

# CME

## CONTINUING MEDICAL EDUCATION

# EFFICACY OF A MEDICAL NUTRIMENT IN THE TREATMENT OF CANCER

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### STATEMENT OF PURPOSE

Natural products are being used as supplements by cancer patients, with or without the knowledge of their cancer treatment team. It is important to know which of these products show efficacy against diseases such as cancer, and which are ineffective. It is also essential to define the mechanism(s) of action of natural products, especially as relevant to cancer prevention or treatment. The purpose of this article is to review the use of one such natural product, a fermented wheat germ extract (Aveamar), in the treatment regimen of cancer patients. Aveamar has shown efficacy in both animal cancer models and human clinical trials with cancer patients, but more well-controlled trials in humans are necessary to assess the full poten-

tial of Aveamar in cancer treatment. Aveamar exerts its anti-cancer effect via an array of mechanisms, likely because there are many undefined components in this product that modulate numerous biological systems in cancer patients.

### TARGET AUDIENCE

This activity is designed to meet the educational needs of physicians and other healthcare professionals who diagnose, treat and manage patients who have or are at risk for cancer.

### OBJECTIVES

Upon completion of this article, participants should be able to:

1. Define Aveamar and discuss its safety profile.
2. Describe the mechanisms by which Aveamar exerts its anti-cancer activity.
3. Review the efficacy of Aveamar in cancer prevention and therapy, both in animal models and human clinical trials. (*Altern Ther Health Med.* 2007;13(2):56-63.)

### DISCLOSURE

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Gary L. Johannung, PhD, and Feng Wang-Johanning, MD, PhD, have no real or apparent conflicts of interest to report.

**T**he American Cancer Society estimated in 2006 that more than 2.4 million new cancer cases, including basal and squamous cell skin cancers, would be diagnosed in the United States that year.<sup>1</sup> Cancer patients in the United States generally are treated using conventional therapy, which includes surgery, chemotherapy, radiotherapy, and newer, more targeted therapies such as

immunotherapy, gene therapy, angiogenesis inhibitors and targeted therapies.<sup>2</sup> With improved diagnosis and treatment, the 5-year survival rate of cancer patients will likely increase, and these cancer survivors will try to find treatments to prevent cancer recurrence and to advance longevity after a diagnosis of cancer. A recent study found that a large percentage of breast and prostate cancer patients use some form of complementary therapy, with

vitamins being used by 63% and 37% of breast and prostate cancer patients, respectively, and diet and nutrition therapy being used by 84% and 46% of these patients.<sup>3</sup> This study suggests that cancer patients have a strong desire to seek out alternative diet and nutritional therapies to augment their conventional cancer therapy. The challenge to physicians and healthcare providers, then, is to provide information to cancer patients about which therapies are likely to be beneficial to them in preventing recurrence of cancer and promoting their well-being.

This review will critically evaluate the efficacy of a plant extract that is currently being evaluated in clinical trials for treatment of cancer patients. The product is a fermented extract of wheat germ called Avemar. This article discusses how the extract is made, whether it is safe, its mode of action, and, finally, the use of Avemar in clinical cancer trials.

### WHAT IS AVEMAR, AND HOW IS IT PRODUCED?

The wheat grain kernel consists of 3 parts. The endosperm is the embryo of the kernel. It makes up 83% of the kernel and is the source of energy for new wheat plants if the kernel is planted and sprouts. It is high in starch and gluten (the protein in wheat flour), but relatively low in vitamin and mineral content. The endosperm is used to make white flour. The bran (14%) consists of the thin outer layers of the wheat kernel and contains vitamins, minerals, and fiber. The germ makes up only 2%-3% of the wheat kernel and is the most nutritious part of the wheat kernel. Nutrients are concentrated in the germ, and it is rich in vitamins, minerals, proteins, and fats. Wheat germ contains high levels of tocopherol and B vitamins. It is separated from the other wheat components by the milling process. Whole-wheat flours are made by milling the whole kernel; that is, all 3 of the above parts of the wheat kernel.

In addition to the nutrients listed above, wheat germ can be subjected to fermentation with *Saccharomyces cerevisiae* (yeast) to yield the benzoquinones 2,6-dimethoxy-benzoquinone (DMBQ) and 2-methoxy-benzoquinone.<sup>4</sup> These benzoquinones are present in unfermented wheat germ as glycosides; yeast glycosidase activity present during fermentation leads to release of the benzoquinones as aglycones. Avemar is an aqueous extract of wheat germ, fermented with *Saccharomyces cerevisiae* for 18 hours at 30°C.<sup>5</sup> After fermentation, water is decanted, and the product is spray-dried, homogenized, encapsulated, and formulated. The wheat germ fermentation end-product, which is suitable for human consumption, is a dried extract standardized to contain methoxy-substituted benzoquinones (2-methoxy-benzoquinone and 2,6-DMBQ) at a concentration of 0.04%. Since Avemar is a complex mixture, additional, as yet poorly characterized molecules remain in the product.

Nobel laureate and Hungarian scientist Dr Albert Szent-Györgyi initially proposed the use of methoxy-substituted benzoquinones like those present in Avemar as anticancer agents. He hypothesized that disorders of metabolism might play important roles in cancer development, and found that high redox potential quinones such as those discussed above could block cell replication<sup>6</sup> and suggested that they might prove to be useful in reversing disorders of cellular metabolism. Avemar was developed by the

Hungarian biochemist Máté Hidvégi and was registered in Hungary as medical nutriment no. 503 in 2002. It is approved there as a non-prescription medical nutriment for cancer patients. Avemar also has been registered as a special nutriment for cancer patients in the Czech Republic and Bulgaria and is on the Australian register of Therapeutic Goods. It is currently available in 10 countries. In the United States and a number of other countries, Avemar is classified as a dietary supplement. It is manufactured in a Good Manufacturing Practices (GMP) facility by Biomedicina First Hungarian Corporation for Cancer Research and Oncology in Budapest and is distributed in the United States as Avé, a dietary supplement instant-drink mix.

### IS AVEMAR SAFE TO CONSUME?

Several studies have been carried out to evaluate the safety of Avemar in doses used for treatment of cancer and autoimmune diseases. Boros et al discussed some of the studies in animals and humans that provide an indication of its safety;<sup>7</sup> studies in these species to date suggest few adverse effects of Avemar. Acute and subacute toxicology tests carried out in a Good Laboratory Practice (GLP) setting revealed minimal side effects. Toxicity studies in the rat and mouse demonstrated an acute oral LD<sub>50</sub> of Avemar in male and female mice and rats of greater than 2,000 mg/kg. The no-observable adverse effect level, which is the greatest concentration or amount of Avemar that causes no detectable adverse alteration, was 2,000 mg/kg/day in rats, and in a subchronic study with mice and rats was found to be 3,000 mg/kg/day. There is a wide therapeutic window for Avemar. Doses toxic to normal cells are more than 50 times higher than the dosage recommended for therapy, which suggests that a wide range of therapeutic dosages can be tested before the product becomes toxic.

The US Food and Drug Administration recently granted Avemar a status of Generally Recognized As Safe (GRAS), which allows it to be used in foods, drinks, and dietary supplements. Significant side effects have not been reported, but mild and transient diarrhea, nausea, flatulence, soft stool, constipation, dizziness, and increase in body weight can accompany the consumption of Avemar. Hematologic evaluations of hospitalized cancer patients in Hungary found that the white blood cell count, lymphocyte count, neutrophil granulocyte count, monocyte count, eosinophil granulocyte count, hemoglobin level, red blood cell count, erythrocyte sedimentation rate, hematocrit, platelet count, and prothrombin level were normal after 1-5 years of Avemar treatment.<sup>7</sup>

### MECHANISM OF ACTION OF AVEMAR

Since Avemar is a plant extract, the exact chemical composition is not known, and the constituent(s) that is active against cancer has not yet been identified. The methoxy-substituted benzoquinones are good candidates for the active ingredients in Avemar, but studies have shown that these may not be the important compounds in Avemar possessing immunostimulatory activity. As discussed in more detail in the "Immunomodulation"

section of this article, Avemar in mice shortened the survival time of skin grafts in comparison to controls. However, DMBQ given in a dose equivalent to the lower Avemar dose did not have any effect on skin grafts whereas a DMBQ dose equivalent to the higher dose of Avemar actually elongated the graft survival. In addition, the higher DMBQ dose was toxic and resulted in the death of 5 experimental animals during the study.<sup>8</sup>

Avemar has documented anticancer activities, which will be discussed in the next section. Many cancer patients are using Avemar as a cancer treatment, so it is important to understand its mode of action, both from the standpoint of providing an explanation for any untoward effects that might develop with its use, as well as to identify potential novel pathways that lead to beneficial effects in cancer patients. What are some of the potential mechanisms that modulate the anticancer effects of Avemar? The molecular targets of Avemar, discussed below, include apoptosis induction via poly (ADP-ribose) polymerase (PARP) and other pathways, the immune system, major histocompatibility complex (MHC) class I, ribonucleotide reductase (RNR), cyclooxygenase (COX-1 and COX-2) enzyme activity, intracellular adhesion molecule (ICAM) 1, tumor necrosis factor alpha (TNF- $\alpha$ ) production, and transketolase (TK). This is a relatively large number of molecular targets, which suggests that several as yet undefined components of Avemar may promote its antineoplastic action. The discovery of individual active compounds in Avemar should thus be pursued to find which components are responsible for each biological effect.

#### **Cell Cycle, Induction of Apoptosis and Poly Polymerase Cleavage**

Avemar influences apoptosis (programmed cell death) via several molecular pathways. Since apoptosis involves the killing of cancer cells, a major mechanism of Avemar action is apoptosis induction. Probably the most significant effect on apoptosis is cleavage of PARP. As discussed below, Avemar activates downstream caspase-3 proteases, resulting in cleavage of PARP and subsequent prevention of DNA repair in cancer cells.

The cytotoxic effects of Avemar have been documented in several studies, and cell death generally occurred by apoptosis and in some cases, necrosis. Avemar treatment decreased the number of Jurkat T-cell leukemia cells that accumulated a formazan dye (MTT), and this decrease was greater at higher Avemar doses, indicating that Avemar decreases cancer cell viability.<sup>9</sup> Cell cycle analysis by flow cytometry after propidium iodide (PI) staining revealed that cells treated with 0.7 and 1 mg/mL Avemar had an increase in the sub-G1 region of the cell cycle, which is associated with apoptosis, and a significant decrease in the S phase, and these changes became prominent at 48 and 72 hours following Avemar treatment. The effective dose of Avemar for inhibiting tumor metastasis formation in cancer patients in clinical trials is 0.5 to 1 mg/mL,<sup>10</sup> so this dosage is physiologically relevant. Avemar caused an increase in apoptosis, as measured by flow cytometry after PI and annexin V staining, in Jurkat cells, beginning at doses of 0.5 mg/mL. Doses of 0.5 and 1 mg/mL showed a greater apoptotic response at 72 hours than at 24 hours

of treatment, and doses of 5 and 10 mg/mL Avemar showed a time-independent maximal effect, with approximately 90% of cancer cells undergoing apoptosis. Laser scanning cytometry experiments showed that Avemar-treated cells had undergone apoptosis, not cell death by necrosis. The authors of this study used a caspase inhibitor to see whether the phosphatidylserine externalization characteristic of caspase action is reversed in Avemar-treated cells. Movement of phosphatidylserine from the inner to the outer plasma membrane of the cell is a characteristic that distinguishes apoptosis from necrosis. The caspase inhibitor Z-VAD.fmk did indeed block the Avemar-induced increase in apoptosis in cells treated with 1 mg/mL Avemar for 72 hours, thus demonstrating that the apoptosis resulting from Avemar treatment is due to caspase activation. To further investigate the involvement of caspases in Avemar action, the effect of Avemar at doses of 0.3, 0.5, and 0.7 mg/mL on cleavage of PARP was determined, and cleavage of PARP was observed at Avemar doses above 0.5 mg/mL and was especially evident at a 0.7-mg/mL Avemar concentration. PARP plays an important role in DNA repair, and its cleavage leads to DNA fragmentation, resulting in the apoptosis that accompanies Avemar treatment.

Breast cancer cells also respond to Avemar by inducing apoptosis. Marcsek et al reported that viability of the breast cancer cell lines MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative) began to decrease when the cells were treated with levels of Avemar between 0.625 and 1.25 mg/mL,<sup>11</sup> levels roughly the same as those cytotoxic to Jurkat cells in the studies described above. Cell cycle S phase and apoptosis were determined by flow cytometry based on PI and anti-5-bromo-2'-deoxyuridine (BrdU) fluorescence. Avemar strongly enhanced apoptosis of MCF-7 cells 24 and 48 hours after treatment, and this effect on apoptosis was even greater in cells treated with a combination of Avemar and the estrogen receptor modulator tamoxifen. In contrast to what was observed with Jurkat cells, the percentage of MCF-7 cells in the S phase of the cell cycle increased after 24 hours of Avemar treatment, followed by a decrease to control levels in cells treated for 48 hours.

Colon cancer HT-29 cells showed decreased colony formation in clonogenic assays, with an IC<sub>50</sub> value for Avemar (concentration of Avemar that results in 50% of the colony formation observed in controls) of 0.118 mg/mL,<sup>12</sup> which is considerably lower than the levels of 0.5 to 1 mg/mL that show clinical efficacy. When vitamin C was co-administered with Avemar, the IC<sub>50</sub> was lowered still further, with a value of 0.075 mg/mL when 100  $\mu$ M vitamin C was added. Vitamin C was used here because it was previously demonstrated that vitamin C influenced the effects of Avemar when these 2 compounds were co-administered. Similar to what was found in the studies with Jurkat cells, Avemar increased the percentage of cells in the G<sub>0</sub>-G<sub>1</sub> phase of the cell cycle and led to an arrest of the cell cycle in the G1 phase, with a subsequent depletion of cells in the S and G<sub>2</sub>-M phases. In contrast to the effect of Avemar almost exclusively involving apoptosis in Jurkat cells, in HT-29 colon cancer cells, Avemar in concentrations of 0.8 to 3.2 mg/mL induced predominantly

necrosis rather than apoptosis, although apoptosis did begin to increase at high Avemar concentrations.

One important aspect of Avemar as it relates to cell death is that it does not induce apoptosis in normal cells such as peripheral blood mononuclear cells.<sup>13</sup>

### **Immunomodulation and Inhibition of MHC-I**

Avemar has a stimulatory effect on cellular immune response, an effect first observed in mice.<sup>8</sup> In this study, Avemar increased the amount of <sup>3</sup>H thymidine incorporated into mouse spleen lymphocytes in response to Concanavalin A, which means that it stimulated lymphoblastic transformation. Further, an attempt was made to determine whether Avemar could influence the rejection period of skin grafts. A skin allograft from one mouse species was implanted into another, thymectomized mouse species. Thymectomized mice treated with Avemar at doses ranging from 0.03 g/kg to 3.0 g/kg had significantly shorter graft survival times than thymectomized mice not treated with Avemar. This indicates that Avemar stimulated cellular immune response of the recipient mice so that they rejected the skin grafts from donors more quickly than recipients not treated with Avemar.

Tumor cells can avoid the adaptive immune response of cytotoxic T lymphocytes by downregulating major histocompatibility complex class I (MHC class I) expression on the cell surface. Fajka-Boja et al found that Avemar treatment decreased major histocompatibility complex class I (MHC class I) antigen expression on the cell surface of lymphoid tumor cells. The cells included Jurkat leukemic T cells and 2 mutant phenotypes of this cell line (one of which was CD45 deficient), the Burkitt lymphoma B cell lines BL-41 and Raji, and the myelo-monocytic cell line U937.<sup>15</sup> They found that 4-hour treatment of Jurkat cells with 2 mg/mL Avemar caused a 90% decrease in cell surface MHC class I molecules and that the DMBQ component of Avemar caused a 70% decrease in MHC class I expression. In Raji cells treated with Avemar and DMBQ under the same conditions, MHC class I was downregulated by 69% and 30%, respectively. The wheat germ agglutinin present in Avemar was not responsible for the downregulation of cell surface MHC class I. When tyrosine phosphatase activity was inhibited with vanadate, MHC class I inhibition was greater than with Avemar alone, whereas blockage of the Avemar-induced Ca<sup>2+</sup> influx with the Ca<sup>2+</sup> chelator EGTA led to less downregulation than was observed with Avemar alone. Vanadate also increased Avemar-induced apoptosis, while EGTA decreased apoptosis due to Avemar treatment. These changes in apoptosis mirror the effects of Avemar on MHC class I downregulation.

In this same study, various other aspects of T and B cell metabolism were considered to help explain how protein phosphorylation and Ca<sup>2+</sup> transport influence MHC class I downregulation in response to Avemar treatment. The authors first found that treatment of either B or T cell lines with Avemar at 5 mg/mL for 10 minutes led to reproducible tyrosine phosphorylation of specific proteins. The pattern of tyrosine phosphorylation in response to Avemar was different from the pattern when an antibody to the T cell receptor was used to stimulate the Jurkat T cells. Proteins with

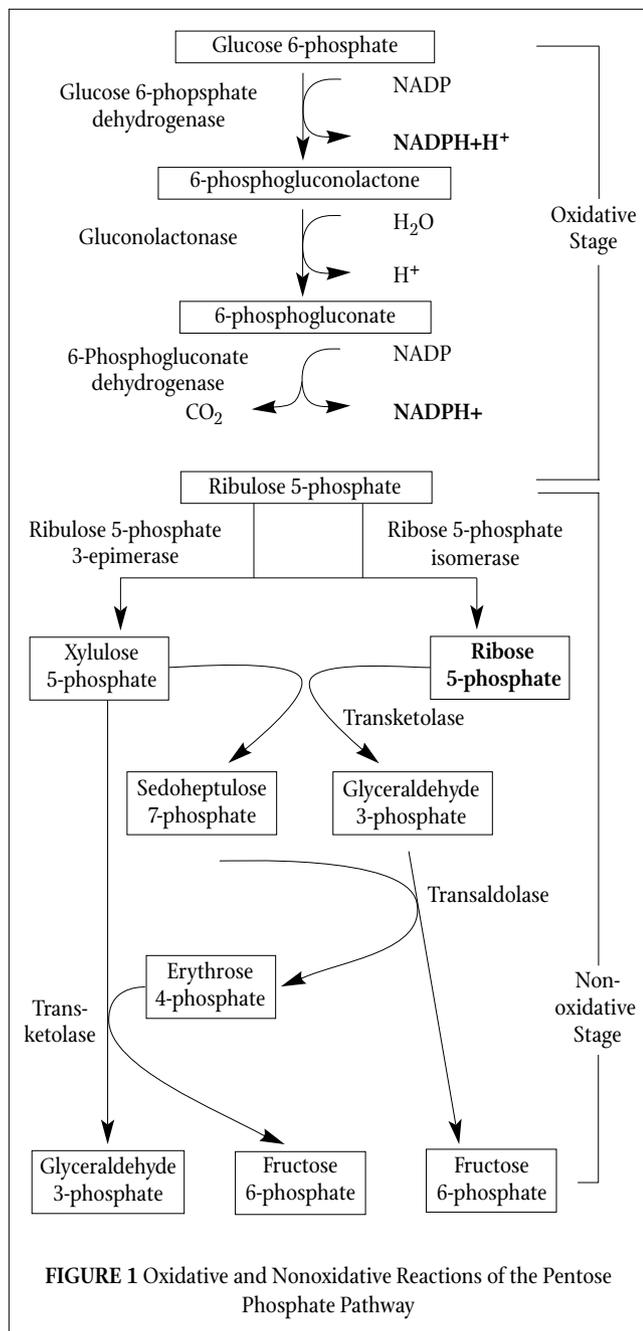
molecular weights of 76, 63, and 38 kDa were uniquely expressed in Jurkat cells treated with Avemar. This suggests that Avemar uses a mode of stimulation that is independent of the T cell receptor in Jurkat cells. Likewise, when the B cell line BL-41 was stimulated with Avemar at the same concentration and time used for Jurkat cells, the protein phosphorylation pattern was different from when these cells were B cell receptor-stimulated. The 63-kDa protein was again expressed in BL-41 cells but not in BL-41 cells stimulated with the B cell receptor, which indicates that the 63-kDa protein plays a specific role in response to Avemar that is independent of the B or T cell receptors. CD45 is a cell surface receptor expressed on leukocytes that plays a key role in leukocyte signaling.<sup>14</sup> Avemar at 5 mg/mL inhibited the tyrosine phosphatase activity of CD45, but this inhibition was found to be due to the wheat germ agglutinin present in Avemar. Finally, Avemar treatment at a concentration of 5 mg/mL caused a transient (25 to 125 seconds after treatment) 3-fold increase in the intracellular Ca<sup>2+</sup> concentration. This increase was blocked by the extracellular Ca<sup>2+</sup> chelator EGTA, showing that the Ca<sup>2+</sup> influx was from the intracellular space.

MHC class I downregulation by Avemar in T and B cell lines may make them susceptible to natural killer (NK) cell activity. NK cells are a key component of anticancer immune defense, and NK killing is blocked by MHC class I proteins expressed on the cell surface.<sup>15</sup> The overexpression of MHC class I molecules on the cell surface is one means by which cancer cells evade eradication by the immune system by avoiding the attack of NK cells. If Avemar decreases the expression of MHC class I proteins on the cancer cell surface, this should make the cancer cells more susceptible to NK cell killing.

### **Pentose Phosphate Pathway, Glucose, and Nucleic Acid Metabolism**

The expression of genes that promote and suppress cancer, such as oncogenes and tumor suppressor genes, is modified in tumors; these genes play critical roles in cancer cell proliferation, differentiation, and death. Another difference between normal and tumor cells is the modification of biochemical pathways in tumors. Warburg first observed anaerobic metabolism and fermentation of glucose even in the presence of oxygen, a phenomenon known as aerobic glycolysis, in tumors. This metabolic abnormality leads to the production of large amounts of lactate by the tumor, a process known as the Warburg effect.

The pentose phosphate pathway operates in the cytoplasm and is also capable of oxidizing glucose. This pathway has 2 sets of reactions, an oxidative branch and a nonoxidative branch. The oxidative branch of the pentose phosphate pathway converts glucose to ribulose and generates NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) via an initial reaction catalyzed by glucose-6-phosphate dehydrogenase (Figure 1). The ribulose can then be converted to ribose. This direct glucose oxidation pathway produces reducing equivalents in the form of NADPH. Large amounts of NADPH are required for biosynthetic reactions such as fatty acid and steroid biosynthesis. The enzyme ribonucleotide reductase also requires NADPH as the electron



source for conversion of ribonucleotides to deoxyribonucleotides.

The nonoxidative branch of the pentose phosphate pathway converts the ribulose produced in the oxidative branch to ribose-5-phosphate as well as fructose and glyceraldehyde (Figure 1). In this branch, ribose-5-phosphate for nucleotide and nucleic acid synthesis can be produced without the requirement of NADP. Ribose is needed to make the nucleotides required for DNA and RNA synthesis in proliferating cancer cells, and cancer cells use the nonoxidative branch of the pentose phosphate pathway for ribose synthesis. In fact, one of the hallmarks of cancer cells is that they use glucose primarily for synthesis of ribose. In these rapidly dividing cells, the nonoxidative branch is reversed, and the glycolytic

substrates fructose-6-phosphate and glyceraldehyde-3-phosphate are used as substrates for the biosynthesis of ribose. A key regulatory enzyme in the nonoxidative branch of the pentose phosphate pathway is transketolase, a thiamine-dependent enzyme. A large fraction (more than 85%) of the ribose used for nucleic acid synthesis in cancer cells comes from the nonoxidative pathway.<sup>16</sup> When transketolase activity is inhibited, tumor cell proliferation is decreased,<sup>17</sup> whereas the addition of thiamine to activate transketolases led to tumor growth stimulation.<sup>18</sup>

Avemar has specific effects on glucose metabolism and the pentose phosphate pathway, and these effects likely explain some of the preventive and therapeutic effects of Avemar against tumor growth. Boros et al<sup>10</sup> determined the effect of Avemar on ribose formation, glucose uptake, and fatty acid synthesis in cultured MIA pancreatic adenocarcinoma cells. They used [1,2-<sup>13</sup>C<sub>2</sub>] stable glucose isotopic tracer as a tool to study glucose metabolism. In this system, <sup>13</sup>C incorporation from glucose labeled on carbons 1 and 2 into ribose molecules labeled on the first carbon of ribose represent ribose produced by the oxidative branch of the pentose phosphate pathway, while ribose molecules labeled on the first 2 carbons represent ribose synthesis via the nonoxidative branch.<sup>19</sup> Thus, the ratio of ribose labeled on the first carbon to that labeled on the first 2 carbons in nucleic acids provides an estimate of the relative metabolism of glucose in the oxidative versus the nonoxidative branches of the pentose phosphate pathway. This approach revealed that increasing doses of Avemar had an inhibitory effect on glucose consumption but did not affect lactate production in these pancreatic cancer cells. The decreased glucose consumption may be in part responsible for decreased proliferation of Avemar-treated cells. Furthermore, there was a dose-response inhibitory effect of Avemar on ribose synthesis from glucose in both the mRNA and rRNA fractions of these cells. Avemar also strongly decreased the isotope uptake through the nonoxidative branch of the pentose phosphate pathway and increased oxidation of the first carbon of glucose via the oxidative branch of the pentose phosphate pathway. Acetyl groups for fatty acid synthesis were enriched in <sup>13</sup>C, and this correlated with a substantial increase in the fraction of palmitate, the most abundant fatty acid in membranes, in cells treated with Avemar.

Another study in Jurkat cells confirmed that <sup>13</sup>C incorporation from glucose into the ribose of RNA was decreased in a dose-dependent fashion after treatment with Avemar.<sup>9</sup> This decreased conversion of glucose to ribose was accompanied by decreased activity of glucose-6-phosphate dehydrogenase and transketolase (Figure 1). In support of the studies described above for pancreatic adenocarcinoma cells, these authors also found decreased glucose uptake and increased (but nonsignificant) carbon flux through the pentose phosphate pathway in Jurkat cells treated with Avemar.

Ribonucleotide reductase (RR) is the enzyme that converts ribonucleotides to deoxyribonucleoside triphosphates, which are required for *de novo* DNA synthesis. RR is upregulated in cancer cells so that they can effectively synthesize the DNA required for rapid proliferation of these cells.<sup>20</sup> Because of the importance of RR in cancer cell proliferation, it is a target for chemotherapeutic

agents, and several of these RR inhibitors, such as 3-AP (Triapine), gemcitabine, and GTI-2040, have entered clinical trials.<sup>21</sup> To evaluate the effect of Avemar on RR activity, HT-29 colon cancer cells were treated with Avemar in doses of 0.4, 0.8, and 1.6 mg/mL for 24 hours and then pulsed with <sup>14</sup>Cytidine for 30 minutes in an *in situ* assay. This was followed by total genomic DNA extraction and measurement of radioactivity in the DNA samples to determine how active RR was in converting cytidine into DNA. The incorporation of label into DNA was found to decrease in a dose-dependent manner, with the highest level of Avemar giving only 13.5% of the incorporation that was observed in controls not treated with Avemar.<sup>12</sup> The dNTP pool sizes were decreased with Avemar treatment, which would be expected if RR were inhibited, thus confirming the results showing that Avemar inhibits RR. RR inhibition by Avemar would help explain how Avemar inhibits proliferation of cancer cells.

These studies collectively suggest that Avemar normalizes pathways of glucose metabolism that have become deranged in cancer. It reverses the increase in nucleotide synthesis from glucose that is observed in cancer cells, and instead redistributes glucose for the synthesis of fatty acids and lipids, which may have the beneficial effect of promoting weight gain in cancer patients. Decreased availability of ribose for nucleic acid synthesis, coupled with inhibition of RR, both of which are observed in cancer cells treated with Avemar, would provide a double obstacle for nucleic acid synthesis by cancer cells. Importantly, the effects of Avemar on tumor cell glucose metabolism are not observed in normal cells unless the Avemar dose is greatly increased.

### Avemar Induces Cytokine Production

Cytokines are crucial early mediators of inflammation and overall immune response. Cytokines play an important role in the promotion of pathological conditions that can lead to chronic inflammation and other disorders. The cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induces hemorrhagic necrosis in tumors and is one of the major cytokines capable of killing tumor cells. In humans, TNF- $\alpha$  is produced mainly by activated macrophages and epithelial cells, and is a key mediator of apoptosis, inflammation, and immunity. It directly stimulates apoptosis and is capable of inhibiting angiogenesis in tumor cells. TNF- $\alpha$  induces interleukin-1 $\alpha$  (IL-1 $\alpha$ ), IL-1 $\beta$ , IL-6, IL-8, ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1) expression via the activation of the transcription factor NF $\kappa$ B. The promoter regions of these genes contain an NF $\kappa$ B-responsive element. NF $\kappa$ B regulates the expression of proinflammatory molecules in response to cytokines, oxidative stress, and infectious agents.

Telekes et al evaluated the effect of Avemar on proinflammatory cytokine production in myeloid (monocyte-macrophage lineage: MonoMac6, THP-1, P388D, and RAW264.7) and nonmyeloid (WEHI 164, WC1, Raji, Sci1, BCL1, CDC-HMEC, and HeLa) cell lines.<sup>22</sup> Prior to studies using cytokines, they evaluated whether Avemar influenced the growth of these cell lines. Since cancer patients consume Avemar for relatively long periods, the cells were grown in the presence of Avemar for 72 hours prior to

conducting each experiment and then for an additional 24 hours during the experiment. Avemar at concentrations ranging from 8  $\mu$ g/mL to 1,000  $\mu$ g/mL did not alter the growth rate and survival (as measured by the MTT assay) of the nonmyeloid cell lines WC1 (TNF-sensitive) and Raji. However, Avemar did strongly inhibit growth and survival of P388D mouse myeloid leukemia cells, especially in the presence of the TNF-inducing agents bacterial lipopolysaccharide (LPS) and phorbol-12 myristate 13-acetate (PMA). The effect of Avemar in this and the other studies is specific for myeloid cells; the nonmyeloid cells showed little or no response to Avemar.

A series of experiments showed that Avemar increased TNF- $\alpha$  production, but not the TNF- $\alpha$  sensitivity of target tumor cells. LPS and PMA treatment is used to optimize TNF- $\alpha$  production. The myeloid cells in this study all showed increased LPS/PMA-induced TNF- $\alpha$  production in the presence of Avemar, and the increase was dependent on the dose of Avemar. However, when WC1 cells, which are highly TNF-sensitive, were incubated with Avemar for 24 hours and then exposed to a serial dilution of TNF- $\alpha$  in the presence of Avemar, the cell survival was not influenced by Avemar treatment. This finding suggests that Avemar did not alter the TNF- $\alpha$  sensitivity of target tumor cells. The WC1 parental cell line WEHI 164 also exhibited similar TNF sensitivities in both the presence and the absence of Avemar.

As noted above, TNF- $\alpha$  activates NF $\kappa$ B, which in turn influences the expression of the cell adhesion molecules ICAM-1 and VCAM-1 in endothelial cells. Human microvascular endothelial cells were exposed to TNF- $\alpha$  in the presence or absence of Avemar in doses of 100, 200, and 500  $\mu$ g/mL for 24 hours, and the expression of ICAM-1 and VCAM-1 was measured by flow cytometry using anti-ICAM-1 and anti-VCAM-1 fluorescent antibodies. Avemar treatment led to a dose-dependent increase in the production of ICAM-1, and when TNF- $\alpha$  was added with Avemar, the production was increased further over the level observed with Avemar alone. However, Avemar did not have any effect on VCAM-1 expression or on TNF-mediated induction of VCAM-1.<sup>22</sup> The upregulation of ICAM-1 by Avemar may be significant because endothelial cells in the vasculature of tumors have decreased expression of ICAM-1 compared to normal endothelial cells. Leukocytes commonly infiltrate solid tumors, and in so doing may trigger spontaneous regression in some cancers. Since leukocytes require ICAM-1 to leave the vascular system to infiltrate the tumor tissue, and since Avemar increased ICAM-1 expression in microvascular endothelial cells, Avemar may assist leukocyte infiltration into tumors.

In this same study, Avemar upregulated the expression of a number of cytokine genes in LPS- and/or PMA-treated myeloid cultures, including the production of mRNAs coding for IL-1 $\alpha$ , IL-2, IL-5, and IL-6 by 3.7- to 8.4-fold. The results of all these studies suggest that Avemar is involved in signaling pathways usually triggered by inflammatory cytokines. One possibility is that Avemar-treated activated macrophages overproduce proinflammatory cytokines and possibly other cytotoxic mediators that block macrophage survival,<sup>22</sup> but this prospect has not been investigated in detail.

## CANCER TREATMENT WITH AVEMAR

Avemar has been tested for efficacy and toxicity in animals and human clinical trials. The gold standard for clinical trials is the randomized, placebo-controlled, double-blind clinical trial, and these types of trials in cancer patients treated with Avemar have not been reported. However, the clinical trials that have been done in humans are promising and have shown efficacy with few harmful side effects. The results of animal and human clinical trials aimed at treating cancer with Avemar are summarized here.

### Animal Carcinogenesis Models

Zalatnai et al found that Avemar inhibited experimental colon carcinogenesis in a rat model.<sup>23</sup> They induced colon carcinogenesis in male F-344 rats by injecting the animals subcutaneously with the colon carcinogen azomethane. Rats were injected 3 times, at 1-week intervals. Avemar was given by gavage (stomach tube) at a dose of 3 g/kg body weight once a day. The treatment group of rats (designated group 3) received Avemar daily, beginning 2 weeks prior to the first injection of azomethane. Controls were rats treated with neither Avemar nor azomethane (group 1), rats treated with azomethane but not Avemar (group 2) and rats treated with Avemar but not azomethane (group 4). Rats were killed and tumors were counted at the end of the 32-week study. In addition, aberrant crypt foci, which are preneoplastic lesions of the colon, were counted. Tumors developed in the colon of 83% of the azomethane-treated rats, but in Avemar-treated rats this percentage was significantly decreased, to 44.8%. The average number of tumors per animal was also decreased from 2.3 in azomethane-treated rats to 1.3 in Avemar-treated rats. The tumor diameter did not differ among treatment groups, but the number of aberrant crypt foci per cm<sup>2</sup> of colon surface was significantly lower in Avemar-treated rats. This was one of the first demonstrations that Avemar can prevent cancer in laboratory animals, and the authors speculated that the mechanism of cancer prevention might be immunomodulation. Another possible mechanism whereby Avemar might prevent colon carcinogenesis is inhibition of cyclooxygenases. Modulation of the inflammation produced by the expression of cyclooxygenases (COX-1 and COX-2) is turning out to be a promising area of research for cancer prevention and therapy; COX-2 inhibitors show anti-inflammatory and anti-cancer activities.<sup>24</sup> In vitro experiments aimed at determining the Avemar concentration that inhibits COX-1 and COX-2 showed that the IC<sub>50</sub> values for Avemar (Avemar concentration that gives 50% inhibition of COX activity) were 0.1 mg/mL for COX-1 and 0.3 mg/mL for COX-2. These Avemar concentrations, as noted earlier, are similar to levels of 0.5 to 1.0 mg/mL that show efficacy in cancer patients. Thus, Avemar is a strong but nonselective inhibitor of the cyclooxygenases.

Experiments were carried out by the same group of researchers in mice using Lewis lung carcinoma (3LL-HH), B16 mouse melanoma, C38 colorectal tumor cells, and HCR-25, a human colon carcinoma xenograft, with and without Avemar treatment.<sup>25</sup> Tumors were implanted, and 24 hours later Avemar was administered by gavage at a daily dose of 3 g/kg body weight. Control mice

received tap water in the same volume as the Avemar-treated group. There was a significant (71%) decrease in the number of liver metastases from 3LL-HH lung carcinoma cells that were injected into the spleen. Avemar treatment for 50 days decreased liver metastases from the spleen of mice implanted with the HCR-25 cells. Avemar treatment significantly decreased (by 85%) the number of metastases from B16 melanoma cells inoculated into the muscle. A final study by this group aimed to determine whether treatment with Avemar at 3 g/kg body weight would influence outcome in animals treated with the antineoplastic agents 5-fluorouracil and dacarbazine (DTIC), which are used in standard chemotherapy regimens in cancer patients. The Avemar + DTIC treatment reduced the number of lung metastases from B16 melanoma cells inoculated into the muscle to a level lower than did DTIC treatment alone. The combined Avemar + 5-fluorouracil likewise synergistically decreased the number of liver metastases from C38 colorectal carcinoma cells implanted in the spleen.<sup>25</sup>

### Clinical Studies in Humans

#### Colorectal Cancer

One of the first clinical trials of Avemar was an open-label comparative cohort trial in colorectal cancer patients.<sup>26</sup> The control cohort had 104 patients who received adjuvant therapy (if necessary) alone. The therapy was a 5-fluorouracil-based standard chemotherapy regimen and/or radiation therapy following surgery. The Avemar cohort had 66 patients who received adjuvant therapy (if necessary) plus 9 g of Avemar in 150 mL of water, once or twice daily, depending on their body weight. The patients were divided into control or treatment groups based on their own preference, and those patients who refused to take the preparation formed the control cohort. Thus, no direct randomization or stratification was performed. Serum assays for methoxy-substituted benzoquinones (MBQ and DMBQ) were used as a specific monitoring technique of Avemar administration and of compliance. The median follow-up of all patients was 9 months. The primary endpoint of the study was progression-free survivals of the 2 cohorts. Tumor progression was defined as an increase of at least 25% in the overall area of the tumor size or the appearance of any new lesions. The patients consuming Avemar had significantly more advanced disease; as many as 27.3% of the Avemar patients already had stage IV of the disease (metastatic), while this value for the control patients was only 3.8%.

The results showed that progression-related events, including new recurrent disease, new metastatic lesions, and death, were significantly more frequent in the control cohort than in the Avemar group. The overall percentage of patients with progression events (new recurrent disease, new metastatic lesions, death) was 42.3% in the controls and 16.7% in the Avemar cohort. In addition, the cumulative probabilities of both progression-free and overall survivals were greater in the Avemar patients than in the controls. There were no serious adverse events, and the greatest side effect was an unpleasant taste. However, this side effect was not great enough to lead to violation of compliance, and no variation in serum levels of methoxy-substituted benzoquinones

was found in the patients. Other minor side effects in the Avemar cohort were diarrhea, nausea and vomiting, flatulence, soft stools, and constipation.

### **Pediatric Cancer Patients**

Infections are a threat to children receiving immunosuppressive chemotherapy. Avemar was tested simultaneously with chemotherapy—and on its own following chemotherapy—in an open-label, matched-pair pilot clinical study to determine whether there was any beneficial impact on the development of treatment-related febrile neutropenia in pediatric cancer patients.<sup>27</sup> Control patients did not receive Avemar supplementation. Eleven pairs of patients with different pediatric malignant solid tumors were enrolled in the study. At baseline, the staging of the tumors was the same in each pair, except for 2 cases in which patients in the Avemar group had worse prognoses that included metastasis at baseline. In addition to standard anticancer treatments, 1 patient in each pair received 6 g/m<sup>2</sup> of Avemar dissolved in water twice daily throughout the study, while the other patient (control) received standard anticancer treatments without Avemar.

Results during the follow-up period revealed no progression of the malignant disease, but the number and frequency of febrile neutropenic events at the endpoint of the study were significantly different between the 2 groups. The Avemar-supplemented patients had 30 febrile neutropenic episodes (24.8% frequency), and the control group had 46 episodes (43.4% frequency), a statistically significant difference between the groups. These results suggest that continuous supplementation with Avemar helps to reduce the incidence of treatment-related febrile neutropenia in children with solid cancers.

### **Malignant Melanoma of the Skin**

An open-label, pilot-scale, randomized, controlled, phase II clinical study was carried out to test the possible value of supportive therapy with Avemar in high-risk patients with stage III melanoma of the skin.<sup>28</sup> In a postsurgical adjuvant setting, the effect of DTIC plus an up to 12-months-long regimen of continuous Avemar administration (Avemar study group, 22 patients) was compared to DTIC treatment without Avemar (control group, 24 patients). Avemar was administered in a dose of 9 g per day daily for up to 12 months after entry to the study. At the endpoint of the trial, progression-related events were present significantly more frequently in the control group than in the Avemar group. The cumulative probability of progression-free survival and the time to progression both showed a significant difference in favor of patients consuming Avemar. There were also fewer side effects in patients receiving the combined treatment than in those of the DTIC-only group. The results indicate that continuous supplementation with Avemar in DTIC-treated high-risk melanoma patients is beneficial in terms of progression-free survival.

Avemar appears to show greatest efficacy as a supplement to cancer patients during the course of their standard anticancer therapeutic regimens. Other clinical trials of Avemar supplementation in cancer patients are ongoing, and as results from these

trials emerge over the next few years, we should gain a clearer understanding of the value of Avemar as a medical nutriment in the treatment of cancer.

### **REFERENCES**

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. *CA Cancer J Clin*. 2006;56(2):106-130.
2. National Cancer Institute. Cancer topics: Types of treatment. Available at: <http://www.cancer.gov/cancertopics/treatment/types-of-treatment>. Accessed January 29, 2007.
3. Hann DM, Baker F, Roberts CS, et al. Use of complementary therapies among breast and prostate cancer patients during treatment: a multisite study. *Integr Cancer Ther*. 2005;4(4):294-300.
4. Cosgrove DJ, Daniels DG, Greer EN, Hutchinson JB, Moran T, Whitehead FK. Isolation of methoxy- and 2,6-dimethoxy-p-benzoquinone from fermented wheat germ. *Nature*. Jun 7 1952;169(4310):966-967.
5. Hidvegi M, Raso E, Tomoskozi-Farkas R, et al. MSC, a new benzoquinone-containing natural product with antimetastatic effect. *Cancer Biother Radiopharm*. 1999;14(4):277-289.
6. Pethig R, Gascoyne PR, McLaughlin JA, Szent-Gyorgyi A. Interaction of the 2,6-dimethoxysemiquinone and ascorbyl free radicals with Ehrlich ascites cells: a probe of cell-surface charge. *Proc Natl Acad Sci USA*. 1984;81(7):2088-2091.
7. Boros LG, Nichelatti M, Shoenfeld Y. Fermented wheat germ extract (Avemar) in the treatment of cancer and autoimmune diseases. *Ann NY Acad Sci*. 2005 Jun;1051:529-542.
8. Hidvegi M, Raso E, Tomoskozi Farkas R, Lapis K, Szende B. Effect of MSC on the immune response of mice. *Immunopharmacology*. 1999;41(3):183-186.
9. Comin-Anduix B, Boros LG, Marin S, et al. Fermented wheat germ extract inhibits glycolysis/pentose cycle enzymes and induces apoptosis through poly(ADP-ribose) polymerase activation in Jurkat T-cell leukemia tumor cells. *J Biol Chem*. Nov 29 2002;277(48):46408-46414.
10. Boros LG, Lapis K, Szende B, et al. Wheat germ extract decreases glucose uptake and RNA ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells. *Pancreas*. 2001;23(2):141-147.
11. Marcsek Z, Kocsis Z, Jakab M, Szende B, Tompa A. The efficacy of tamoxifen in estrogen receptor-positive breast cancer cells is enhanced by a medical nutriment. *Cancer Biother Radiopharm*. 2004;19(6):746-753.
12. Illmer C, Madlener S, Horvath Z, et al. Immunologic and biochemical effects of the fermented wheat germ extract Avemar. *Exp Biol Med* (Maywood). 2005;230(2):144-149.
13. Fajka-Boja R, Hidvegi M, Shoenfeld Y, et al. Fermented wheat germ extract induces apoptosis and downregulation of major histocompatibility complex class I proteins in tumor T and B cell lines. *Int J Oncol*. 2002;20(3):563-570.
14. Thomas ML, Brown EJ. Positive and negative regulation of Src-family membrane kinases by CD45. *Immunol Today*. 1999;20(9):406-411.
15. Lopez-Botet M, Bellon T. Natural killer cell activation and inhibition by receptors for MHC class I. *Curr Opin Immunol*. 1999;11(3):301-307.
16. Boros LG, Puigjaner J, Cascante M, et al. Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res*. 1997;57(19):4242-4248.
17. Rais B, Comin B, Puigjaner J, et al. Oxythiamine and dehydroepiandrosterone induce a G1 phase cycle arrest in Ehrlich's tumor cells through inhibition of the pentose cycle. *FEBS Lett*. 1999;456(1):113-118.
18. Comin-Anduix B, Boren J, Martinez S, et al. The effect of thiamine supplementation on tumour proliferation. A metabolic control analysis study. *Eur J Biochem*. 2001;268(15):4177-4182.
19. Boros LG, Cascante M, Lee WN. Metabolic profiling of cell growth and death in cancer: applications in drug discovery. *Drug Discov Today*. 2002;7(6):364-372.
20. Takeda E, Weber G. Role of ribonucleotide reductase in expression in the neoplastic program. *Life Sci*. 1981;28(9):1007-1014.
21. Shao J, Zhou B, Chu B, Yen Y. Ribonucleotide reductase inhibitors and future drug design. *Curr Cancer Drug Targets*. 2006;6(5):409-431.
22. Telekes A, Kiss-Táoth E, Nagy T, et al. Synergistic effect of MSC on proinflammatory cytokine production and Ras-mediated cell activation. *Ann NY Acad Sci*. Jun 2005;1051:515-528.
23. Zalutnai A, Lapis K, Szende B, et al. Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats. *Carcinogenesis*. 2001;22(10):1649-1652.
24. Meric JB, Rottey S, Olausson K, et al. Cyclooxygenase-2 as a target for anticancer drug development. *Crit Rev Oncol Hematol*. 2006;59(1):51-64.
25. Nichelatti M, Hidvegi M. Experimental and clinical results with MSC (a dried extract from fermented wheat germ) in animal cancer models and in cancer patients. *Nogygyaszati Onkologia*. 2002;7:40-41.
26. Jakab F, Shoenfeld Y, Balogh A, et al. A medical nutriment has supportive value in the treatment of colorectal cancer. *Br J Cancer*. Aug 4 2003;89(3):465-469.
27. Garami M, Schuler D, Babosa M, et al. Fermented wheat germ extract reduces chemotherapy-induced febrile neutropenia in pediatric cancer patients. *J Pediatr Hematol Oncol*. 2004;26(10):631-635.
28. Demidov LV, Manzhuk LV, Kharkevitch GY, Artamonova EV, Pirogova NA. Antimetastatic effect of MSC in high-risk melanoma patients. 18th UICC International Cancer Congress, Oslo, Norway, 30 June-5 July, 2002. *Int J Cancer*. 2002;100(S13):408.