the repression of gene transcription is paving the way toward a better understanding of the epigenetic changes that occur in cancer. Most of the genome contains transcriptionally repressive chromatin that is associated with heavily methylated DNA.^{1,2} Normally, the unmethylated CpG regions, particularly those around gene promoter sites, do not have these proteins. The superimposition of methylation on the start sites of genes does not lead to silencing of transcription until chromatin proteins are recruited to the region.⁷

Studies have identified chromatin-associated protein complexes that link DNA methylation to transcriptional silencing.^{1,2} The DNA in these transcriptionally silent regions is packaged into compacted nucleosomes containing histones that are deacetylated by enzymes called histone deacetylases (HDACs).^{1,8-10} The deacetylated histone

state maintains the nucleosomes in a transcriptionally silent state.⁸⁻¹⁰

In addition, DNA methylation itself appears to play a central role in the transcriptionally silent state associated with chromatin (see Figure).¹ Methylcytosine-binding proteins (MBPs) bind to DNA at methylated cytosine sites. The MBPs recruit HDACs to methylated DNA in regions of transcriptional silencing.¹¹⁻¹⁴ The HDACs help maintain the histones around the start site of the gene in the deacetylated state. DNA methyltransferases (DNMTs), which actually cause methylation, can also recruit HDACs. In this way, the proteins may work together to silence the genes.

Antitumor Effect of Loss of DNMT Activity

Emerging data from animal research have indicated that the removal of DNMTs reduces methylation and has a highly potent antitumor effect. Laird and colleagues conducted work with a mouse model engineered for the *APC* mutations that cause colon cancer.¹⁵ Mice that have background *APC* mutations develop colon polyps at a very high rate shortly after birth. The investigators also engineered some of these mice so as to knock out 1 allele of the gene for DNMT1, which is believed to be the most important of the DNMTs for maintaining DNA methylation and is responsible for most of the DNA methylating enzyme activity in cells. In contrast to the animals with both copies of the *DNMT1* gene, those with 1 allele of this gene knocked out had half the number of colon polyps. Recently, Laird et al have also been able to engineer mice with both alleles knocked out and have found that these animals do not develop any colon polyps.¹⁶

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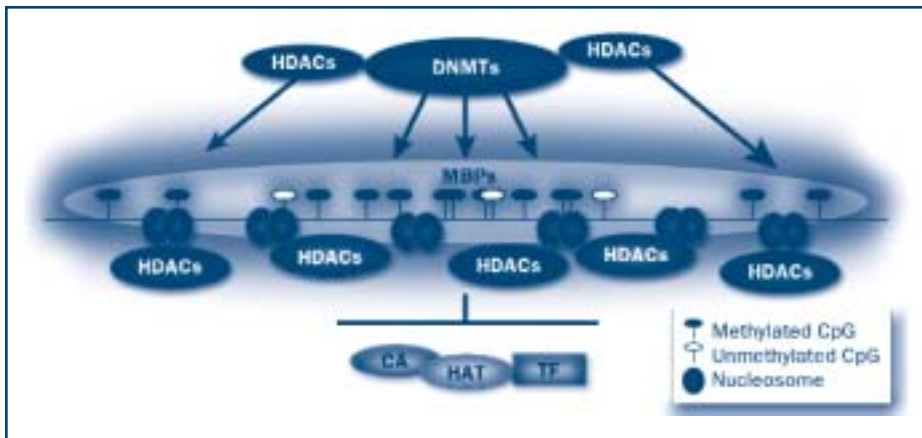


Figure. In this schematic showing the typical chromatin configuration of transcriptionally silent DNA, most CpG sites along the DNA are methylated and bound by methylcytosine-binding proteins (MBPs) present in complexes that include histone deacetylases (HDACs). Deacetylated histones, resulting from HDAC activity, are organized into tightly compacted nucleosomes. DNA methyltransferases (DNMTs), which catalyze the methylation and can also complex with HDACs and potentially target them to the region, can access this area, but transcription activator complexes—consisting of a coactivator protein (CA), a histone acetyltransferase (HAT), and a transcription factor (TF)—are blocked.

Our group, in collaboration with the laboratories of Vogelstein and Kinzler, conducted experiments in colon cancer cells in which both copies of *DNMT1* were knocked out.¹⁷ This eliminated 95% of the DNMT activity, reducing it to 5% of baseline, but did not reactivate or demethylate any of the abnormally hypermethylated and silenced genes studied. On the other hand, simultaneous deletion of *DNMT1* and *DNMT3b*, a gene encoding for another enzyme that catalyzes DNA methylation, resulted in the cells losing most of their DNMT activity and caused the abnormally hypermethylated genes to become demethylated and transcriptionally active.¹⁷ This finding illustrates the potentially complementary activities of the DNMTs and also provides genetic evidence of the importance of promoter hypermethylation in the silencing of key tumor-suppressor genes.

Therapeutic Implications: Actions of 5-Azacytidine

It is uncertain whether all cancer cells behave like the colon cancer cells described above. If so, however, these observations suggest opportunities for therapeutic intervention in cancer through inhibition of all DNMT activity.

5-Azacytidine is a powerful inhibitor of all the DNMTs, since it is incorporated directly into the DNA and RNA of dividing cells. This agent reverses hypermethylation at the start site of genes and reactivates key genes, such as antitumor genes or those with tumor-suppressor-like effects.¹

Increasing the dose of 5-azacytidine beyond its optimally effective concentration does not necessarily increase the efficiency with which genes in treated cells are reactivated¹ and can, moreover, induce toxicity not necessarily related only to demethylation of DNA. Therefore, our group has been interested in reducing the 5-azacytidine dose as much as possible, then complementing it with an inhibitor of HDACs, which form another component of the block in expression of hypermethylated genes. As noted earlier, HDACs retain proteins around the start site of the gene in the deacetylated state.

The rationale for this combined approach is based on laboratory observations by our group using cultured cancer cells, and on the subsequent work of others, indicating that inhibition of HDACs alone in an extensively hypermethylated and inactive gene will not reactivate it.¹⁸ High-dose 5-azacytidine, on the other hand, will activate the gene. In the setting of reduced

5-azacytidine doses that alone have little effect on expression of the gene, HDAC inhibitors become synergistic with 5-azacytidine to reactivate hypermethylated genes.¹⁸

On the basis of these laboratory observations, Drs. Michael Carducci and Steven Gore have initiated clinical trials to test whether combination treatment for cancer with 5-azacytidine and the HDAC inhibitor phenylbutyrate might be a useful approach. Their goal is to titrate down the dose of the 5-azacytidine to a point where it still provides inhibition of DNMT activity but avoids toxicities—and also to determine whether phenylbutyrate will be synergistic in this regimen for gene reactivation. As this work progresses, we hope to gain a better understanding of the efficacy of therapy directed toward reversal of gene silencing.

Whatever therapeutic approach is used to combat inappropriate gene silencing, continuous or repeated inhibition of the repressive chromatin will probably be necessary for chronic gene reactivation. The repressed gene transcription associated with promoter hypermethylation is a heritable state. If this repression is blocked and the inhibitor is then removed, it will revert to its inactive state, and the presence of transcriptionally repressive chromatin may recur as long as the tumor clone is present. Thus, chronic therapy may be required—and this, again, suggests the importance of using the lowest doses of 5-azacytidine possible.

Conclusions

Our knowledge of the role of epigenetic changes in carcinogenesis is at an evolutionary stage, and much more remains to be learned. The research conducted thus far suggests that the processes implicated in gene silencing may represent productive targets for therapeutic intervention. 5-Azacytidine has many promising attributes in this regard, since it reverses hypermethylation at the start site of genes and reactivates genes involved in tumor suppression. Further work will be needed to gain a fuller understanding of the mechanisms of action of 5-azacytidine as well as its optimal dosing. Future studies will examine the potential for enhanced responses when 5-azacytidine is used with standard combination therapies.

Clinical trials with biologic end points are also warranted. These efforts promise to shed more light on the efficacy of using the reactivation of epigenetically silenced genes as a method of treating cancer. ■

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Multipotent Adult Progenitor Cells

BY MORAYMA REYES, PHD, ROBERT E. SCHWARTZ, BE, BALKRISHNA JAHAGIRDAR, MD, YUEHUA JIANG, MS, AND CATHERINE M. VERFAILLIE, MD

Stem Cell Institute, University of Minnesota, Minneapolis, Minn

Almost 6 years ago, we were asked to develop a therapy for patients with Hurler disease, or mucopolysaccharidosis I, based on genetically modified mesenchymal stem cells (MSCs) to correct bone and cartilage problems that persist even after successful hematopoietic cell transplantation. MSCs were first described by Fridenshtein et al¹ more than 25 years ago as cells that adhere to culture plates when bone marrow is plated in the presence of fetal calf serum (FCS). Since the initial description of MSCs, several groups—in particular, Caplan and colleagues²⁻⁵—have further developed MSC culture systems, adjusted culture conditions to induce their differentiation to limb-bud mesoderm in vitro, and begun to apply MSC therapies in vivo.⁶

Because of prior disease and other potential infectious agents, the United States Food and Drug Administration (FDA) and comparable regulatory bodies in Europe prefer that cells created for human therapies be cultured in the absence of animal product. Therefore, we attempted MSC derivation in the absence of FCS, necessitating enrichment of MSCs independent of their characteristic of adhesion to culture plates in the presence of FCS.

A Nonhematopoietic Stem Cell From Bone Marrow

Because more than 99% of bone marrow consists of hematopoietic cells, we removed all nucleated hematopoietic cells (CD45+) as well as erythroid progenitors (glycophorin A [GlyA]+) by depleting CD45+ and GlyA+ cells from human bone marrow mononuclear cells. By trial and error, we demonstrated that presumed MSCs could best be cultured when CD45- and GlyA-depleted cells were cultured on fibronectin in the presence of 2 cytokines—epidermal growth factor and platelet-derived growth factor—as well as ascorbic acid, insulin, and dexamethasone.⁷

Because we wanted to demonstrate that single MSCs can generate all limb-bud mesodermal cell types, we attempted to subclone cells at 1, 5, or 10 cells per well. This led us to demonstrate that when presumed MSCs are cultured at low cell densities, prolonged proliferation is possible—beyond 60 to 70 cell doublings—suggesting that these cells were not subject to the Hayflick phenomenon. Indeed, we found that these presumed MSCs express telomerase, that telomeres are longer in cells recovered from these MSC cultures than in other nucleated cells in bone marrow or blood, and that these telomeres do not shorten in culture.⁷ The phenotype of MSCs in our culture system was different from that defined by Caplan and colleagues and others.^{2,8,9} Specifically, MSCs in our culture were HLA class I–negative, CD44–negative, and endoglin (SH2)–negative, whereas MSCs described by others express these antigens.⁷

A Cell Type Different From the Classical MSC

We demonstrated that these presumed MSCs differentiate into osteoblasts, chondroblasts, adipocytes, and skeletal myoblasts when conditions defined by Caplan et al for such lineage differentiation are applied.^{5,10} In contrast to MSCs, cells in our cultures express the vascular endothelial growth factor (VEGF) receptors Flk1 and Flt1.⁷ This led us to test whether addition of VEGF would induce an endothelial phenotype.¹¹⁻¹³ We demonstrated that addition of VEGF to cells from our MSC cultures, replated at high density, induce differentiation to mature endothelial cells that are positive for CD34, CD36, Tie, Tek, and von Willebrand factor (vWF).¹⁴ Like primary endothelial cells, those generated from MSCs uptake low-density lipoprotein (LDL), release vWF when exposed to hista-

mine, upregulate HLA antigens when induced with inflammatory cytokines, upregulate VEGF and the VEGF receptor under hypoxia, make vascular tubes when plated on extracellular matrix (ECM), and contribute to neoangiogenesis *in vivo*.

This, then, was the third observation—the first having been the ability of cells in our culture system to proliferate without obvious senescence, and the second their cell surface phenotype—suggesting to us that the cells in our cultures were different from “classical” MSCs.

Because hematopoietic cells and endothelial cells are thought to be derived from a common progenitor from mesoderm, termed the hemangioblast,^{13,15} which is Flk1-positive, we next attempted to induce hematopoietic differentiation from cells in our MSC culture. Until now, such differentiation has not been achieved. We demonstrated repeatedly, however, that cells with neuroectodermal morphology could be generated. Labeling these cells with antibodies against neurons, astrocytes, and oligodendrocytes showed that those with neuroectodermal morphology were positive for these markers. This was likely due to the fact that our culture conditions contained, aside from hematopoietic cytokines, 2 neural-stem-cell-supportive cytokines: EGF and bFGF^{16,17}—the latter present in medium conditioned by hematopoietic supportive stromal feeders.¹⁸ Since then, we have shown that specific addition of cytokines that support neural stem cells as well as their differentiation to mature neuronal elements induces differentiation to cells with neural morphology and staining pattern and with functional voltage-gated sodium channels (M.R., unpublished data, 2002).

Endodermal Cell Differentiation

Once we had shown that cells in our culture may differentiate not only into mesodermal cell types but also into neuroectodermal types, we tested whether they could also differentiate into endodermal cells. We found that addition of FGF-4¹⁹ and HGF²⁰ caused differentiation to cells with epithelial morphology, expressing mature hepatocyte markers.²¹ In addition, these hepatocyte-“like” cells derived from MSC

can produce urea and albumin, uptake LDL, store glycogen, and contain phenobarbital-inducible cytochrome P450.

Using retroviral marking, we also showed that differentiation into cells with limb-bud mesodermal, visceral mesodermal, neuroectodermal, and endodermal characteristics occurs at the single-cell level.^{7,14,21} Thus, these studies demonstrated that the presumed MSCs in our culture system are significantly more multipotent than classically defined MSCs. We therefore have termed cells in our culture system multipotent adult progenitor cells, or MAPCs. We demonstrated that the low-density culture and low concentrations of FCS or no FCS are imperative to obtain cells that proliferate without obvious senescence, are CD44- and HLA class I-negative, and differentiate into cell lineages outside the limb-bud mesoderm.

MAPC Generation and Differentiation

Attempts to obtain MAPCs from murine bone marrow using culture conditions established for human MAPCs were unsuccessful. When, however, we added leukemia inhibitory factor to the rodent cultures, MAPCs could be generated.²² Like human MAPCs, murine MAPCs are CD44-negative and MHC class I-negative. We also showed that murine MAPCs, like human MAPCs, can be maintained *in vitro* without telomere shortening. Murine MAPCs also differentiate *in vitro* at the single-cell level into cells with endothelial, neuroectodermal, and endodermal characteristics.²² Furthermore, when single-mouse MAPCs are introduced in the blastocyst, one third of the animals born are chimeric, and chimerism is detected in most, if not all, somatic tissues (see **Figure**).²² When murine MAPCs are infused in postnatal recipients, 1% to 9% engraftment is seen in the hematopoietic system, liver, intestine, and lung—where cells acquire phenotypic characteristics of the organ in which they engraft.²²

Thus, like many other recent studies, ours suggest that adult-tissue-specific stem cells may have greater potential for differentiation than previously believed. The mechanism underlying this apparent ability

of adult stem cells to undergo lineage switching remains unclear. Although there is evidence that multiple stem cells for different tissues coexist in certain organs,^{23,24} we showed both *in vitro* and *in vivo* that multilineage differentiation is possible starting from single cells.

Two recent papers have shown that fusion can be induced between 2 different cell types, with transfer of genetic information from 1 cell type to a second.^{25,26} However, our *in vitro* differentiation conditions do not require that MAPCs be cocultured with a second cell type—excluding the possibility that the *in vitro* lineage switching is due to fusion of MAPCs with, for instance, neural cells or hepatocytes. Moreover, MAPCs are euploid, not tetraploid as seen in the work of Terada et al and Ying et al.^{25,26} Studies are also ongoing to establish whether *in vivo* lineage switching—in the chimera experiment or in postnatal transplantation experiments—is due to cell fusion.

This leaves 2 further possible explanations for what we have observed in our studies: either (1) our culture conditions favor dedifferentiation of MSCs or (2) rare, more multipotent cells remain in marrow even after birth. Interestingly, MAPCs share at least some characteristics with embryonic stem cells—including the need for leukemia inhibitory factor in cultures of mouse MAPCs²⁷ but not human MAPCs;²⁸ expression of stage-specific embryonic antigen (SSEA) 1 on mouse MAPCs;^{29,30} and presence of the transcription factor Oct-4,³¹ known to be important for maintaining undifferentiated embryonic stem cells. However, since it is not clear from our studies whether SSEA1 and Oct-4 were present in fresh bone marrow cells or induced during culture, it remains to be seen whether their presence is due to dedifferentiation or to persistence of more primitive cells into adulthood.

Conclusions

Studies are ongoing to determine conclusively whether MAPCs are the result of dedifferentiation *in vitro* or persisting pluripotent stem cells. Irrespective of the mechanism underlying the greater differentiation potential of MAPCs, we believe they

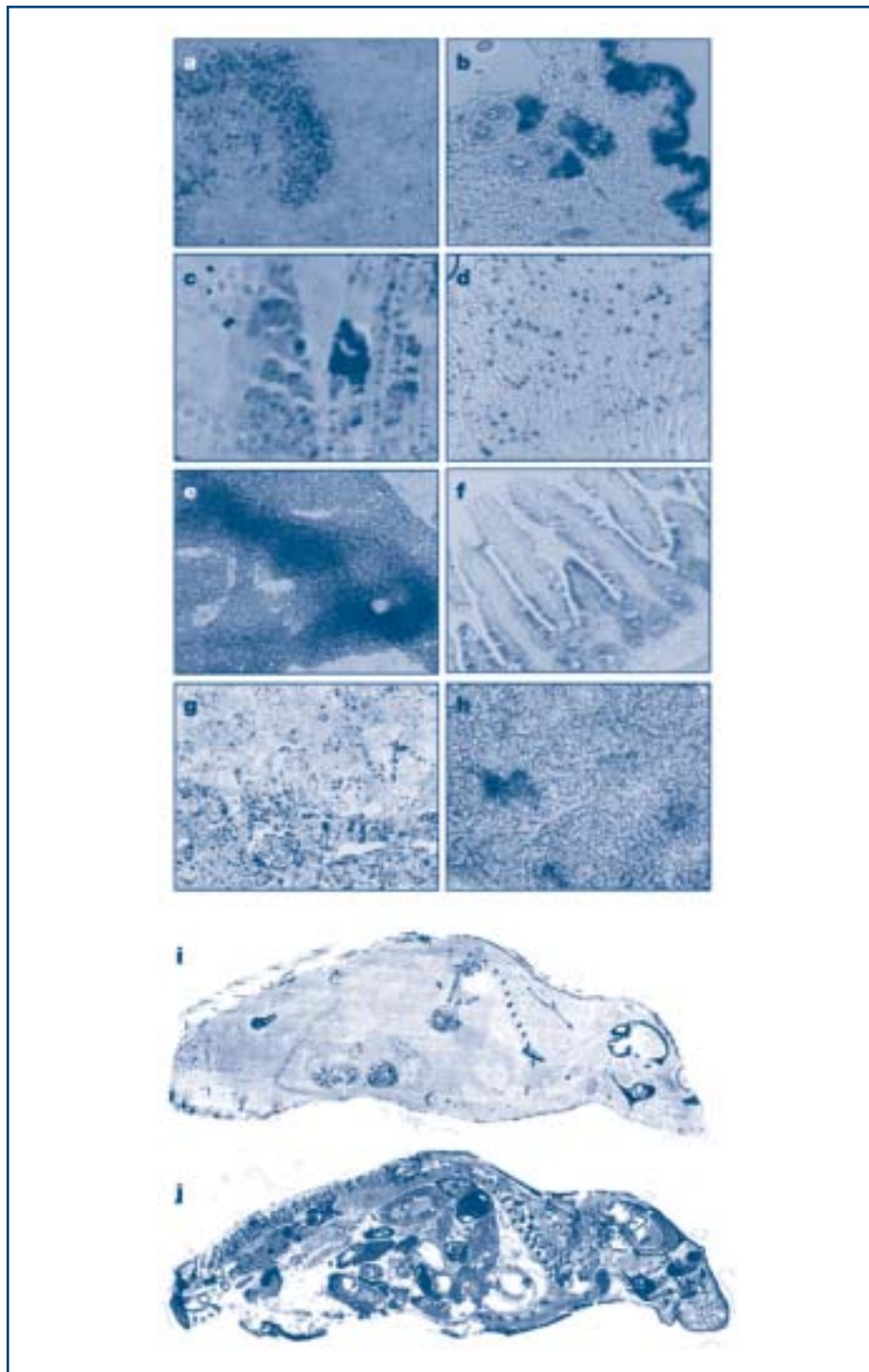


Figure. Chimerism detection by X-gal staining and anti- β -gal staining in animals generated from blastocysts microinjected with a single ROSA26 MAPC. **a-h:** Images are from X-gal-stained individual organs from a 45% chimeric mouse, determined by Q-PCR for Neo on tail clip. Tissue sections were from brain (**a**), skin (**b**), skeletal muscle (**c**), myocardium (**d**), liver (**e**), small intestine (**f**), kidney (**g**), and spleen (**h**). **i, j:** Images are from an X-gal-stained section through a mouse that was not chimeric (**i**) or was 45% chimeric (**j**). Magnification $\times 20$. Reprinted with permission from Jiang et al.²² Copyright 2002 Macmillan Publishers Ltd.

hold clinical promise for the management of genetic and/or degenerative disorders. As with all novel therapies, extensive studies will be needed to demonstrate safety and efficacy with rodent as well as large-animal models, to create clinically suitable culture methods for undifferentiated MAPCs and possibly differentiated progeny, and to determine whether therapies should be devised using autologous or allogeneic cell sources. ■

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CASE REPORT

Immunotherapy With GM-CSF Plus IL-2 Following a Third Allogeneic PBSC Transplant in an AML Patient

M. DOLORES CABALLERO, MD, PHD, ISABEL GONZÁLEZ-FRAILE, MD, LOURDES VÁZQUEZ, MD, PHD, CONSUELO DEL CAÑIZO, MD, PHD, JOSÉ A. PÉREZ-SIMÓN, MD, PHD, RICARDO LÓPEZ-PÉREZ, PHD, RAMÓN GARCÍA-SANZ, MD, PHD, MARCOS GONZÁLEZ, MD, PHD, AND JESÚS SAN MIGUEL, MD, PHD

Servicio de Hematología, Hospital Clínico Universitario, Salamanca, Spain

The prognosis for patients with relapsing leukemia after allogeneic stem cell transplantation is generally poor. A small proportion of patients are eligible for a second transplant with myeloablative conditioning, but this is associated with high treatment-related morbidity and mortality.¹ Nonmyeloablative preparative regimens can reduce this toxicity while preserving the graft-vs-leukemia (GVL) effect.² Nevertheless, the efficacy of this alternative strategy for relapsing patients after allogeneic transplantation is still unknown. There is increasing evidence for the immune system's role in controlling minimal residual disease (MRD).

Accordingly, several different strategies to enhance both allo- and autoreactivity against leukemic cells are currently under investigation. Donor lymphocyte infusion is the most commonly used strategy, but therapy with cytokines such as interleukin-2 (IL-2) or granulocyte-macrophage colony-stimulating factor (GM-CSF) may also have an important role.^{3,4}

Here we describe a patient with acute myeloid leukemia (AML) who has achieved a 42-month remission after a third allogeneic peripheral blood stem cell (PBSC) infusion followed by combination immunotherapy with GM-CSF plus IL-2.

Presentation

This 38-year-old man had AML subtype M1 (FAB), with dyshematopoiesis and normal cytogenetics. He reached complete

remission after second-line chemotherapy, and a related major ABO-mismatched allo-geneic transplant was performed. His condi-tioning regimen consisted of oral busulfan (16 mg/kg) and cyclophos-phamide (120 mg/kg). Methotrexate (days 1, 3, 6, 11) and cyclosporine were used as graft-vs-host disease (GVHD) prophylaxis. A total number of 8.96×10^6 /kg CD34+ cells were infused. Bone marrow studies showed full chimerism from day 28, and flow cytometry analysis indicated MRD between 0.1% and 0.23%. On day 63, antral biopsy detected grade II intestinal acute GVHD, and steroid treatment with prednisone 1 mg/kg/d led to complete res-olution. A transbronchial biopsy on day 130 revealed bronchiolitis (in the context of chronic GVHD), and therefore, cyclo-sporine was continued.

The patient had thus far remained ane-mic because of hemolysis related to major ABO mismatch. He did not respond to erythropoietin and remained transfusion dependent on day 205. His hepatic func-tion began to deteriorate, and a hepatic biopsy on day 210 revealed chronic GVHD. At the same time, a decrease in peripheral blood counts prompted a bone marrow study showing morphological relapse. When there was no response to cyclosporine withdrawal, a second allo-geneic transplant from the same donor was scheduled. To reduce conditioning toxicity, a nonmyeloablative preparative regimen (fludarabine 120 mg/m² plus melphalan

Immunotherapy After Third Transplant

140 mg/m²) was used. The total number of CD34+ PBSCs infused was 5×10^6 /kg; GVHD prophylaxis consisted of cyclosporine alone.

Thirty days after the second allogeneic transplant, the patient was independent of red blood cell (RBC) transfusions for the first time since the first transplant. On day 15, a bone marrow examination showed absence of blast cells (MRD 0.24%) and full chimerism. On day 14, a gastric and cutaneous biopsy revealed acute grade II GVHD, and prednisone (2 mg/kg) was started, with partial response.

On day 148 following the second transplant, bone marrow examination detected a second relapse with mixed chimerism (90% of donor's cells). Cyclosporine treatment was stopped, without response, and it was decided to initiate a palliative treatment with low-dose subcutaneous cytosine arabinoside (20 mg/m²/d). Surprisingly, blast cells disappeared from the bone marrow 14 days later. At this time, since the patient's general condition was acceptable, a third PBSC infusion from the same donor was performed—24 hours after the last cytosine arabinoside dose, with no GVHD prophylaxis. A total number of 5.1×10^6 /kg CD34+ cells were infused.

On day 1 following the third transplant, immunotherapy with IL-2 and GM-CSF began. The GM-CSF dose was 5 m g/kg/d from day 1 to day 6, afterward decreasing to 3 m g/kg/d. When platelet levels reached $>20 \times 10^9$ /L (day 16), IL-2 was initiated at a dose of 0.5×10^6 IU/m² and gradually increased to 2×10^6 IU/m²/d—combined with GM-CSF 5 m g/kg for 1 week each month. Immunotherapy continued until day 66, when it was interrupted because of fever and low platelet levels, then restored on day 102 and maintained up to day 135, after which it was stopped definitively because of a second episode of fever and low platelets. On day 363, after a third infusion due to extensive chronic GVHD (cutaneous, oral, hepatic, ocular, and gastrointestinal), daily cyclosporine treatment was begun at a dose necessary to maintain blood levels between 150 and 300 ng/mL.

The **Table** shows the patient's evolution over the 3 transplants. At last follow-up—

TABLE. ENGRAFTMENT AND TOXICITY

	Ablative Transplant	Mini-allograft	Low-Dose Ara-C + GM-CSF + IL-2
500 granulocytes	Day 13	Day 13	Day 5
1000 granulocytes	Day 15	Day 13	Day 6
20,000 platelets	Day 24	Day 11	Day 16
RBCT independence	Not reached	Day 30	Day 10
WHO toxicity grade	II (hepatic)	I (hepatic), III (stomatitis)	I (stomatitis)
Acute GVHD grade	II (intestinal, day 63)	II (intestinal and cutaneous, day 14)	None
Chronic GVHD	Pulmonary (day 130), hepatic (day 210)	None	Extensive (day 363)
Hospitalization following PBSC infusion	30 days	30 days	23 days

Ara-C = cytosine arabinoside; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL-2 = interleukin-2; RBCT = red blood cell transfusion; WHO = World Health Organization; GVHD = graft-vs-host disease; PBSC = peripheral blood stem cell.

3.5 years after the third transplant—he has a good performance status (ECOG-1), good engraftment, full chimerism, and the lowest MRD level since diagnosis ($<0.0014\%$, just at the detection limit).

Discussion

GM-CSF and IL-2 each have several effects on immune and nonimmune cells. It has been observed that the efficacy of hematopoietic transplantation in patients with hematologic malignancies is a function not only of high-dose chemoradiotherapy but also of immune mechanisms.⁵ In allogeneic transplantation, these mechanisms are linked to the GVHD and the GVL effects, both mediated by T-reactive cells.⁶ It is not only T cells that are important in the elimination of tumor cells, however; endoge-

nously generated activated killer (AK) lymphocytes circulating after hematopoietic transplantation are also relevant. AK cells can act directly, killing malignant cells in a major histocompatibility complex (MHC) nonrestricted fashion, or by inducing secretion of cytokines such as gamma interferon and tumor necrosis factor, produced by T cells and natural killer cells. It has been shown that combination therapy with IL-2 and GM-CSF enhances AK cell function. Thus, these drugs may play a role in the antileukemic effect of hematopoietic transplantation.⁵

Previous studies suggest that the absence of GVL effect in some patients is related to abnormal antigen presentation to the dendritic cells, and that this defect might be overcome by using GM-CSF after stem cell

infusion. GM-CSF is thus a potential molecule for inducing antitumor immunity. It has been speculated that this effect may be due to the ability of GM-CSF to promote differentiation of CD34+ cells into dendritic cells.⁷

In this patient, immunotherapy with IL-2 and GM-CSF was initiated in hypoplastic bone marrow without blasts, achieving a third response significantly longer than the previous ones (16 months vs 7 and 5 months, respectively). Moreover, after the first 2 transplants the level of residual leukemic cells with leukemic phenotype always remained greater than 10^{-3} . We have seen that above this threshold the incidence of relapses is very high, whereas after immunotherapy the MRD level is as low as 10^{-5} —a value that corresponds to low-risk patients.⁸

Several conclusions can be drawn from this case. First, the use of mini-allografts to rescue patients in early relapse after allogeneic transplantation is feasible. Second, the GVL effect is complex. In this patient, it did not work in spite of acute and chronic GVHD following the first transplant and

acute GVHD after the mini-allograft—but it did work after a third infusion from the same donor when GM-CSF and IL-2 were used to enhance the antitumor immune response. GVHD was less severe after the second and third infusion, and RBC engraftment was achieved. Both these aspects suggest better tolerance after the 2 later infusions.

In summary, this case illustrates the need for further investigation of new strategies to increase immune response against residual leukemic cells. ■

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Report on Malignant Lymphoma

Notes and Reflections From the Eighth Lugano International Conference

BY GARETH MORGAN, MD, PHD, FRCP, FRCPATH

Unlike many malignant diseases, malignant lymphoma is highly responsive to chemotherapy. Unfortunately, the outlook for many of these patients remains poor and could be considerably improved. The development of new agents for treating lymphoma has been slow in the past, but recently a number of novel therapeutic approaches have begun to show promise. The challenge for the cancer physician treating these diseases is to move into the clinic the concept of personalized medicine, in which laboratory approaches are used to fully characterize the biology of the tumor and to subsequently select a rational treatment strategy. The Eighth International Conference on Malignant Lymphoma, held last June in Lugano, Switzerland, provided an ideal forum for inte-

grating these new approaches into an accessible format for clinicians and scientists alike.

Developments in pathological classifications can prove complex for many clinicians. Matters were greatly simplified, however, by the introduction of the Revised European-American Lymphoma (REAL) classification in 1994 and the World Health Organization (WHO) classification of tumors of lymphoid tissues in 2001. One concept underlying these approaches is that of defining clinical disease entities. As newer technologies become available, we can understand more fully how to subdivide these entities and thus define and understand their normal cellular counterparts. An additional challenge is targeting the use of these treat-

ments on the basis of an understanding of the tumor biology—an approach now becoming possible with advances in technology.

Novel Diagnostic Approaches Based on Gene Chip Technology

The Lugano meeting offered a number of presentations that developed and illustrated these themes of disease definition and treatment targeting. The first could be considered a solid-phase approach to cytogenetics. Carsten Schwänen, of Deutsches Krebsforschungszentrum in Heidelberg, Germany, reported on automated genomic profiling for recurrent chromosomal aberrations in B-cell chronic lymphocytic leukemia (B-CLL). A recently developed microarray-based screening technique known as Matrix-CGH could become a powerful tool for identifying many chromosomal prognostic markers at one time, he suggested.

According to Dr. Schwänen, molecular cytogenetic analysis of B-CLL has significantly contributed to the identification of genomic imbalances with prognostic relevance. Matrix-CGH is capable of screening for such genomic gains and losses in a single experiment, applying comparative genomic hybridization to defined DNA targets. This would make it possible to assess copy-number changes of hundreds of target regions in an integrated, fully automated procedure—greatly facilitating risk-adapted therapy decisions.

Dr. Schwänen's group initially constructed a B-CLL chip containing 455 PAC- and BAC-DNA fragments, then selected PACs and BACs for chromosomal regions frequently altered in CLL (3q26, 6q21, 8q24, 10q24, 11q22-q23, 12q13, 13q14, 17q13, and 18q21), as well as for 60 oncogenes and 29 tumor-suppressor genes. They analyzed DNA samples of 20 CLL patients and compared the results with data from fluorescence in situ hybridization (FISH) studies. The Matrix-CGH technology confirmed all aberrations shown by FISH in 85% of these patients.

CLL: A Single Disease Entity?

Another Lugano presentation examined the use of gene profiling in a “class discovery” fashion to define CLL subtypes. Louis M. Staudt, of the National Cancer Institute's Center for Cancer Research in Bethesda, Md, has used gene-expression profiling of purified CLL cells from untreated patients to test the hypothesis that CLL might represent more than a single disease entity. In some patients, CLL follows a progressive course and requires early treatment. In others, however, the course of the disease is relatively stable, and treatment may not be necessary for many years, if ever. Somatic mutations in CLL-cell immunoglobulin (Ig) genes were recently seen to be associated with the less progressive form of the disease, while an absence of such mutations is associated with the more progressive form.

Despite the overall similarity of cases, Dr. Staudt and his colleagues found that Ig-mutated CLL and Ig-unmutated CLL differed in the expression of several hundred genes. In combination, these genes provided an Ig mutational status prediction correctly assigning 93% of CLL cases. The most predictive gene, *ZAP70*,

encodes a tyrosine kinase previously thought to be restricted in expression to T lymphocytes. In Dr. Staudt's study, *ZAP70* mRNA was seen to be 4- to 5-fold more highly expressed in Ig-mutated than in Ig-unmutated CLL—a finding he believes could prove clinically useful.

Regardless of Ig mutational status, all CLL cases were found to share a common gene-expression signature distinguishing CLL from other normal and malignant B-cell types. This would suggest that CLL is a single disease, the study concluded, with a common mechanism of oncogenic transformation and/or a common precursor cell type in all cases.

The technologies described by Dr. Schwänen and Dr. Staudt are, of course, applicable to the full range of malignancies, some of which are discussed later in this report.

Marginal Zone Lymphoma

The clinical relevance of marginal zone lymphoma is rapidly increasing. The basic concepts underlying the normal cellular origin of the marginal zone cell were outlined by P. G. Isaacson of University College London, UK. A marginal zone of IgM+, IgD- (or weak+) B cells is well known in the spleen, and isolated marginal zone cells are believed to be present in peripheral lymph nodes. Well-defined marginal zones, however, are found only in mesenteric lymph nodes.

According to Dr. Isaacson, when the concept of marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) was first described, its normal cell counterpart was thought to be the follicle center cell. Later, however, when the marginal zone cells were identified as a component of Peyer's patches, it became clear that the MALT lymphoma normal cell counterpart was the marginal zone B cell—and that this lymphoma should be characterized as “extranodal marginal zone.”

MALT lymphomas account for 7.5% of all lymphomas. When disseminating to lymph nodes, MALT lymphomas localize in the marginal zone, where they are indistinguishable in appearance from primary nodal marginal zone lymphoma. It is not clear, in Dr. Isaacson's view, whether the normal cell counterpart of this rare lymphoma is in fact the isolated marginal zone cell thought to be present in lymph nodes.

Splenic marginal zone lymphoma is histologically, immunophenotypically, and genotypically different from both MALT lymphoma and nodal marginal zone lymphoma—and thus, Dr. Isaacson believes, there is some doubt as to its precise normal cell counterpart.

Marginal Zone and Monocytoid B Cells

The discussion of marginal zone lymphoma was further developed by Harald Stein, of the Free University of Berlin, Germany, who compared and contrasted the splenic marginal zone B (MZB) cell and the monocytoid B (MB) cell. MZB cells are regularly found in the spleens of humans and mice and are generally thought to be the first-line defense against blood-borne encapsulated pathogens, with a low triggering threshold and rapid antibody response.

Although there is no agreement as to their origin and homogeneity, the protective function of MZB cells was recently underscored by an association of their absence in the spleens of Wiskott-Aldrich syndrome patients with an inability to mount immune responses to encapsulated bacteria, according to Dr. Stein.

The function and exact origin of MB cells, on the other hand, remain unknown. Although in humans they show the closest resemblance to splenic and nodal MZB cells in terms of cytology and homing, they are not found in normal lymphoid tissue but occur regularly in toxoplasma-induced lymphadenopathy.

Dr. Stein and his colleagues analyzed new immunophenotypic data for MZB and MB cells, compared them with data from molecular genetic and functional studies, and concluded that the 2 differ in origin, differentiation stage, and function. They believe this may also hold true for lymphomas developing by malignant transformation of MZB and MB cells.

Clinical Features of Marginal Zone Subtypes

Pathological discussion returned to the clinical arena as Elias Campo, of the University of Barcelona, Spain, identified 3 subtypes of marginal zone lymphoma according to their origins—in extranodal tissue, lymph nodes, or spleen. The primary extranodal (MALT) and nodal subtypes have relatively similar pathological features, characterized by a proliferation of mature B cells with centrocytoid/monocytoid morphology and frequent plasma cell differentiation. According to Dr. Campo, these cells proliferate in the marginal zone of reactive follicles with adjacent epithelial structures in mucosal sites.

Despite their similar morphological and phenotypic features, however, the 2 subtypes appear to have different pathogenetic mechanisms and clinical behavior. The extranodal subset is associated with chronic infectious or autoimmune inflammatory disorders, with tumor cells frequently showing IgH gene somatic hypermutations and evidence of antigen selection. Tumor progression involves at least 2 mutually exclusive pathways. Tumors with the t(11;18) are karyotypically stable and do not evolve into large B-cell lymphoma (LBCL), whereas those without this translocation are often chromosomally unstable and do evolve into LBCL.

Tumors of the nodal subtype, on the other hand, are negative for t(11;18) and may originate from both naïve and memory B cells, with or without intraclonal variation. They are also more aggressive.

Finally, the splenic subtype of marginal zone lymphoma is characterized by a biphasic proliferation of mature IgD B cells, with frequent leukemic and bone marrow involvement. Although the cell of origin is not yet definitively characterized, it may include naïve and postgerminal B cells associated with different cytogenetic alterations, including 7q deletions.

Molecular Pathogenesis of MALT Lymphomas

We are now beginning to understand the molecular pathways underlying these entities, and this will eventually allow us to approach them with specific treatments based on their molecular

abnormalities. An example of this new understanding is the failure of gastric marginal zone lymphoma with a t(11;18) to respond to *Helicobacter pylori* eradication. The molecular pathway to this tumor was expertly delivered in Lugano by Randy D. Gascoyne, of the British Columbia Cancer Agency of Vancouver, Canada, and Toulouse, France.

He described 2 possible pathways of extranodal (or MALT) lymphoma development suggested by recent data on differences in chromosomal instability. One, the t(11;18) pathway, is stable both cytogenetically and histologically. The other involves gains at chromosome 3q26-27—or more rarely a t(1;14)—and produces a MALT lymphoma at risk for transformation to mucosal diffuse large B-cell lymphoma (DLBCL). This latter type shares clonal cytogenetic alterations with de novo DLBCL at mucosal or epithelial sites.

Low-grade MALT lymphomas are unusual in having 2 distinct alterations not seen in nodal or splenic marginal zone lymphomas, according to Dr. Gascoyne. Yet these alterations have been found in tumors from several different mucosal sites. Most frequent is the t(11;18), occurring in 30% to 40% of cases. This alteration results in a variable-sized fusion of 2 novel genes—*API2* on chromosome 11, which is an inhibitor of apoptosis, and *MLT* on chromosome 18, which is a paracaspase. The t(11;18) has not been noted in DLBCLs.

The second cytogenetic alteration is the t(1;14), found in 1% to 2% of cases. This translocation brings *BCL10* under the control of enhancer elements of the *IGH* locus, resulting in overexpression of BCL10. Although mutations were initially thought to be important in pathogenesis, they have only rarely been found. Thus, overexpression of wild-type BCL10 appears to be more important. A search for normal binding partners of BCL10 has revealed MALT1, a protein product of one of the genes involved in the t(11;18), suggesting a common pathogenesis.

Dr. Gascoyne noted that *BCL10* transgenic animals develop “profound splenic marginal zone hyperplasia” and that although *BCL10* knockout mice have normal apoptotic signaling, their NF- κ B activity has been seen to be defective after engagement of the antigen receptor complex.

Therefore, in his view, 2 quite distinct chromosomal translocations are involved in deregulating the same signaling pathway. On the one hand, a MALT lymphoma may develop following a t(1;14)—resulting in upregulation of wild-type *BCL10*, which activates I kappa kinase (IKK) and causes translocation of NF- κ B into the nucleus, where it induces transcriptional upregulation of a number of target genes. On the other hand, a t(11;18) may produce an *API2-MLT* fusion, which bypasses normal BCL10 to activate IKK directly and similarly causes increased nuclear translocation of NF- κ B. Either translocation results in the nuclear expression of BCL10 protein—which would correlate with resistance to antibiotic therapy.

PET With 18-Fluorodeoxyglucose

A persistent clinical question is when to stop treatment. Despite the excellent information provided by CT and MRI scans, residual masses are often present whose behavior is impossible to predict. This is disturbing for both physician and patient, but in the

future 18-fluorodeoxyglucose positron emission tomography (FDG-PET) may provide help in both Hodgkin's and non-Hodgkin's lymphoma. Two reports addressed this issue in both disease types.

A study by M. Hoffman et al, of Medizinische Hochschule Hannover in Germany, assessed the impact of FDG-PET in the clinical management of 120 patients with Hodgkin's lymphoma and 180 patients with non-Hodgkin's lymphoma. Results were correlated with morphological imaging by CT and MRI. These investigators found that FDG-PET influenced management in 70% of cases—especially in upstaging disease status prior to ther-

apy, prolonging or changing treatment, and determining resumption of treatment. In almost a third of cases scanned for recurrence, FDG-PET clearly identified lesions that CT and MRI had “visualized but not specified as recurrence.”

A similar study led by N. G. Mikhael at Guy's and St. Thomas' Cancer Centre in London evaluated the prognostic value of interim and post-treatment FDG-PET scanning in 65 adults with Hodgkin's lymphoma. This team saw higher predictive values—both negative and positive—with FDG-PET than with CT. The prognostic advantage was consistent with results they had previously reported for non-Hodgkin's lymphoma. ■

ASCO/EHA News

The 38th Annual Meeting of the American Society of Clinical Oncology (ASCO) was held in Orlando, Florida, in May 2002, and the 7th Congress of the European Hematology Association (EHA) met in Florence, Italy, in June 2002. The following pages contain news coverage of selected highlights of scientific presentations at these meetings.

Multiple Myeloma: Targeting the Bone Marrow Microenvironment

Osteolytic bone destruction is a characteristic feature of multiple myeloma, causing some of the most distressing clinical features of the disease, including bone pain, hypercalcemia, spinal cord compression, and decreased mobility from femoral fractures. As a result, many multiple myeloma patients require treatment with analgesics, bone surgery, and radiation therapy—not to cure the disease but rather to alleviate bone-related morbidity and improve quality of life. Recent research into the interaction between bone marrow stroma and myeloma cells suggests that bone destruction may be more than just a sequela of multiple myeloma and may, in fact, be a direct contributor to the pathogenesis of the disease.

“Over the past several years, we have learned more about the key players at the molecular levels that lead to osteoclast activation,” said James Berenson, MD, of Cedars-Sinai Medical Center in Los Angeles, Calif, speaking at ASCO. “We have learned that osteoclasts as well as osteoblasts can produce cytokines that drive myeloma. Therefore, strategies to reduce osteoclast function may result in a reduction in tumor burden in these patients. We are seeing some hints of this from recent clinical trials using anti-bone-resorptive therapies.”

Understanding Myeloma Bone Disease

The bone destruction seen in multiple myeloma is due to excessive stimulation of osteoclasts (the cells responsible for bone resorption), a process that results from complex interactions among osteoclasts, tumor cells, and the bone marrow microenvironment, Dr. Berenson reported. Key players in this arena include NF- κ B, RANK (receptor for the activation of NF- κ B), RANKL (the ligand for RANK), and osteoprotegerin (see **Figure**). NF- κ B is a gene transcription factor that, among other things, stimulates differentiation of precursor cells into mature osteoclasts. This action is dependent on the binding of RANKL (present on the surface of stromal cells and osteoblasts) to RANK (present on the surface of osteoclasts), and can be inhibited by osteoprotegerin, a soluble decoy receptor that binds to RANKL and disrupts RANK/RANKL interactions.

This delicate balance between RANKL and osteoprotegerin, which regulates normal bone development, is disrupted in multiple myeloma patients. Interestingly, said Dr. Berenson, there is evidence to suggest that myeloma tumor cells also express RANKL, in which case blockage of RANK/RANKL interactions could have an inhibitory effect on tumor growth.

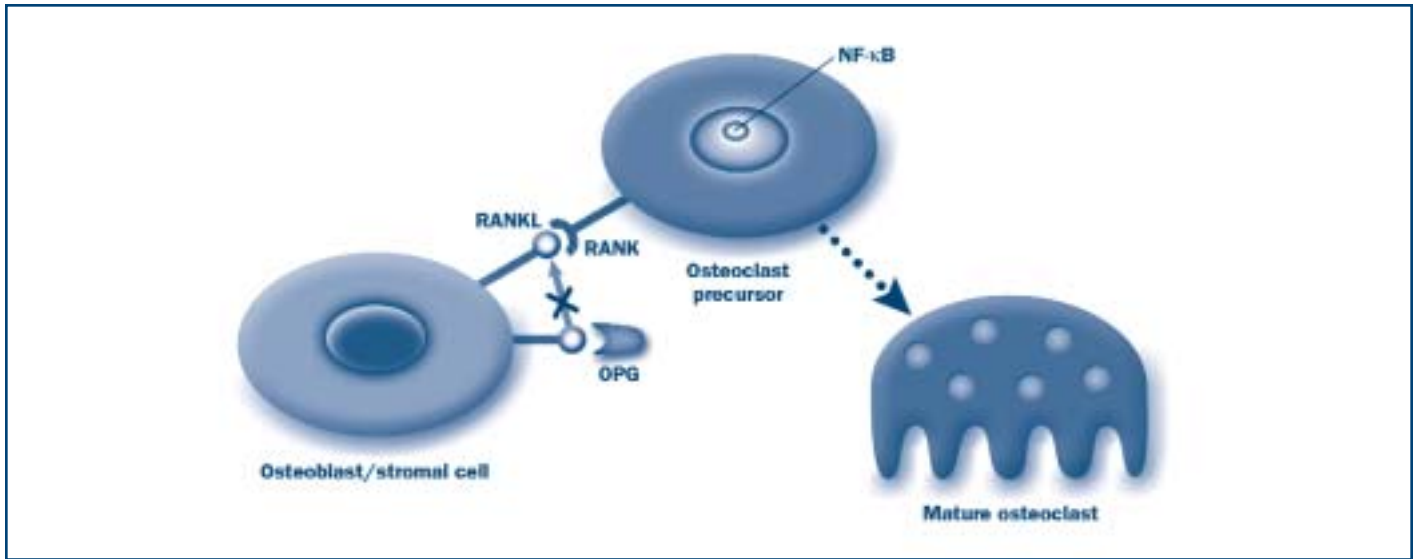


Figure. The bone destruction seen in multiple myeloma is due to excessive stimulation of osteoclasts. Key players in this arena include NF- κ B, RANK (receptor for the activation of NF- κ B), RANKL (the ligand for RANK), and osteoprotegerin (OPG). NF- κ B is a gene transcription factor that stimulates differentiation of precursor cells into mature osteoclasts. This action is dependent on the binding of RANKL to RANK and can be inhibited by OPG, a soluble decoy receptor that binds to RANKL and disrupts RANK/RANKL interactions.

Identification of the osteoclast stimulatory factors responsible for enhanced bone loss in myeloma patients remains an area of intense study. In vitro evidence suggests that several cytokines, including interleukin-6 (IL-6), IL-1b, and tumor necrosis factor- α , may be involved in osteoclastogenesis, although direct evidence of this in myeloma patients is lacking. Recently, a study from Han and colleagues at the University of Texas Health Science Center in Houston has pointed to the possibility that the cytokine human macrophage inflammatory protein-1 α (hMIP-1 α) may also have a role in myeloma bone disease, Dr. Berenson said. This study showed that in human bone marrow cultures, hMIP-1 α increased osteoclast formation by acting directly on osteoclast progenitor cells and by enhancing the osteoclastogenic effects of IL-6 and RANKL.

Emerging Treatment Strategies

Although much work remains to be done to fully understand the pathogenesis of myeloma bone disease, current research is already having a significant impact on the development of new therapeutic strategies for myeloma patients. A study from the Norwegian University of Science and Technology has shown that serum osteoprotegerin levels are reduced in myeloma patients with lytic bone disease, Dr. Berenson noted. These low levels of osteoprotegerin were associated with the degree of radiographically assessed skeletal destruction—suggesting that osteoprotegerin may be a useful therapeutic agent against myeloma bone disease.

According to Dr. Berenson, a recombinant construct of osteoprotegerin (AMGN-0007) has shown promise as a treatment for myeloma bone disease in a phase I clinical trial. This study randomized myeloma patients to a single subcutaneous dose of

AMGN-0007 (0.1, 0.3, 1.0, or 3.0 mg/kg) or standard intravenous pamidronate (90 mg). Biological activity of the drugs was measured by levels of urinary N-telopeptide of collagen (NTX), a marker of bone resorption. Results of this study show that AMGN-0007 produced a rapid and sustained drop in NTX levels of about 60%. Another promising drug, a human monoclonal antibody to RANKL (AMG-162), is under investigation in a phase I trial of myeloma and breast cancer patients with lytic lesions. Data from this trial may be made available at this year's annual meeting of the American Society of Hematology (ASH) or at ASCO in 2003, according to Dr. Berenson.

Kenneth Anderson, MD, of the Dana-Farber Cancer Institute in Boston, also noted that several other drugs—including thalidomide and its immunomodulatory analogs (IMiDs), the proteasome inhibitor PS-341, and arsenic trioxide—may directly target multiple myeloma cells in the bone marrow microenvironment, thereby overcoming classical drug resistance. According to Dr. Anderson, these agents represent a new treatment paradigm in multiple myeloma and have significant potential to improve patient outcome.

For example, one study presented at ASCO by Daniel Farray, MD, of the Cleveland Clinic in Ohio, demonstrated that slow incremental dosing of thalidomide produced marked treatment benefits in multiple myeloma patients; 38% of patients achieved a response and 30% had stabilization of disease after a median treatment duration of 260 days. Another study, presented by Paul Richardson, MD, of the Dana-Farber Cancer Institute, showed similarly positive results for PS-341. In this study, 85% of multiple myeloma patients were found to have stabilized or responded after only 2 cycles of treatment (21 days per cycle).

Bisphosphonates: Bone Protectors and Tumor Fighters?

Bisphosphonates, including alendronate, clodronate, etidronate, and pamidronate, are also being evaluated as supportive therapy for myeloma patients with lytic bone disease. These agents, which inhibit bone turnover by decreasing the resorption of bone, have shown efficacy in treating postmenopausal osteoporosis, cancer-induced hypercalcemia, and Paget's disease of bone. Notably, Dr. Berenson added, there is evidence to suggest that bisphosphonates may also prove to have antitumor effects.

"We showed several years ago that there is a marked suppression of the cytokine IL-6 in the bone marrow microenvironment in the presence of bisphosphonates, particularly nitrogen-containing ones," he said. In addition, a study by Derenne and colleagues (INSERM U463, Nantes, France) showed that the bisphosphonates zoledronate and pamidronate induce myeloma cell apoptosis. The potential of bisphosphonates to reduce tumor activity is further supported by a study presented at EHA by Lucia Villalón and colleagues (Hospital Ramón y Cajal, Madrid, Spain), which found that pamidronate stimulates gamma delta T cells in patients

with multiple myeloma or Paget's disease. Gamma delta T cells are a minor subset of T cells that have been shown, in vitro, to lyse certain hematopoietic tumor cells, including Burkitt lymphoma cell line Daudi and myeloma cell line RPMI 8226. The study authors emphasized, however, that further studies are needed to assess more fully the antitumor properties of bisphosphonates.

Regardless of whether bisphosphonates possess significant antitumor activity, clinical trials have established their effectiveness in the prevention and treatment of bone disease in multiple myeloma, Dr. Berenson pointed out. Although the data are still modest, these trials have shown that oral clodronate, intravenous pamidronate, and intravenous zoledronic acid are superior to placebo in reducing skeletal complications. New ASCO Clinical Practice Guidelines recommend the use of intravenous pamidronate or zoledronic acid rather than clodronate for treating lytic bone disease in myeloma patients, because the clinical trials for pamidronate and zoledronic acid included time to first skeletal event as a primary end point and provided a more complete assessment of bony complications. ■

New Therapies for Myelodysplastic Syndromes

Emerging therapies and their place in the overall treatment scheme for myelodysplastic syndromes (MDS) were addressed at EHA both in regular sessions and at a satellite symposium. "With the advent of small molecules and molecular biology—and the understanding of some of the key lesions that may be operative in MDS—it has become possible for the first time to try to think about some of the therapeutic strategies that may be adopted in various risk groups of MDS," said symposium chairman Prof. Ghulam Mufti, of Guy's, King's, and St. Thomas' School of Medicine in London. "It is very important that we understand the heterogeneity of the disease, because treatment strategies for the subgroups are different," he stressed.

DNA Methylation Inhibitors

The observation that 5-azacytidine inhibits DNA methyltransferase led to the first studies of this agent in patients with MDS, according to Lewis Silverman, MD, of Mount Sinai Medical Center, New York, NY. (See article by Stephen Baylin on page 1 and commentary by John Byrd on page 19.)

In early studies conducted in the United States by the Cancer and Leukemia Group B, this agent was administered in the hospital as a continuous intravenous infusion (75 mg/m²) for 7 days every 28 days for 4 months. "Probably fortuitously at the time, we proposed that the treatment be given for 4 months rather than what might have been considered standard chemotherapy of only 1 or 2 months' duration." In subsequent trials, 5-azacytidine was administered as a subcutaneous bolus at the same dosage and schedule, which Dr. Silverman termed

"a significant treatment advantage" because it did not require hospitalization. Overall, response rates were about 50% in both the intravenous and the subcutaneous studies, with comparable low toxicity.

A later crossover study conducted by the same group in some 200 patients with all the French-American-British (FAB) subgroups of MDS compared 5-azacytidine with supportive care, reporting a 60% response rate with 5-azacytidine and a 5% response rate in the supportive care arm.

Interest is also increasing in the use of decitabine (5-aza-2'-deoxycytidine) as a therapy for cancers such as MDS in which epigenetic silencing of critical regulatory genes has occurred, according to Dr. Silverman. For example, in an early study in patients with MDS, Wijermans and colleagues in the Netherlands administered decitabine at a dose of 50 mg/m², escalated to a maximum of 75 mg/m², as a 4-hour infusion every 8 hours for 3 days, repeated every 6 weeks.

Because of unacceptable toxicity at the higher dose levels, in a subsequent trial decitabine was administered according to the same schedule at a dose of either 15 mg/m² or 45 mg/m². The overall response rate was 49%, with a trilineage response of 14%. In both studies, the median duration of response was 7 months, with median survival of about 11 months in the first study and 15 months in the second. With the lower dose, the mortality rate was 8%. The incidence of treatment-related pancytopenia is about 30%.

Other investigators have found that low-dose decitabine ameliorates cytopenias, inducing trilineage responses in about 50% of

patients with high-risk MDS—and moreover that repeated low-dose courses of the drug induce cytogenetic remissions in elderly MDS patients with preexisting chromosomal abnormalities, Dr. Silverman said.

Arsenic Trioxide

At clinically relevant concentrations, the investigational agent arsenic trioxide (ATO) has been shown to inhibit growth and induce apoptosis in a variety of malignant cell lines. Early studies in patients with MDS suggest some benefit, according to Hussain I. Saba, MD, of the University of South Florida in Tampa, and other investigators.

One open-label, 2-stage, multicenter phase II study now under way in the United States has assessed 32 MDS patients—15 classified as International Prognostic Scoring System (IPSS) intermediate-1 (lower) and 17 considered intermediate-2 (higher). Treatment included ATO 0.25 mg/kg administered intravenously over 1 hour each day for 5 days in 2 weeks, followed by a 2-week break for multiple treatment cycles. Early outcome analysis demonstrated trilineage activity in patients with both high-risk and low-risk disease, Dr. Saba reported. About 65% of low-risk patients achieved disease control, and 33% of high-risk patients had hematologic improvement. Adverse events included hematologic toxicity and leukopenia.

Other Investigational Drugs

Other drugs directed at specific molecular or biological targets in MDS are under development and await the determination of their roles, if any, in therapy. For example, the aminothiol amifostine induces responses in about 35% of patients, predominantly a reduction in the transfusion requirement, and the response appears to be increased when combined with hematopoietic growth factors, according to Prof. Mufti. *Ras* may play a role in MDS, and activation of this gene and its signaling pathways may require farnesylation. Several farnesyl transferase inhibitors have been developed and are now in phase II trials in MDS patients. At lower doses, these agents are reasonably well tolerated. Available data suggest a possible role for angiogenesis in MDS, and a number of antiangiogenesis agents are in clinical trials, including

thalidomide and anti-vascular endothelial growth factor (VEGF) antibodies, Prof. Mufti said.

New Approaches to Transplantation

The only way to cure MDS is eradication of the MDS clone, and the best treatment option for that is allogeneic stem cell transplantation when patients are in an early stage of disease, according to Prof. Theo M. de Witte, of University Medical Centre in Nijmegen, the Netherlands. Overall survival following early-stage transplantation is now about 50%. Reduced-intensity conditioning treatment before transplantation is being explored, and preliminary findings suggest a survival advantage.

Standard MDS Therapeutic Guidelines Proposed

The Italian Society of Haematology has developed evidence- and consensus-based guidelines for MDS therapy that will soon be published in *Haematologica*, according to Giovanni Barosi, MD, of the IRCCS Policlinico San Matteo in Pavia, Italy. The committee for the development of practice guidelines concluded that watchful waiting is never recommended for patients younger than 20 years of age and only rarely for those younger than 55 (if mild anemia, stable low-risk status, and favorable cytogenetics exist in patients younger than 40).

The guidelines recommend frontline allogeneic stem cell transplantation for patients younger than 55 with an IPSS risk of intermediate-2 to high or with a low to intermediate-1 progressive disease. Patients younger than 40 with stable intermediate-1 disease after 3 months of follow-up are likewise candidates for frontline transplantation.

Allogeneic transplantation (not as a first-line therapy) is also recommended for adults below 40 years of age with low-risk disease that is stable after 3 months of follow-up but accompanied by moderate-to-severe anemia or severe neutropenia and unfavorable cytogenetics. If no suitable donor is available for allogeneic transplantation, patients under 65 years of age with an IPSS risk of intermediate-2 to high are candidates for chemotherapy. Patients younger than 40 with a low to intermediate-1 risk are also candidates for chemotherapy if cytogenetics are unfavorable, according to these guidelines. ■

Imatinib Update: High-Dose Therapy and Newly Diagnosed Patients

The introduction of imatinib mesylate (STI-571, Gleevec[®]) as an oral treatment for chronic myelogenous leukemia (CML) in May 2001 was widely hailed as a milestone in cancer research. Not only did this tyrosine kinase inhibitor produce dramatic remission rates in interferon-resistant CML patients, but it provided a clear demonstration that targeted cancer therapeutics had

moved from the confines of the laboratory to the clinical arena. Not surprisingly, imatinib was quickly dubbed a “magic bullet” and a “miracle pill” by the news media, with *Newsweek* headlining one article “A Cure for Cancer?” Perhaps even less surprising was the inevitable disappointment of CML patients as it became obvious that the answer to *Newsweek’s* question was no.

Diminished expectations notwithstanding, imatinib remains an important treatment option for patients with CML. Results from the International Randomized Interferon vs STI-571 (IRIS) study, reported at ASCO, showed that imatinib is superior to interferon plus cytarabine as initial therapy for CML patients. Results from other studies presented at ASCO suggest that the use of higher doses of imatinib (800 mg) may increase drug efficacy and may be useful in patients who have developed resistance to the standard 400-mg dose. “Imatinib should now be considered as the standard first-line treatment for CML,” said Brian Druker, MD, of Oregon Health and Science University in Portland.

IRIS: Initial Therapy for CML Patients

In a seminal study by Hagop M. Kantarjian and colleagues (see *Frontiers in HematOncology*, Volume 1, Issue 1), imatinib was shown to produce a complete hematologic response in 95% of patients with late-chronic-phase CML in whom prior interferon therapy had failed. In addition, major cytogenetic response was seen in 60% of patients, and complete cytogenetic response was seen in 41% of patients. A key goal of IRIS was to determine whether the benefits of imatinib therapy would be similarly evident in newly diagnosed CML patients, as well as to assess the efficacy of imatinib compared with interferon-based therapy, according to Dr. Druker.

The IRIS results presented at ASCO were based on data collected up to the end of January 2002, with a median follow-up time of 14 months. In this study, a total of 1106 patients were randomized to receive either imatinib 400 mg/d or a combination of subcutaneous interferon (target dose, 5 MIU/m²/d) plus subcutaneous cytarabine (20 mg/m²/d for 10 days per month). The primary end point was time to progression defined as either death, progression to accelerated or blast phase, rapidly increasing WBC, or loss of either a complete hematologic or major cytogenetic response. At the time of this analysis, 90% of imatinib patients remained on their original treatment, whereas 9% had discontinued treatment and 1% had crossed over to interferon. Only 30% of interferon patients, on the other hand, remained with their original treatment, while 31% had discontinued treatment and 39% had crossed over to imatinib, Dr. Druker reported.

A significantly superior rate of progression-free survival at 12 months was observed for imatinib patients (97%) vs interferon patients (80%). More importantly, only 1.5% of imatinib patients progressed to accelerated phase or blast crisis, compared with 7% of interferon patients. “Of note, this analysis uses a strict intent-to-treat principle that may actually overstate the rate of progression-free survival for interferon, because approximately 40% of the interferon-treated patients crossed over, and we know that imatinib is an effective salvage therapy for patients who fail interferon,” Dr. Druker said.

Moreover, 68% of imatinib patients achieved a complete cytogenetic response (absence of detectable Ph chromosome-pos-

itive cells in metaphase), as compared with 7% of interferon patients. When the study data were analyzed with a Kaplan-Meier approach to adjust for the high discontinuation and crossover rate of interferon, response-rate differences between treatment groups remained large and statistically significant in favor of imatinib. According to Dr. Druker, toxicity profiles of the 2 treatment arms were consistent with those in the published literature.

Raising the Imatinib Dose

IRIS clearly established the clinical benefits of imatinib in patients with newly diagnosed CML. Could treatment outcomes be improved in this patient population by increasing the dose of imatinib? To answer this question, Kantarjian and colleagues are currently analyzing preliminary results from 2 sequential trials, one assessing the standard 400-mg dose of imatinib and one assessing an 800-mg dose in patients with newly diagnosed CML in chronic phase.

Susan O’Brien, MD, of the M.D. Anderson Cancer Center in Houston, Tex, presented results from these 2 trials at ASCO. Both doses of imatinib produced high cytogenetic response rates at 3 and 6 months, she said. The complete cytogenetic response rate for imatinib 400 mg was 36%, compared with 52% for imatinib 800 mg. At 6 months, the complete cytogenetic response rate was more than 50% for both doses and slightly higher for the 800-mg dose, although this difference was not statistically significant. In addition, PCR analysis of the ratio of BCR-ABL over normal ABL protein showed that a significant fraction of patients treated at both doses had values less than 0.5.

The data also suggest that cytogenetic responses are faster for patients treated with the 800-mg dose. Dr. O’Brien stressed, however, that the much shorter follow-up currently available for the 800-mg study makes it difficult to determine whether the 800-mg dose of imatinib will provide a better cytogenetic response rate over the long run. “However, it is very striking that at 6 to 9 months, the only patients so far who are PCR-negative are those treated with the 800-mg dose,” she said.

The potential benefits of high-dose imatinib were also seen in a study of CML patients resistant or intolerant to interferon who were treated with imatinib 400 mg twice daily (800 mg/d). Data available for 35 patients as of March 2002 show that 87% of patients had achieved a major cytogenetic response at 3 months, according to Jorge Cortes, MD, also of the M.D. Anderson Cancer Center. These results compare favorably with those reported using a dose of 400 mg/d (major cytogenetic response of 37% at 3 months) and warrant a formal comparison of high-dose and standard-dose imatinib, Dr. Cortes concluded. ■

SUGGESTED READING

Kantarjian H, Sawyers C, Hochhaus A, et al, for the International STI571 CML Study Group. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*. 2002;346:645-652.

HIGHLIGHTS FROM THE LITERATURE

Combination Therapy With Thalidomide Plus Dexamethasone for Newly Diagnosed Myeloma

Rajkumar VS, Hayman S, Gertz MA, et al. *J Clin Oncol*. 2002;20:4319-4323.

Rationale

The current standard of care for multiple myeloma patients with good performance status is combination nonalkylator chemotherapy for 4 to 6 months, followed by high-dose therapy with autologous stem-cell transplantation. A preferred regimen is vincristine, doxorubicin, and dexamethasone (VAD). Despite relatively high response rates (55% to 65%), however, this infusional therapy is associated with significant toxicity and places patients at increased risk for infection, thrombosis, and sepsis due to an indwelling central venous catheter.

Recent evidence suggests that the addition of dexamethasone to thalidomide can provide clinical benefit for patients who have previously failed treatment with thalidomide alone. This study was con-

ducted to determine whether thalidomide and dexamethasone combined could provide an orally administered, less toxic alternative to VAD and other similar regimens.

Methods

Fifty patients (median age 61 years, range 33 to 78 years) with newly diagnosed multiple myeloma were scheduled to receive thalidomide 200 mg/d for 2 weeks and 200 mg/d dose increases every 2 weeks to a maximum of 800 mg/d as tolerated. Dexamethasone 40 mg/d was given orally in monthly cycles on days 1 to 4, 9 to 12, and 17 to 20 (odd cycles) and on days 1 to 4 (even cycles). Patients were evaluated every 4 weeks for response. After 4 cycles, candidates for high-dose therapy were allowed to terminate study treatment and pursue stem-cell collection and transplantation.

Main Results

Confirmed response—a greater than 50% reduction in serum and urine M protein—was seen in 32 patients, yielding a response rate of 64% (95% CI, 49% to 77%). Overall, 46 patients (92%) achieved a

greater than 25% reduction in M protein. After 4 cycles of therapy, 31 patients have proceeded to stem-cell collection, and 26 have undergone stem-cell transplantation. Early in the study, the dosing schedule was altered when unexpected skin toxicities occurred in 2 patients. Subsequent participants were kept at a constant thalidomide dose of 200 mg/d, reduced to 50 to 100 mg/d if they experienced grade 2 or higher toxicity. Major grade 3 or 4 toxicities were observed in 17 patients—most frequently deep vein thrombosis (6), constipation (4), rash (3), and dyspnea (2). Three deaths early in the trial were caused by pancreatitis, pulmonary embolism, and infection.

Conclusions

Combination therapy with thalidomide plus dexamethasone is a feasible and active regimen in the treatment of multiple myeloma. This combination merits further investigation as a possible oral alternative to infusional chemotherapy regimens currently used for induction therapy prior to autologous stem-cell transplantation. ■

Randomized Controlled Trial of 5-Azacytidine in Patients With MDS: A Study of the Cancer and Leukemia Group B

Silverman LR, Demakos EP, Peterson BL, et al. *J Clin Oncol*. 2002;20:2429-2440.

Rationale

Myelodysplastic syndrome (MDS) is characterized by morphologic features of dyspoieses, a hyperproliferative bone marrow, and peripheral-blood cytopenias involving one or more lineages. The French-American-British (FAB) classification system cat-

egorizes MDS into 2 broad groups (with related subgroups) based on the presence of excess blasts. Patients exhibiting refractory anemia with excess blasts (RAEB) are considered at higher risk of mortality from bone marrow failure or transformation to acute leukemia than patients exhibiting refractory anemia (RA). The majority of MDS patients with RAEB or related FAB subtypes die within 1 year from progressive bone marrow failure because of hemorrhage or infection. In addition, 35% to 40% of patients transform to acute leukemia refractory to current therapies.

Currently, the standard of care for high-risk (FAB, RAEB, and subtypes) MDS patients is supportive care with antibiotics

and periodic transfusions. This study evaluates the effectiveness of the addition of 5-azacytidine (Aza C), compared with standard therapy, in terms of patient response, mortality or leukemic transformation, and quality of life.

Methods

A total of 191 patients satisfying FAB classification criteria for MDS were randomized by FAB subtype into 2 groups, with 1 receiving standard supportive care and the other receiving Aza C (75 mg/m²/d subcutaneously for 7 days every 28 days) with standard supportive care. Patients with FAB classification RA and refractory anemia with ringed sideroblasts (RASB) were required to

meet at least 1 of the following entry criteria: symptomatic anemia requiring RBC transfusion at least 3 months prior to study entry; thrombocytopenia with 2 or more platelet counts $\leq 50 \times 10^9/L$ or a significant clinical hemorrhage requiring platelet transfusions; and neutropenia with an ANC $< 1 \times 10^9/L$ and an infection requiring intravenous antibiotics. Patients receiving standard supportive care whose disease worsened were permitted to cross over into the Aza C arm.

Patients were evaluated in terms of complete response (CR), partial response (PR), improvement, median time to leukemic transformation or death, transformation to acute myeloid leukemia (AML) as first event, and quality of life (QOL) assessment using the European Organization for Research and Treatment of Cancer (EORTC) and Mental

Health Inventory (MHI) questionnaires in conjunction with telephone interviews.

Main Results

In the Aza C group, 60% of patients responded to therapy (CR = 7%, PR = 16%, improved = 37%), compared with 5% for the supportive care group (improved = 5%, $P < .001$). Median time to leukemic transformation or death was 21 months for the Aza C group, compared with 13 months for the supportive care group ($P = .007$). Transformation to AML as first event occurred in 15% of patients in the Aza C arm and 38% of patients in the supportive care arm ($P = .001$). Patients in the Aza C group scored significantly higher on QOL measures than patients in the supportive care group. (Please see adjacent summary for additional detail on QOL assessment.)

Conclusions

The present study demonstrates that Aza C is effective therapy for MDS patients within the profiles and subgroups studied. Aza C improves bone marrow function, significantly decreases and delays transformation to AML, and improves survival and QOL, as compared with supportive care. (For more on QOL benefits, see adjacent summary.)

Although Aza C is active in the regimen studied, other doses and schedules and/or combinations with other signal transduction modulators or cytokines may improve its efficacy, and further study is needed. However, data from this study suggest that Aza C should be considered the treatment of choice for MDS patients satisfying the study's entry criteria. ■

Impact of 5-Azacytidine on the Quality of Life of MDS Patients Treated in a Randomized Phase III Trial: A Cancer and Leukemia Group B Study

Kornblith AB, Herndon JE, Silverman LR, et al. *J Clin Oncol*. 2002;20:2441-2452.

Rationale

Patients with myelodysplastic syndrome (MDS) receiving palliative care often suffer fatigue, dyspnea, and reduced physical functioning and generally suffer from psychological stress. Quality of life (QOL) data from patients participating in the randomized controlled trial of 5-azacytidine (Aza C) in patients with MDS, a study of the Cancer and Leukemia Group B (see preceding summary for patient population information), were collected to assess potential QOL benefits of Aza C treatment in patients with MDS.

Methods

(See accompanying summary of Silverman et al for inclusion criteria and treatment reg-

imens.) QOL assessment was conducted using European Organization for Research and Treatment of Cancer (EORTC) QOL Questionnaire C30, Mental Health Inventory (MHI), and patient perception of improvement — based on an 11-point visual analog scale (0 = no improvement, 10 = complete improvement).

QOL assessments were conducted at the following times: study entry, before randomization, day 50 (corresponding to completion of 2 Aza C cycles and 6 days before bone marrow test for treatment response assessment), day 106 (corresponding to the completion of 4 Aza C cycles and 7 days before treatment response reevaluation), and day 182 (6 months after study entry). QOL assessment was discontinued when patients treated with Aza C either progressed or withdrew from the study. Patients who crossed over into the Aza C treatment arm were restarted on the QOL assessment at the time of crossover.

At study entry, patients were given a QOL questionnaire packet and asked to complete it at home. This was followed up by a 30- to 40-minute telephone interview conducted by 2 trained nurse interviewers. QOL packets were mailed to patients 7 to 10 days prior to the scheduled interview for subsequent assessments.

Main Results

Of the 99 patients initially randomized to the Aza C group, 56% ($n = 56$) remained on active treatment at day 182; 16% ($n = 16$) had died; 22% ($n = 22$) had terminated protocol treatment because of treatment failure, toxicity, or transformation to acute myeloid leukemia (AML); and 5% ($n = 5$) refused to complete QOL questionnaires. Of the 91 patients initially randomized to supportive care, 47% ($n = 43$) remained on study with QOL data collected through day 182 (13% [$n = 12$] of these patients remained on supportive care); 34% ($n = 31$) remained on Aza C after crossover; 23% ($n = 21$) had died; 26% ($n = 24$) had terminated protocol treatment because of treatment failure, toxicity, or transformation to AML; and 4% ($n = 5$) refused to complete questionnaires.

Patients in the Aza C arm experienced significantly greater improvement in fatigue (EORTC, $P = .001$), dyspnea (EORTC, $P = .0014$), physical functioning (EORTC, $P = .0002$), positive effect (MHI, $P = .0077$), and psychological distress (MHI, $P = .015$) over the course of the study period than patients receiving supportive care. Differences in reported fatigue and psychological state were greatest between the 2 groups

through day 106 (completion of 4 cycles of Aza C). Differences were maintained after controlling for number of RBC transfusions. Crossover design and the number of patients remaining on supportive care (13%, $n = 12$) through day 182 limit analysis to linear regression models.

Conclusions

The results demonstrate that patient QOL was significantly improved by treatment with Aza C when compared with that of patients receiving supportive care. The significant improvement in fatigue, dyspnea, physical functioning, and psychological state noted in patients receiving treatment

with Aza C and the previously noted clinical findings (see preceding summary for details) of greater treatment response and delayed time to transformation to AML or death, compared with patients receiving supportive care ($P < .001$), establish Aza C as an important treatment option for MDS patients. ■

COMMENTARY

Hypomethylating Agents in Myelodysplasia: Hope for the Future

By John C. Byrd, MD

Myelodysplasia (MDS) is a devastating disease that occurs more frequently in the elderly. Its symptoms include fatigue, infection, and bleeding as a consequence of disease-related cytopenias. The majority of patients with MDS die as a direct consequence of the disease or progress to develop acute myeloid leukemia. While allogeneic stem cell transplantation offers the opportunity to cure myelodysplasia, the application of this modality is often limited by the absence of an appropriate donor or by the patient's advanced age. Therapies for MDS outside of allogeneic stem cell transplant have not been successful. Agents that target methylation are currently under clinical investigation in MDS and offer the opportunity to reverse a molecular aberration central to this disease. These therapies include decitabine and 5-azacytidine, which both have proven efficacy.

How do we establish one or more of the hypomethylating agents as an accepted therapy for a disease when no standard treatment exists? Regulatory agencies generally require randomized trials that incorporate a "supportive care" arm to ensure that the new therapy is not harmful when phase II data do not demonstrate curative potential. Performance of trials with these new agents can be challenging, as patients are not often keen on randomization to a placebo or no therapy. This is particularly true when the underlying disease symp-

toms impair quality of life so significantly and the therapy being investigated has documented treatment efficacy.

The open-label, randomization/crossover design employed by Silverman et al in their study of 5-azacytidine vs supportive care as standard therapy illustrates what I believe to be the most ethical study design that is capable of attracting large numbers of patients and provides all individuals participating access to the therapeutic drug. This crossover design limits the likelihood of demonstrating a survival advantage, as all patients except in the case of early death could go on to receive therapy. This multicenter Cancer and Leukemia Group B (CALGB) study demonstrated significant benefits in end points such as complete response, partial response, "improvement" (ie, mono- or bilineage response or $\geq 50\%$ reduction of baseline transfusion needs), time to leukemic transformation or death, and patient quality-of-life assessment.

While an overall survival trend favored patients randomized to early 5-azacytidine vs supportive care followed by 5-azacytidine (median, 20 months vs 14 months, $P = .10$), the beneficial effect of treatment with 5-azacytidine later impaired the ability of this group to distinguish a survival benefit. This is best demonstrated by a comparison of patients originally randomized to 5-azacytidine with those in the supportive care subgroup who crossed over to active treatment late or not at all ($P = .03$).

These corroborating data provide support that 5-azacytidine is an effective therapeutic approach for MDS.

In an accompanying editorial to Dr. Silverman's study, Dr. Kantarjian rightly raises the question of whether improved survival should invariably be the "ultimate treatment end point" in trials of this sort. The oncology community has made strides in demonstrating the clinical value of surrogate end points, he observes, and regulatory agencies have begun to take notice. MDS is one disease where relief of symptoms is a worthwhile end point. Indeed, the CALGB trial did prospectively measure benefits that reflected positively on the quality of patients' lives.

The concurrent report by Kornblith et al of the randomized 5-azacytidine vs supportive care trial's quality-of-life component noted significant improvements in the domains of fatigue, dyspnea, physical functioning, and psychological well-being vs distress. Translated into clinically meaningful and understandable terms, these findings underscore the important interrelationship between the improvement of physical status seen with 5-azacytidine and the improved psychological state of patients receiving this treatment. MDS is clearly a devastating disease, and as confirmatory efficacy trials of the different hypomethylating agents mature, hope exists that one or more of these therapies will be readily available in the future. ■

Anticoagulants in Thrombosis and Cancer: The Missing Link

Mousa SA. *Expert Review in Anti-cancer Therapy*. 2002;2:227-233.

Literature Review

Observations of decreased mortality among cancer patients receiving low-molecular-weight heparin (LMWH) therapy for deep vein thrombosis (DVT) over those receiving standard heparin therapy suggest that LMWH might modify tumor progression either directly or indirectly. Although cancer patients are at increased risk of DVT or venous thromboembolism (VTE) from a variety of independent factors—including chemotherapy, immobilization, surgery, and the use of central venous catheters that contribute to a hypercoagulable state—studies have demonstrated that LMWH interferes with processes associated with tumor growth and metastasis. These processes may include fibrin formation;

binding of angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor; modulation of tissue factor; and release of tissue-factor pathway inhibitor. Additional studies have shown increased incidence of newly diagnosed malignancy among patients with unexplained VTE in the first 6 to 12 months following the thromboembolic event.

Since tumor fibrin is a consistent feature of the tumor stroma and is deposited shortly after tumor inoculation, it is believed that the ability of normal or malignant tissue to generate fibrin may influence metastasis.

In addition, excessive and sustained angiogenesis, resulting from a deficiency in endogenous angiostatic mediators and overproduction of positive regulators of angiogenesis, has been observed in tumor angiogenesis, psoriasis, rheumatoid arthritis, diabetic retinopathy, and age-related macular degeneration. In vivo effects of the LMWH tinzaparin, warfarin, anti-VIIa,

and recombinant tissue factor pathway inhibitor (r-TFPI) on angiogenesis were evaluated with the chick chorioallantoic membrane (CAM) model. Significant, comparable, concentration-related inhibitory effects were noted on endothelial cell tube formation for tinzaparin, anti-VIIa, and r-TFPI. Moreover, tinzaparin, warfarin, anti-VIIa, and r-TFPI all effectively blocked fibroblast growth factor 2 (FGF2)-induced angiogenesis in the CAM model by 80% to 100%.

Preclinical studies also have indicated that the LMWH tinzaparin may be very effective at causing the release of natural TFPI from endothelial cells, suggesting a potential role for tinzaparin and its releasable TFPI in preventing tumor metastasis.

While the precise mechanism by which anticoagulants affect tumor metastasis is not fully understood, it is nonetheless clear from the evidence that there are a number of potential benefits from anticoagulant therapy beyond the management of DVT and VTE. ■

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