Glutamine, Cancer, and Its Therapy
V. Suzanne Klimberg, MD, John L. McClellan, Little Rock, Arkansas

OBJECTIVE: This overview on glutamine, cancer and its therapy discusses some of the in vitro and in vivo work on glutamine and tumor growth, and summarizes animal and human data on the potential benefits of glutamine in the tumor-bearing host receiving radiation or chemotherapy.

BACKGROUND: Glutamine is the most abundant amino acid in the body. A tumor can act as a "glutamine trap," depleting host glutamine stores and resulting in oothioxia. In vitro evidence of the dependence of tumor growth on glutamine has deterred its use in the clinic setting.

METHODS: Data from a variety of investigations studying glutamine's interaction with the tumor-bearing host receiving radiation or chemotherapy were compiled and summarized.

RESULTS: A large body of evidence in vivo suggests that supplemental glutamine does not make tumors grow but in fact results in decreased growth through stimulation of the immune system. When given with radiation or chemotherapy, glutamine protects the host and actually increases the selectivity of therapy for the tumor.

CONCLUSION: Further prospective randomized trials are needed to demonstrate the safety and efficacy in humans undergoing radiation and chemotherapy.

Glutamine, Cancer, and Its Therapy
Glutamine is the most abundant amino acid in the blood, comprising 50% of the whole body pool of free amino acids.1 Of the GLN pool 75% resides in skeletal muscle, with most of the remaining stores in the liver.13 Glutamine transports almost one third of circulating amino acids and nitrogen; it is also the principal carrier of nitrogen from skeletal muscle to visceral organs. It is necessary for nucleotide synthesis and also acts as a fuel, which is, carbon-for-carbon, as efficient as glucose.14 Concentrations of GLN in blood and skeletal muscle decrease markedly after injury and catabolic states, such as advanced malignant disease.10,13,15 Under such conditions, GLN stores may be depleted by greater than 50%, while plasma levels fall 20% to 30%. This reduction in the intracellular pool results from an accelerated release of GLN from muscle and severe host GLN depletion in all tissues.10,16

Catabolic states such as major surgery, sepsis, and cancer are characterized by alterations in the interorgan exchange of amino acids by net skeletal muscle breakdown and negative nitrogen balance.1,2 Glutamine (GLN) is a nonessential amino acid that serves not only as a primary respiratory fuel but as a necessary substrate for nucleotide synthesis in most dividing cells. Glutamine is required by enterocytes, lymphocytes, and fibroblasts as well as by rapidly growing tumors.6 Welbourne demonstrated that in kidney tissue with oxidative stress, GLN becomes rate limiting in glutathione (GSH) synthesis. In addition, a number of studies have examined the ability of exogenous glutamine to promote bowel rescue after injury from methotrexate,8 radiation injury,9 and other catabolic states such as progressive tumor growth by supporting gut glutamine metabolism.10 Several studies indicate that the disruption of interorgan glutamine flux by progressive tumor growth may contribute to host cachexia. Provision of supplemental oral glutamine alleviates depletion of muscle GLN stores. The provision of dietary GLN to the cancer-bearing host nearly doubles the tumoricidal action of methotrexate while reducing morbidity and mortality.11 Animals experience less gut toxicity and improved hematological parameters, decreased sepsis, and improved survival. Similar results have been seen with radiation therapy. Further observations have demonstrated a reduction in tumor growth with GLN alone through its effect on the immune system.12 These observations suggest that GLN may have important therapeutic as well as nutritional advantages for oncology patients. This overview reviews the available data regarding glutamine, cancer, and its therapy, and discusses the possible mechanisms by which GLN facilitates decreased tumor growth and increased tumoricidal effectiveness of radiation and chemotherapy while ameliorating host toxicity.

HOST GLN AND GSH METABOLISM
Glutamine is the principal fuel utilized by most rapidly growing tumors.2 Such tumors have high glutaminase activity, the principal enzyme of GLN degradation.14 Advanced malignant disease results in muscle GLN depletion.
and weight loss. This GLN depletion is most profound in skeletal muscle, the principal tissue of GLN storage. As the tumor grows, it becomes a major GLN consumer, behaving as a "GLN trap" that may contribute to host GLN and GSH depletion. Concern existed in the past about providing supplemental GLN to the host with cancer since it was thought to stimulate tumor growth. In vitro evidence has demonstrated a linear relationship of tumor glutaminase activity and tumor volume doubling time and a negative correlation between growth of hepatomas and intracellular glutamine. On the contrary, in vivo experiments have shown that supplemental GLN does not increase tumor growth when given via either the oral or intravenous route. Recent studies with both sarcoma and breast models demonstrate decreased tumor growth with oral GLN supplementation.

GLUTAMINE FACILITATES CHEMOTHERAPY WHILE REDUCING HOST TOXICITY

In 1988 Fox and colleagues demonstrated that the morbidity and mortality of methotrexate administered to rats was ameliorated by the oral administration of glutamine. Subsequently, it was demonstrated that oral GLN, when given alone, does not stimulate tumor growth in a rat sarcoma model. Clinical application of these findings has been inhibited by concern that glutamine would not only "protect" the host but also the tumor, thereby reducing chemotherapeutic effectiveness of methotrexate. It was therefore necessary to examine the influence of enteral glutamine supplementation on the tumoricidal effectiveness of methotrexate.

Fisher 344 rats implanted with a fast-growing sarcoma (MCA) were assigned to one of four regimens: glutamine plus methotrexate (GLN + MTX), glutamine plus saline (GLN + CTRL), glycine plus methotrexate (GLY + MTX), or glycine plus saline (GLY + CTRL). Two doses of methotrexate (5 mg/kg) or isotonic saline were given by intraperitoneal injection on days 26 and 33 of the study. At sacrifice, 40 days after implantation, the GLN + MTX demonstrated a 45% decrease in tumor volume from the control group as compared with a 28% decrease in the GLY + MTX group (P < 0.05). Tumor weights were similarly decreased. Blood cultures from the GLY + MTX group were uniformly positive as compared with the GLN + MTX group (100% versus 33.3%; P < 0.05). Survival was significantly decreased in the GLY + MTX group versus GLY + CTRL (44.4% versus 83.3%; P < 0.05) but not the GLN + MTX group versus GLN + CTRL group. There was no significant difference between groups in tumor glutaminase activity or morphometry. Tumor and blood concentrations of MTX were not measurable. Therefore, a model of high-dose MTX was designed to measure intracellular tumor MTX levels. Rats pair-fed for 2 days with chow and 1 gm/kg GLN or GLY by gavage received a single intraperitoneal dose of 20 mg/kg MTX and were killed either 24 or 48 hours after initiation of MTX. Concentrations of MTX in tumor tissue were significantly increased at both 24 and 48 hours and were associated with differences in serum methotrexate only at 48 hours. Tumor morphometrics were not different between the groups but tumor volume loss was significantly greater even at 24 hours.

These studies demonstrated that glutamine not only increased intracellular MTX tumor concentration but also increased the tumoricidal effectiveness as demonstrated by decreased tumor volume. Repeat experiments at 24 hours after high-dose MTX examined tissue and tumor GSH levels and calculated tissue oxidant injury as measured by the ratio of oxidized to reduced GSH. All host tissue had significantly elevated GSH content whereas the tumor tissue levels were significantly decreased. Figure 1 demonstrates a significant increase in oxidant injury to the tumor by MTX whereas host tissues were spared.

Dietary glutamine prevents acute and chronic radiation injury

When animals are subjected to whole abdominal radiation (XRT) after 1000 cGy XRT to the abdomen, there is a 50% 10/day mortality rate. With glutamine supplementation, this can be converted to a 100% survival. Rats receiving glutamine-enriched elemental diets after or before radiation will have a significant increase versus the control group in the jejunal villous number, villous height, and the number of metaphase mitoses per crypt. This is correlated with a decrease in the number of culture-positive mesenteric lymph nodes and general increase in well-being. Clinically, the dose-limiting toxic effect of radiotherapy on the gut is not acute mucosal injury but the late reactions of fibrosis, stricture, and necrosis. These effects are believed to be mediated at least in part by vascular injury and are responsible for significant morbidity and mortality.

Based on the preliminary data with acute radiation injury, the effects of supplemental oral GLN on long-term XRT injury in the small intestine were evaluated. After receiving orchietomies and placement of a loop of small bowel in the scrotal sac, rats were randomized to isocaloric, isonitrogenous elemental diets with 1 g/kg/day of GLN or GLY by gavage and then pair-fed chow. After 2 days of prefeeding, a single dose of 2,000 rads was delivered to the scrotum via a collimated beam. Control rats were not irradiated but received identical diets. Rats were sacrificed 2 months postirradiation at a time when chronic XRT enteropathy is stable. Chronic radiation injury was assessed with an injury score using eight histopathologic parameters es-
The light microscopic findings of the ileum of a radiated rat that did not receive glutamine are on the left. The villous tips are ulcerated. Villous fusion and chronic inflammation are present. On the right is the light microscopy of the ileum of an irradiated rat that received glutamine. The mucosal surface is intact without evidence of ulceration.

Established by Hauer-Jensen and colleagues (ulcerations, epithelial atypia, serosal thickening, vascular sclerosis, fibrosis, thickening of the intestinal wall, lymph congestion, and ileitis cystica profunda). A known correlation exists between calculated injury scores and observed morbidity and mortality rates in this animal model. Supplemental GLN prevented chronic radiation injury as demonstrated by injury scores that were similar to those of unirradiated bowel (P = not significant). Elevated injury scores in XRT + GLY correlated with gross intestinal thickening and fibrosis as well as significant decreases in villous number/cm, a tenfold decrease in gut GLN extraction and a 30% decrease in GSH content of the irradiated bowel when compared with XRT + GLN (Figure 2). Provision of GLN during abdominal or pelvic XRT may accelerate healing of the irradiated bowel, prevent injury, and decrease the long-term complications of radiation enteropathy.

**GLUTAMINE SUPPORTS HOST WHILE DECREASING TUMOR GLUTATHIONE AFTER RADIATION**

Evidence existed that intravenous glutamine increases the content of GSH in liver and has been shown to exert a protective effect against oxidant injury. Concern existed about providing dietary GLN to the tumor-bearing host since it may increase tumor GSH content and thus protect the tumor against radiation or other types of chemotherapy other than MTX. In an experiment designed to examine this question, 10 days after implantation of a MCA sarcoma, animals were randomized to receive one of four regimens: GLN + XRT, GLN only, GLY + XRT, or GLY only. Rats received isonitrogenous, isocaloric diets consisting of 1 gm/kg GLN or GLY by gavage and were pair-fed chow. After 4 days of gavage, tumors in the XRT group were irradiated via collimated beam with 2,000 cGy. Two weeks after receiving radiation, all rats were sacrificed. Provision of glutamine-enriched diet decreased tumor GSH content while increasing gut GSH levels. Grossly, there was equal tumor volume loss in both GLN and GLY supplemented rats. Tumor growth parameters appeared to be identical. The finding of decreased GSH in the irradiated tumor of the glutamine-supplemented rat was thought to indicate sensitization to fractionated doses of XRT. To test this, similarly treated animals were subjected to 1,000 rads of XRT fractionated over a 5-day period. Indeed, significantly increased tumor kill in the GLN-supplemented group following XRT was observed.

**GLN, GSH AND THE IMMUNE SYSTEM**

Glutamine is a primary fuel utilized by rapidly dividing host cells, including lymphocytes. Studies have shown that protein malnutrition and GLN depletion have a significant effect on host cell immunity, particularly in the immunological function of the gastrointestinal tract stimulating secretory IgA and decreasing bacterial translocation. These same phenomena are seen with advanced malignant disease as well as other catabolic states. Likewise, GLN is necessary for the in vitro growth and maximal function of T cells and natural killer (NK) cells. Natural killer cells, a subpopulation of cytotoxic lymphocytes present in normal individuals, are capable of spontaneous cytolytic activity against a variety of tumor cells. Unlike T cells, tumor cell killing is mediated by a non-MHC-restricted mechanism, allowing NK cells to act spontaneously at first contact. The cytokine IL-2 stimulates the activation and proliferation of human and rodent NK cells both in vitro and in vivo. This activation enhances NK cytotoxicity and, at the same time, expands the repertoire of tumor cell killing such that activated NK cells are able to kill a wider array of tumor cell types. Activated NK cells also participate in other host functions through their production of cytokines such as interferon. There is a substantial body of experimental data indicating that NK cells may serve a major role in tumor surveillance and may be the first line of defense against the blood-borne phase of tumor metastases. The role of GLN in lymphocyte activity is to provide both nitrogen and carbon for precursor synthesis of purines and pyrimidines and also for energy production. However, the rate of GLN use by lymphocytes is markedly in excess of these precursor re-
GLUTAMINE AND CANCER THERAPY/ KLIMBERG

Figure 3. Effect of glutamine (GLN) concentration on natural killer cell cytotoxicity (NKCC) in vitro. Natural killer cell activity is expressed in lytic units; one lytic unit is defined as the number of effector cells per 10⁶ mediating 20% target cell lysis. The GLN-free culture medium was supplemented with 0, 0.2, 0.5, 0.7, and 2 mmol/L GLN for a 3-day period as reflected in the top figure. The bottom figure shows the GLN-free culture was supplemented with 0.65, 1.5, and 3.0 mmol/L glutathione (reduced) and assayed for NKCC at 18 hours. Each patient's standard deviation in this assay did not exceed 10% (reprinted with permission).

Effect of GLN and GSH on IL-2 Augmented NK Cytotoxicity in Vitro

Liang et al. have demonstrated that GSH regulates IL-2 activation of cytotoxic T-cells. Juretic et al. demonstrated that a GLN deficit affects the generation of lymphokine-activated killer (LAK) cells. Recent work demonstrated the in vitro dependence of GLN and GSH on IL-2 augmentation of NK cytotoxicity (Figure 3). Changes in GLN concentration within the physiological range produced a significant rise in cell-mediated cytotoxicity that plateaued above 1 mM GLN. In a similar fashion, peripheral blood mononuclear cells stimulated with IL-2 in GLN-free culture media supplemented with 0, 0.65, 1.5 or 3.0 mM GSH were assayed for cytotoxic activity. When GSH was added to the media in place of GLN, an earlier maximal activation of anti-JY cytotoxicity was seen at 18 hours for GSH versus 3 days for GLN. Figure 3 demonstrates that GSH, added in vitro, obviated the need for GLN and followed an activation curve similar to that of GLN.

Effect of GLN and GSH on IL-2 Augmented NK Cytotoxicity in Vivo

After demonstrating an in vitro dependence of IL-2-activated NK activity on GLN and GSH, the effects of oral GLN supplementation on GLN and GSH metabolism, IL-2-augmented NK activity, and tumor growth in a rat sarcoma model were evaluated. Two days before tumor implantation, rats were randomized to receive GLN (1 g/kg/day; TUM + GLN) or isonitrogenous glycine (TUM + GLY) by gavage; all rats were pair-fed isocaloric isonitrogenous chow diets. Rats were sacrificed at 21 days post-tumor implantation; tumors were measured and processed for glutaminase activity, GSH content, and tumor morphometrics. No significant differences were observed in mean body weight or chow and gavage intakes between the GLN-fed and GLY-fed rats at tumor cell implantation or at sacrifice. Splenic lymphocytes were incubated with human...
recombinant IL-2 in vitro for 3 days. Cells were then assayed for cytolytic activity against NK sensitive, YAC-1 murine tumor target cells. Blood GLN and GSH levels were measured. A second set of rats was treated similarly except that ketamine was given twice weekly specifically to suppress the activation of NK cells.31 Natural killer cell activity in the TUM + GLN group increased by 30% compared with the TUM + GLY group (Figure 4). This increased NK activity was associated with a 30% decrease in tumor growth. Ketamine, which specifically blocks augmented NK activity, completely reversed the higher NK activity and decreased tumor growth in the GLN-treated group (Figure 4), demonstrating that GLN was acting via augmentation of NK activity.

In order to extend and expand the observations on oral GLN efficacy in other tumor systems, the effects of GLN on NK activity and breast cancer growth, using MTF-7 rat mammary carcinoma cells, were examined.22 Fisher 344 rats were implanted subcutaneously with MTF-7 cells and randomized to one of two groups: 1UM + GLN, or 1UM + freamine (FA). Freamine, a mixture of essential and nonessential amino acids that does not contain GLN was substituted for GLY as a better control in this experiment because of the concern that GLY might increase GSH levels and thereby potentially obscure a more significant effect of GLN. Rats were pair-fed chow and gavaged with 1 gm/kg/day of GLN or an isonitrogenous amount of FA, starting on day 1 of tumor implantation and continuing until sacrifice. Over the 49-day study period, tumor growth was decreased by 40% in the GLN-supplemented group. This decrease in growth was associated with a 2.5-fold increase in IL-2-augmented NK activity. In addition, 3 rats in the TUM + FA group but none in TUM + GLN had axillary metastases at the time of sacrifice (P = 0.1).

Taken together, these results suggest that upregulation of GSH via supplemental GLN will improve antitumor NK activity and suppress tumor growth both in rat sarcoma and mammary carcinoma models. Although NK activity can be augmented experimentally by interferons and IL-2, these cytokines have not been as useful clinically as anticipated.32 The modest response of cancer patients to cytokine therapy may reflect variations in depletion of host GLN and GSH stores associated with poor nutritional status or tumor progression in these patients.

POSSIBLE MECHANISMS OF ACTION OF INCREASED THERAPEUTIC INDEX OF GLUTAMINE

One mechanism by which GLN can effect MTX-induced tumor kill is through increased uptake, or more likely prevention of efflux, from the tumor via polyglutamation.23 A more inclusive mechanism by which GLN can enhance both radiation and chemotherapy toxicity is by alteration in GSH metabolism.28 Glutathione is a tripeptide that is ubiquitous and acts in a protective role against oxidant injury in normal tissue and as a resistance mechanism against radiation- and chemotherapy-related injury in tumor tissue. Wellbourne demonstrated that when the kidney receives an oxidant stress, GLN becomes rate limiting for GSH synthesis.27 In addition, GSH plays a central role in calcium metabolism, leukotriene biosynthesis, thyroid metabolism, and membrane and channel function and nutrition. Gluta-thione is also important in the protection of critical cellular molecules. Several clinical and experimental studies have shown depletion of GSH in host tissues during shock, sepsis, endotoxemia, multiple trauma, and hemorrhagic shock, and in the malnourished. Toxicity of target tissue is a result of depletion of tissue GSH concentration and protein alkylation. Depletion of greater than 70% of tissue GSH is associated with irreversible cellular damage.

Provision of supplemental oral GLN to the tumor-bearing host enhances the tumoricidal effect of MTX and radiation and is associated with a decrease in intracellular tumor GSH levels and decreased GLN metabolism. Reduced as well as total GSH content of the tumor was significantly decreased only in the GLN-supplemented group receiving MTX, which correlated with significantly greater tumor loss and oxidant injury as measured by the ratio of oxidized GSH (GSSG) to reduced GSH (Figure 1). Although MTX injection caused significant reduction in most host tissues, GLN supplementation restored GSH levels to normal. Decreased host treatment-related injury was demonstrated by a reduced GSSG to GSH ratio. A possible explanation for this dichotomy in GSH metabolism in tumor and host tissues in animals treated with radiation or chemotherapy is proposed to be the ability of GLN to bypass the acidotic block in the GSH-recycling enzyme, oxoprolinase, in host but not in tumor cells (Figure 5).35 Uptake and metabolism of extracellular GSH molecules requires the oxidation of an intracellular GSH molecule. In cells receiving radiation or chemotherapy, intracellular GSH regeneration is slowed because the pH-sensitive oxoprolinase enzyme is inhibited in the resulting acidic environment. This results in depletion of intracellular GSH levels in the tumor. However, in normal cells, the presence of abundant GLN can upregulate the gamma-glutamyl transferase and glutaminase enzymes, providing additional glutamate to bypass the blocked oxoprolinase enzyme. These enzymes in the tumor are not upregulated by supplemental GLN. If no radiation or chemotherapy is given, tumor GSH remains unchanged while host GSH stores increase and tumor growth decreases, possibly through GSH-mediated upregulation of the immune system.12

Baxevanis et al32 have shown that reduced NK cell activity in breast cancer patients is associated with increased prostaglandin (PGE2) synthesis. Prostaglandin E2 has profound effects in vitro and in vivo on cellular immunity and may influence host-tumor interplay. Findings of increased PGE2 production by a variety of tumors have prompted interest in the possibility that tumor cell PGE2 production might constitute another mechanism whereby tumors subvert immune surveillance. Moreover, inhibition of PGE2 synthesis by indomethacin or flurbiprofen is associated with decreased tumor growth. Glutathione is also an inhibitor of prostaglandin synthesis in vitro and in vivo.34 A similar experiment as described before using the MTF-7 tumor widely demonstrates this. Over a 7-week study period, tumor growth was decreased by approximately 40% in the GLN-supplemented group. This decrease in growth was associated with a 2.5-fold greater NK activity in the GLN-fed versus FA fed animals. This was associated with a 25% rise in GSH levels and a proportional 2.5-fold decrease in PGE2 synthesis. These results suggest that, when given alone,
GLN will upregulate GSH, improve NK activity, and suppress tumor growth possibly through the action of GSH on PGE2 synthesis.

It would seem that GSH could be the central mechanism by which GLN induces a number of its beneficial effects. Further work in this area is needed to clarify the mechanisms by which GLN exerts its diverse protective role.

**CLINICAL APPLICATION OF GLN**

**Clinical Trials Using Glutamine and Glutamine Analogs**

Many GLN analogs, which inhibit GLN-requiring enzymes, have been studied as possible chemotherapeutic adjuvant therapies in both animals and humans. Two of the most thoroughly studied analogs include L-DON (6-diazo-5-oxo-L-norleucine) and acivicin (a amino-3-chloro-4,5-diisoxazoleacetic acid). Toxicities exhibited in phase I trials have prohibited their use as adjuvants to chemotherapy.

Studies in humans have demonstrated the safety of GLN-supplemented total parenteral nutrition. There have now been several trials examining the effects of intravenous GLN on morbidity and mortality of bone marrow transplant patients as well as solid tumor patients. Limited non-randomized trials have been published using oral GLN for patients receiving radiation or chemotherapy. The first and perhaps the best prospective, randomized, double-blind trial of GLN in bone-marrow transplant patients was published by Ziegler and colleagues. Forty-five patients who had undergone allogeneic bone marrow transplants were randomized to receive isonitrogenous, isocaloric glutamine-free TPN (standard) or GLN-supplemented TPN (0.57 g/kg/day GLN) for 4 weeks posttransplantation. Glutamine-supplemented patients had better nitrogen balance, significantly decreased clinical infections, and significantly shorter hospital stays.

A similar study in solid tumor patients carried out by Schloerb et al found a significantly shortened hospital stay but no change in infection rate. Skubitz and Anderson published a pilot study using oral GLN (8 gm/day, not per kg) in cancer patients already demonstrating chemotherapy-induced stomatitis. The total number of days of mucositis with the next round of chemotherapy were decreased (2.7 + 0.8 in GLN versus 9.9 + 1.1 in non-GLN, P < 0.001) as well as a decrease in the grade of mucositis. Richards and colleagues in a randomized study evaluated the prophylactic efficacy or oral glutamine (21 g/day) given throughout a standardized radiotherapy protocol (2300 cGy whole-pelvis irradiation). Rectal biopsies revealed significant improvements in histological and morphometric parameters, as assessed by light microscopy. Further trials with oral and intravenous GLN are planned or are under way.

**SUMMARY**

Can GLN be an important adjunct to antitumor radiation and chemotherapy in the clinical setting? Glutamine by decreasing intracellular levels of GSH in tumor cells, makes tumor cells more sensitive to radiation and chemotherapy. At the same time, GLN restores the depressed levels of GSH in normal host tissues, thereby improving the overall host well-being and resulting in decreased morbidity and
mortality associated with cancer and its treatment.\textsuperscript{11} Thus, GLN supplementation effectively increases the therapeutic index of radiation and chemotherapy.\textsuperscript{11,12,13} Glutamine may be superior to other antioxidants, which, unlike GLN, may also enhance tumor GSH, increasing tumor resistance to therapy. The low cost of GLN, its ease of administration, and lack of toxicity make it an ideal adjunct to radiation and chemotherapy.

Cancer, with its concomitant morbidity and mortality, has a high monetary cost. An easy, safe, and inexpensive method of prevention of cancer progression and the side effects of its treatments needs to be explored.\textsuperscript{36}

\section*{REFERENCES}


27. Alverdy JC. Effect of glutamine-supplemented diets on immunity of the gut. JPEN. 1990;14(suppl):109S-113S.


35. Chance WT, Cao L, Fischer JE. Response of tumor and host to hyperalimentation and antiglutamine treatments. JPN. 1990;14:122128.


37. Schl0w PR, Amare M. Total parenteral nutrition with glutamine in bone marrow transplantation and other clinical applications (a randomized, double-blind study). JPN. 1993;17(4):407-413.

