



## Immuno-Modulators & Cancer

Although there are many Chinese and Japanese reports of the use of immuno-stimulants in treating cancer, the Western medical profession is generally unfamiliar with them. This is partly due to the Western preference for rather more aggressive Western techniques and partly due to a lack of awareness. When I recently asked a well-known UK cancer expert his views on immuno-stimulants, he said he was not interested because there was 'no data'. In fact, there are nearly a hundred papers in the scientific literature that document the effects of beta glucans and similar molecules on cancer cells in vitro, in animal models and in clinical trials.

In the following monograph I have generally restricted myself to articles published within the last decade. Any reader who wishes to research the subject in greater detail will find many more articles published prior to 1999.

Some of the papers cited below describe the use of yeast-derived 1-3, 1-6 beta glucans, which can be regarded as complex carbohydrates. Others used very similar molecules derived from mushrooms (Lentinan, Curdlan), barley and even dates. The source doesn't matter that much; what is important is the structure and size of the immuno-stimulant molecules, which must be recognised by beta glucan receptors on specific immune cells if they are to elicit an innate immune system response (Czop & Austen '85). From the patient's point of view, the most important criteria are likely to be cost and the total evidence base; and from this perspective, the yeast-derived beta glucans are lead candidates.

## Natural Killer Cells: Innate vs Acquired Immune protection

The acquired immune system is that part of the immune system we use when we vaccinate against disease; it is the part of the immune system that 'learns' to recognise pathogens, and it has a memory. This level of sophistication, and the fact that it is a more recent defence (evolutionarily speaking) than the innate immune system, lead to its over-emphasis in 19<sup>th</sup> and 20<sup>th</sup> century medicine. It is only recently that the crucial importance of the innate immune system has been recognised. The innate immune system protects us for 99.999% of the time; it is only on rare occasions that pathogens get through this first line of defence, make us sick, and bring the acquired immune system into action

Natural killer cells form a key element in the innate immune system. These cells form in the bone marrow, and then migrate to the "secondary lymphoid tissues" - the tonsils, lymph nodes and spleen. There, the natural killer cells await activation before they react in one of two distinct modes. In one mode they secrete cytokines, chemical messenger proteins which modulate the T and B immune cell responses which are part of the acquired immune system. In the other, they become potent killers of tumors and virus-infected cells. Natural killer cells provide a crucial first defence against many infectious agents and tumor cells, and do so both rapidly and efficiently (Ferlazzo & Munz '04, Ferlazzo et al '04, Munz et al '05).

In most cases it is the natural killer cells that do the job. They burst forth from the tonsils, lymph nodes and spleen, and destroy infected and cancerous cells before the immune system's T and B cells can fully mobilize. Without natural killer cells, threatening conditions can get a strong foothold before the much slower adaptive immune response kicks in.

The best way to up-regulate the natural killer cells is to use 1-3, 1-6 beta glucans or related compounds. This approach has been shown to kill cancer cells in vitro, and to be highly effective in animal models of cancer. A growing body of work shows that this kind of immuno-stimulation has a role in the management of human cancer also. In a recent review by members of the Tumour Immunobiology Program at the University of Louisville, the authors stated: 'Extensive studies in preclinical animal tumour models have demonstrated the efficacy of combined oral particulate yeast beta-glucan with antitumour mAb therapy in terms of tumour regression and long-term survival. It is proposed that the addition of beta-glucan will further improve the clinical therapeutic efficacy of antitumour mAbs in cancer patients.' (Yan et al '05).

## The Data: A. In Vitro

There are large numbers of cancer cell lines, and beta glucans are effective in restraining and killing many of them.

For example, beta glucans inhibit the growth of human breast carcinoma cells. They do this by inducing cell-cycle arrest and apoptosis in the cancer cells (Zhang et al '06).

Different forms of beta glucan have different levels of anti-cancer activity: the most active forms were as potent in killing sarcoma cancer cells in vitro as 5-fluorouracil, a highly toxic chemotherapy drug (Zhang et al '05). They were also highly effective when assayed against gastric carcinoma cells (Wang et al '04), and against prostate cancer cells (Fullerton et al 2000); so much so that the authors concluded: 'This polysaccharide may have a great potential as an alternative therapeutic modality for prostate cancer.' Working in the Leiden University Medical Center, a Dutch team find that beta glucans increase the therapeutic effectiveness of monoclonal antibodies in killing renal cell carcinoma micro-metastases (Sier et al '04).

These findings are interesting, but in vitro data can be very misleading. It is easy to achieve high concentrations of an active in a Petri dish, but Petri dish experiments do not tell us if it is possible to reach the same levels of the active in vivo; or whether the doses needed to obtain those levels might cause toxicity when used clinically. To answer these questions, we must review the animal and human data.

## The Data: B. Animal models

### 2005

1. In one elegant and persuasive study, a research team from the Memorial Sloan-Kettering Cancer Center in New York studied the impact of beta glucans on mice with Hodgkins and non-Hodgkins lymphoma (Modak et al '05). They gave beta glucans singly or together with rituximab, a monoclonal antibody which activates complement, another key element in the innate immune response to cancer cells.

The cancers were significantly suppressed in mice given the combination, and the survival of mice was significantly increased in the combination group as compared to other treatment groups. No clinical toxicity was observed. The authors commented: 'The therapeutic efficacy and lack of toxicity of this combination supports further investigation into its clinical utility.'

2. Working in parallel with the Sloan-Kettering team, scientists from the Tumour Immunobiology Program at the University of Louisville proved that the mode of action of beta glucans involved complement (Hong et al '03, Hong et al '04, Allendorf et al '05); thus complementing the Sloan-Kettering findings. They found that beta glucans promoted tumor regression and survival and concluded: 'These data suggest that the therapeutic efficacy of monoclonal antibodies known to activate complement (e.g., Herceptin, Rituxan, and Erbitux) could be significantly enhanced if they were combined with beta-glucan.'

3. Also in 2005, a Japanese group published the results of a study of the effects of beta glucans on a range of cancers in mice (Kobayashi et al '05). They found that the beta glucans were effective in killing human ovarian cancer cells, and did so by triggering apoptosis in the cells. They also found that although the beta glucans did not kill lung cancer cells, they did reduce the formation of metastases in both the ovarian cancer and lung cancer cell models. The authors concluded: 'Treatment with beta-glucan may be beneficial for cancer patients with or at risk for metastasis.'

4. A Japanese study found that beta glucans delayed tumor growth in tumor-bearing mice, and protected them against radiation injury (Gu et al '05). The authors concluded: 'These results suggest that beta-glucan may be a promising adjunct treatment for cancer patients receiving radiotherapy'.

5. Another Japanese scientist was rather more forthcoming about beta glucan's anti-tumour effects (Ebina '05). In his paper, he states that while several beta glucan preparations inhibit tumour growth, the most effective beta glucans 'cured primary and metastatic tumours in a double grafted tumour system.'

### 2004

6. At New York Medical College, beta glucans were tested on canine and human cancer cell lines (Konno '04). They were highly effective against the canine and the human cancer cell lines, either potently inhibiting cell growth and/or directly killing the cancer cells. The author concluded 'This is a potent natural agent that could be useful in treating canine cancers as well as other veterinary cancers.'

7. A Libyan group is the first to report on the anti-tumour effects of beta glucans derived from dates (Ishurd et al '04).

8. At the British Columbia Cancer Agency in Vancouver, beta glucans are found to increase the effectiveness of photodynamic therapy for solid tumours, via complement activation (Korbelik et al '04). The authors concluded: 'This study establishes the potential of complement-activating agents to serve as effective adjuvants to PDT for cancer treatment.'

9. At the University of South Carolina, a research team establishes that beta glucans reduce the metastatic spread of injected melanoma cells in mice, and report that that the beta glucans increase cancer cell killing by the mouse's own macrophages (Murphy et al '04).

## **The Data: C. Clinical findings**

2009 - update: <http://www.ncbi.nlm.nih.gov/pubmed/19515245>

### **2005**

10. A Japanese paper reviews the clinical literature of the use of beta glucans as adjuvants in the treatment of cancer, an approach named 'immunochemotherapy', and describes improved efficacy and quality of life (Hamuro '05).

11. Report of a clinical intervention in 10 cases of malignant effusion, using immunochemotherapy (Kawaoka et al '05). In 11 lesions in the 10 cases, the treatment lead to 7 complete remissions and one partial remission. Authors' conclusion: 'Repetitive intracavitary administration of LTN and OK-432 is effective for malignant effusion.'

### **2004**

12. A Japanese study indicates that beta glucans increase the effectiveness of orthodox chemotherapy in patients with inoperable and advanced gastric cancer, and improve patients' quality of life (Nagahashi et al '04).

13. A fourth Japanese paper reports on two cases of inoperable liver metastases from colorectal cancer Ueda et al '04). The patients receive intra-hepatic arterial infusion of pharmacokinetic modulating chemotherapy (HAI + PMC), plus beta glucans. One patient went into apparently complete remission for 3 months, but then dies; the second is well and without remission at 19 months. The authors conclude: 'HAI with PMC in combination with beta glucans could be one of the most promising treatment strategies for unresectable liver metastases from colorectal cancer'.

14. A patient with peritoneal metastasis of a gastric carcinoma is given immunochemotherapy (Nakayama et al '04). At 5 years and 8 months after surgery, she is alive and without any sign of recurrence.

### **2003**

15. Immunochemotherapy is reported 'safe' in the treatment of patients with advanced recurrent gastric cancer (Kimura et al '03).

16. In a pilot study of 22 patients with unresectable or advanced recurrent gastric cancer, immunochemotherapy is linked to increased survival times and reduced toxicity (Nimura et al '03).

### **1999**

17. A major multi-site intervention study, giving immunochemotherapy to patients with advanced gastric cancer (Nakano et al '99). In the immunochemotherapy group, quality of life and survival times are significantly extended.

### **1994**

18. Another major, multi-site intervention study, giving immunochemotherapy to patients with advanced prostate cancer (Tari et al '94). As with the gastric cancer study, survival time is significantly extended in the immunochemotherapy group.

## **CONCLUSIONS**

**The available data makes a strong case for using beta glucans in cancer treatment programmes.**

**It would be rash to attempt to use beta glucans on their own, and we would not recommend this at all. However, the data indicate that it would be equally rash not to incorporate beta glucans into cancer management; thus upgrading from chemotherapy to immuno-chemotherapy.**

**Cures are not the point here, but extended survival and improved quality of life may well be considered desirable.**

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[Wang Y](#), [Zhang L](#), [Li Y](#), [Hou X](#), Zeng F **Correlation of structure to antitumor activities of five derivatives of a beta-glucan from *Poria cocos sclerotium*.** [Carbohydr Res](#). 2004 Oct 20;339(15):2567-74.

Yan J, Allendorf DJ, Brandley B. **Yeast whole glucan particle (WGP) beta-glucan in conjunction with antitumour monoclonal antibodies to treat cancer.** Expert Opin Biol Ther. 2005 May;5(5):691-702.

Zhang L, Li X, Xu X, Zeng F. **Correlation between antitumor activity, molecular weight, and conformation of lentinan.** Carbohydr Res. 2005 Jun 13;340(8):1515-21.

Zhang M, Chiu LC, Cheung PC, Ooi VE. **Growth-inhibitory effects of a beta-glucan from the mycelium of *Poria cocos* on human breast carcinoma MCF-7 cells: Cell-cycle arrest and apoptosis induction.** Oncol Rep. 2006 Mar;15(3):637-43.

### **Three key papers**

1. Yan J, Allendorf DJ, Brandley B. **Yeast whole glucan particle (WGP) beta-glucan in conjunction with antitumour monoclonal antibodies to treat cancer.** Expert Opin Biol Ther. 2005 May;5(5):691-702.

Beta-glucans, biological response modifiers (BRMs) derived from the cell walls of yeast and other sources, have been demonstrated to prime leukocyte complement receptor 3 (CR3), thus enabling these cells to kill tumours opsonised with complement fragment iC3b. Many tumours activate complement via the classical pathway mediated by antitumour monoclonal antibodies (mAbs) or natural antibodies. Studies into the cellular and molecular mechanisms of action have demonstrated that orally administered yeast beta-glucans are ingested and processed by macrophages. These macrophages secrete the active moiety that primes neutrophil CR3 to kill iC3b-opsonised tumour cells. Extensive studies in preclinical animal tumour models have demonstrated the efficacy of combined oral particulate yeast beta-glucan with antitumour mAb therapy in terms of tumour regression and long-term survival. It is proposed that the addition of beta-glucan will further improve the clinical therapeutic efficacy of antitumour mAbs in cancer patients.

2. Nakano H, Namatame K, Nemoto H, Motohashi H, Nishiyama K, Kumada K.

**A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unresectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group.** Hepatogastroenterology. 1999 Jul-Aug;46(28):2662-8.

BACKGROUND/AIMS: Lentinan is one of the host-mediated anti-cancer drugs which has been shown to affect host defense immune systems. Although the mechanisms involved in the antitumor effects of lentinan have been reported experimentally, the clinical outcome on prolongation of survival and improvement of quality of life in gastric cancer patients with unresectable or recurrent diseases has yet to be clarified. The aim of the present study was to investigate whether administration of lentinan prolonged survival or improved quality of life in these patients. METHODOLOGY: A multi-institutional randomized prospective protocol, consisting of patients administered tegafur and cisplatin (control group), and patients administered lentinan, tegafur and cisplatin (lentinan group), was performed. Quality of life was investigated using a questionnaire survey. RESULTS: Median survival was significantly longer in the lentinan group than in the control group (297 days vs. 199 days,  $p = 0.028$ ). One-year survival rate was greater in the lentinan group than in the control group (49.1% vs. 0%). Total QOL score, especially appetite and sleep quality, was significantly improved with the administration of lentinan. CONCLUSIONS: Lentinan is considered to prolong survival and improved quality of life when gastric cancer patients with unresectable or recurrent diseases are treated in combination with other chemotherapeutic agents.

3. Tari K, Satake I, Nakagomi K, Ozawa K, Oowada F, Higashi Y, Negishi T, Yamada T, Saito H, Yoshida K. **Effect of lentinan for advanced prostate carcinoma** Hinyokika Kyo. 1994 Feb;40(2):119-23. Japanese.

A prospective, randomized multi-center study was conducted to assess the clinical effectiveness of Lentinan, an immunomodulatory agent, in the metastatic prostate cancer. Of seventy-five patients enrolled from July 1987 to June 1992, 69 were eligible. All patients received hormonal therapy and chemotherapy using Tegafur p.o. at a dose of 400-800 mg/day. While 33 patients received Lentinan i.m. for at least three months, the other 36 did not. The dose of Lentinan was 2 mg weekly for inpatients and 4 mg every other week for outpatients. The mean age of treated and control patients was 70

(range; 53-83) and 71 (range; 50-86), respectively. The 50% survival length of treated and control patients was 48 and 35 months, respectively. The five-year survival rate of treated patients was 43% according to the Kaplan-Meier method, while that of control patients was 29% ( $p < 0.05$ ). We conclude that Lentinan is effective in metastatic prostate cancer when incorporated into hormonochemotherapy.

## IN VITRO

Zhang M, Chiu LC, Cheung PC, Ooi VE. **Growth-inhibitory effects of a beta-glucan from the mycelium of *Poria cocos* on human breast carcinoma MCF-7 cells: Cell-cycle arrest and apoptosis induction.** *Oncol Rep.* 2006 Mar;15(3):637-43. Because of the reported immune-enhancing and anti-tumor activities of some mushroom polysaccharides, their applications as biological response modifiers have attracted significant attention. We have purified a water-soluble beta-glucan PCM3-II, comprising mainly 1right curved arrow 3 and 1right curved arrow 4 linkages, from the mycelia of *Poria cocos* (Schw.) Wolf (Fu-ling). In this study, the growth-inhibitory effect of PCM3-II was further explored on the human breast carcinoma MCF-7 cells in vitro. The dose effect of PCM3-II was studied by incubating the breast cancer cells with 12.5-400 microg/ml of the glucan for 72 h. The MTT study showed that PCM3-II reduced proliferation and viability of the MCF-7 cells dose-dependently, so that the cancer-cell growth was decreased by 50% of the control level at 400 microg/ml of the glucan. The time effect of PCM3-II was then investigated by treating the breast cancer cells with 400 microg/ml of the glucan for 24, 48 and 72 h, respectively. Results from the flow cytometry study demonstrated that PCM3-II induced cell-cycle G1 arrest time-dependently and about 90% of the cells in cell cycle were accumulated at G1 phase after 72 h of treatment. The G1 arrest was associated with downregulations of the unscheduled cyclin D1 and cyclin E expressions in the breast cancer cells. Apoptosis was also induced by PCM3-II in the MCF-7 cells, so that the subG1 cells in DNA histogram of the flow cytometry were elevated by 5-fold of the control level at 48 h and by 24-fold at 72 h of treatment. The immunoblot study also showed that the glucan induced depletion of the antiapoptotic Bcl-2 protein, but not the proapoptotic Bax protein, so that the Bax/Bcl-2 ratio was elevated in the breast cancer cells at the time when the most prominent apoptosis was also observed. In conclusion, although the detailed mechanism for the anti-tumor activity of the *P. cocos* beta-glucan still needs further investigation, this study provides preliminary insights into its mode of action and perspectives of its development as a water-soluble anti-tumor agent.

Zhang L, Li X, Xu X, Zeng F. **Correlation between antitumor activity, molecular weight, and conformation of lentinan.** *Carbohydr Res.* 2005 Jun 13;340(8):1515-21.

A (1 $\rightarrow$ 3)-beta-D-glucan having (1 $\rightarrow$ 6) branching (L-FV-IB) from *Lentinus edodes* in water was degraded into seven fractions of different molecular weights by ultrasonic irradiation, and each was further fractionated into three parts, by precipitation from water into acetone at room temperature. The weight-average molecular weight ( $M(w)$ ), radius of gyration ( $\langle S^2 \rangle(z)^{1/2}$ ), and intrinsic viscosity ( $[\eta]$ ) of lentinan and its fractions in 0.9% NaCl aqueous solution and dimethyl sulfoxide ( $Me(2)SO$ ) were determined by size-exclusion chromatography combined with multi-angle laser light scattering (SEC-LLS), LLS, and viscometry. Analysis of  $M(w)$ ,  $[\eta]$ , and  $\langle S^2 \rangle(z)^{1/2}$  in terms of known theory for worm-like chains yielded 2240  $\pm$  100 nm<sup>-1</sup>, and 100  $\pm$  10 nm for molar mass per unit contour length ( $M(L)$ ), and persistence length ( $q$ ), respectively, corresponding with theoretical data for triple-helical chains. The  $[\alpha](D)$  of lentinan in water- $Me(2)SO$  mixtures indicated an order-disorder transition. The results indicated that lentinan exists as a triple helix in 0.9% NaCl aqueous solution and as a single flexible chain in  $Me(2)SO$ . Assays in vivo and in vitro against the growth of Sarcoma 180 solid tumor as well as the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method for lentinan showed that the triple-helix sample exhibited a relatively high inhibition ratio. Interestingly, the triple-helix lentinan with  $M(w)$  of  $1.49 \times 10^6$  exhibited the highest antitumor activity in vivo, having an inhibition ratio ( $\xi$ ) of 49.5%, close to that of 5-fluorouracil ( $\xi = 50.5\%$ ), whereas the bioactivity ( $\xi = 12.3\%$ ) of its single flexible chains almost disappeared. The triple-helix conformation plays an important role in enhancing the antitumor effects of lentinan.

[Wang Y](#), [Zhang L](#), [Li Y](#), [Hou X](#), Zeng F **Correlation of structure to antitumor activities of five derivatives of a beta-glucan from *Poria cocos sclerotium*.** *Carbohydr Res.* 2004 Oct 20;339(15):2567-74.

A water-insoluble (1 $\rightarrow$ 3)-beta-D-glucan isolated from fresh sclerotium of *Poria cocos* was, respectively, sulfated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated, to afford five water-soluble derivatives. Their weight-average molecular masses ( $M_w$ ) and intrinsic viscosities ( $[\eta]$ ) were determined by size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS, and viscometry in phosphate buffer solution (PBS) at 37 degrees C. The antitumor activities, against Sarcoma 180 tumor cell (S-180) and gastric carcinoma cell strain (MKN-45 and SGC-7901) of the native beta-glucan and the five derivatives, were tested in vitro and in vivo. The  $M_w$  values of the five derivatives in PBS were determined to be  $3.8 \times 10^4$ ,  $18.9 \times 10^4$ ,  $16.0 \times 10^4$ ,  $76.8 \times 10^4$ , and  $224.3 \times 10^4$ , respectively. The high  $M_w$  values of the hydroxyethylated and hydroxypropylated derivatives in aqueous solution resulted from aggregation, and their true  $M_w$  values obtained in dimethyl sulfoxide were  $20.1 \times 10^4$  and  $19.1 \times 10^4$ . The sulfated and carboxymethylated derivatives having DS of 1.0-1.3 show good water solubility, and exist as relatively expanded chains in aqueous solution. Interestingly, the native beta-glucan did not show antitumor activity, whereas the sulfated and carboxymethylated derivatives exhibit significant antitumor activities against S-180 and gastric carcinoma tumor cells. This work showed that good water solubility, relatively high chain stiffness, and moderate molecular mass of the derivatives in aqueous solution contribute beneficial to enhancement of antitumor activity.

Modak S, Koehne G, Vickers A, O'Reilly RJ, Cheung NK. **Rituximab therapy of lymphoma is enhanced by orally administered (1 $\rightarrow$ 3),(1 $\rightarrow$ 4)-D-beta-glucan.** *Leuk Res.* 2005 Jun;29(6):679-83.

By activating complement, antitumor monoclonal antibodies coat tumor cells with iC3b. beta-glucans, naturally occurring glucose polymers, bind to the lectin domain of the leukocyte receptor CR3, prime it for binding to iC3b, and trigger cytotoxicity of iC3b-coated tumor cells. We studied the combination of the complement-activating antibody rituximab with barley-derived (1->3),(1->4)-beta-D-glucan (BG) against CD-20 positive lymphoma xenografts in SCID mice. Growth of established subcutaneous non-Hodgkin's lymphoma (NHL) (Daudi and EBV-derived B-NHL) or Hodgkin's disease (Hs445 and RPMI6666) was significantly suppressed in mice treated with a combination of intravenous rituximab and oral BG, when compared to mice treated with rituximab or BG alone. Survival of mice with disseminated lymphoma was significantly increased in the combination group as compared to other treatment groups. No clinical toxicity was observed. The therapeutic efficacy and lack of toxicity of this combination supports further investigation into its clinical utility.

Kobayashi H, Yoshida R, Kanada Y, Fukuda Y, Yagyu T, Inagaki K, Kondo T, Kurita N, Suzuki M, Kanayama N, Terao T. **Suppressing effects of daily oral supplementation of beta-glucan extracted from Agaricus blazei Murill on spontaneous and peritoneal disseminated metastasis in mouse model.** J Cancer Res Clin Oncol. 2005 Aug;131(8):527-38.

**PURPOSE:** The Basidiomycete fungus *Agaricus blazei* Murill has traditionally been used as a health food for the prevention of cancer. **METHODS:** We examined whether beta-(1-6)-D-glucan extracted from *A. blazei* is a potential anticancer agent in an in vitro and in vivo animal model. **RESULTS:** Here we show that (1) beta-glucan had cytotoxic effect against human ovarian cancer HRA cells, but not against murine Lewis lung cancer 3LL cells, in vitro; (2) beta-glucan promotes p38 MAPK activity for suppressing HRA cell proliferation and amplifying the apoptosis cascade; (3) beta-glucan stimulates translocation of the proapoptotic protein, Bax, from the cytosol to mitochondria, cytochrome c release, and subsequent caspase-9 activation; (4) treatment with SB203580, a p38 MAPK-specific inhibitor, suppresses beta-glucan-induced effects, indicating that activation of p38 MAPK is involved in the suppression of cell proliferation and mitochondrial activation-mediated cell death pathway; (5) in mice, oral supplementation with beta-glucan reduces pulmonary metastasis of 3LL cells and peritoneal disseminated metastasis of HRA cells and inhibits the growth of these metastatic tumors in lung or peritoneal cavity, in part, by suppressing uPA expression; and (6) in an in vivo experimental metastasis assay, however, the oral supplementation with beta-glucan after i.v. tumor cell inoculation did not reduce the number of lung tumor colonies. **CONCLUSION:** Treatment with beta-glucan may be beneficial for cancer patients with or at risk for metastasis. The beta-glucan-dependent signaling pathways are critical for our understanding of anticancer events and development of cancer therapeutic agents.

Konno S. **Potential growth inhibitory effect of maitake D-fraction on canine cancer cells.**

Vet Ther. 2004 Winter;5(4):263-71.

Department of Urology, New York Medical College, Munger Pavilion 4th Floor, Valhalla, NY, 10595, USA.

The postulated anticancer effect of D-fraction, the bioactive extract of maitake mushroom, on three types (CF33, CF21, and CL-1) of canine cancer cells was evaluated. The effect of D-fraction on several human cancer cells was also investigated. The effect of other beta-glucan products was likewise examined. D-fraction was highly effective on the canine cancer cells, either potently inhibiting cell growth or directly killing cells. Similar effects were also demonstrated in certain human cancer cells. However, other beta-glucan products relevant to D-fraction had no such effects on canine cancer cells. Therefore, D-fraction is a potent natural agent that could be useful in treating canine cancers as well as other veterinary cancers.

Allendorf DJ, Yan J, Ross GD, Hansen RD, Baran JT, Subbarao K, Wang L, Haribabu B. **C5a-mediated leukotriene B4-amplified neutrophil chemotaxis is essential in tumor immunotherapy facilitated by anti-tumor monoclonal antibody and beta-glucan.** J Immunol. 2005 Jun 1;174(11):7050-6.

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Intravenous and orally administered beta-glucans promote tumor regression and survival by priming granulocyte and macrophage C receptor 3 (CR3, iC3bR and CD11b/CD18) to trigger the cytotoxicity of tumor cells opsonized with iC3b via anti-tumor Abs. Despite evidence for priming of macrophage CR3 by oral beta-glucan in vivo, the current study in C57BL/6 and BALB/c mice showed that granulocytes were the essential killer cells in mAb- and oral beta-glucan-mediated tumor regression, because responses were absent in granulocyte-depleted mice. Among granulocytes, neutrophils were the major effector cells, because tumor regression did not occur when C5a-dependent chemotaxis was blocked with a C5aR antagonist, whereas tumor regression was normal in C3aR(-/-) mice. Neutrophil recruitment by C5a in vivo required amplification via leukotriene B(4), because both C5a-mediated leukocyte recruitment into the peritoneal cavity and tumor regression were suppressed in leukotriene B(4)R-deficient (BLT-1(-/-)) mice.

Gu YH, Takagi Y, Nakamura T, Hasegawa T, Suzuki I, Oshima M, Tawaraya H, Niwano Y. **Enhancement of radioprotection and anti-tumor immunity by yeast-derived beta-glucan in mice.** J Med Food. 2005 Summer;8(2):154-8.

Intraperitoneal injection of beta-glucan was shown to greatly delay mortality in mice exposed to whole-body X-ray radiation and tumor growth in tumor-bearing mice. Since the leukocyte and lymphocyte numbers were increased by a single dose of beta-glucan, the radioprotective effect of beta-glucan is probably mediated, at least in part, by a hemopoietic action in irradiated mice. In addition, both natural killer (NK) and lymphokine-activated killer (LAK) activities were significantly increased by repeated doses of beta-glucan. Augmented immunological activity as seen in increased NK and LAK activity by beta-glucan seems to play a role in preventing secondary infections associated with irradiation, and probably contributes to the attenuated tumor growth in tumor-bearing mice through enhanced anti-tumor immunity. These results suggest that beta-glucan may be a promising adjunct treatment for cancer patients receiving radiotherapy.

Ebina T. **Antitumor effects of intratumoral injection of Basidiomycetes preparations**

Gan To Kagaku Ryoho. 2005 Oct;32(11):1654-6. Japanese.

The antitumor effects of Basidiomycetes preparations in an experimental mouse model, the "double grafted tumor system" were analyzed. Some BRMs prevented metastases by utilizing the anti-tumor immunological cascade reactions, which activates macrophages in the body. The following Basidiomycetes preparations were analyzed: PSK was a hot water extract of cultured mycelia from *Coliolum versicolor* and a protein bound beta-glucan. Matsumax was extracted from mycelia of *Tricholoma matsutake* and was a protein bound (38%)  $\alpha$ -glucan. The *Agaricus* preparation was extracted from fruit bodies of *Agaricus blazei* and a protein-bound (17%)  $\alpha$ -glucan, beta-glucan. *Himematsutake* preparation was extracted from fruit body of *Agaricus blazei* (*Himematsutake*) and a protein bound (5%) glucan. Lentinan was purified from fruit bodies of *Lentinus edodes* and is a purified beta-glucan. PSK cured both primary and metastatic tumors in the double grafted tumor system. Lentinan inhibited the growth of neither primary nor metastatic tumors. Matsumax and *Agaricus* preparation cured primary tumor and inhibited the growth of metastatic tumor. *Himematsutake* preparation inhibited the growth of primary tumor. Immunosuppressive acidic protein (IAP) is produced by activated macrophages. The PSK, Matsumax, *Agaricus* preparation and *Himematsutake* preparation induced IAP but Lentinan did not.

Ishurd O, Zgheel F, Kermagi A, Flefla M, Elmabruk M. **Antitumor activity of beta-D-glucan from Libyan dates.** J Med Food. 2004 Summer;7(2):252-5.

Beta-glucan with antitumor activities was isolated from Libyan dates, and the structure of the purified glucan was characterized using methods such as methylation, periodate oxidation, and acetolysis. Glucans were found to exhibit potent antitumor activity; this activity could be correlated to their (1 $\rightarrow$ 3)-beta-D-glucan linkages. Such antitumor glucans have also been obtained from a number of other sources, such as yeast, fungi, bacteria, and plants. This is the first study to report antitumor activity for date glucan.

Korbelik M, Sun J, Cecic I, Serrano K. **Adjuvant treatment for complement activation increases the effectiveness of photodynamic therapy of solid tumors.** Photochem Photobiol Sci. 2004 Aug;3(8):812-6.

British Columbia Cancer Agency, Vancouver, B.C., Canada.

Phototoxic lesions generated in tumor tissue by photodynamic therapy (PDT) are recognized by the host as a threat to the integrity and homeostasis at the affected site. Among the canonical pathways invoked by the host for dealing with this type of challenge is the activation of the complement system, integrating proteins that serve as molecular sensors of danger signals produced by PDT and those initiating signalling cascades coupled into the network of inflammatory and immune responses. Since the activated complement system is a salient participant of the antitumor response produced by PDT, it is worth exploring whether its manipulation can be exploited for the therapeutic benefit. Using mouse tumor models, the present study examined the potential of representative complement-activating agents to act as effective adjuvants to PDT. Tumor-localized treatment with zymosan, an alternative complement pathway activator, reduced the recurrence-rate of PDT-treated tumors, markedly increasing the percentage of permanent cures. In contrast, a similar treatment with heat aggregated gamma globulin (complement activator via the classical pathway) was of no significant benefit as a PDT adjuvant. Systemic complement activation with streptokinase treatment had no detectable effect on complement deposition at the tumor site without PDT, but it augmented the extent of complement activity in PDT-treated tumors. This finding based on immunohistochemistry analysis explains the results of tumor therapy experiments, which showed that systemic treatment with streptokinase or a similar agent, urokinase, enhances the PDT-mediated tumor response. Zymosan and streptokinase administrations produced no beneficial results with PDT of tumors growing in complement-deficient mice. This study, therefore, establishes the potential of complement-activating agents to serve as effective adjuvants to PDT for cancer treatment.

Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, Ostroff GR, Xing PX, Cheung NK, Ross GD. **Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models.** J Immunol. 2004 Jul 15;173(2):797-806.

Antitumor mAb bind to tumors and activate complement, coating tumors with iC3b. Intravenously administered yeast beta-1,3;1,6-glucan functions as an adjuvant for antitumor mAb by priming the inactivated C3b (iC3b) receptors (CR3; CD11b/CD18) of circulating granulocytes, enabling CR3 to trigger cytotoxicity of iC3b-coated tumors. Recent data indicated that barley beta-1,3;1,4-glucan given orally similarly potentiated the activity of antitumor mAb, leading to enhanced tumor regression and survival. This investigation showed that orally administered yeast beta-1,3;1,6-glucan functioned similarly to barley beta-1,3;1,4-glucan with antitumor mAb. With both oral beta-1,3-glucans, a requirement for iC3b on tumors and CR3 on granulocytes was confirmed by demonstrating therapeutic failures in mice deficient in C3 or CR3. Barley and yeast beta-1,3-glucan were labeled with fluorescein to track their oral uptake and processing in vivo. Orally administered beta-1,3-glucans were taken up by macrophages that transported them to spleen, lymph nodes, and bone marrow. Within the bone marrow, the macrophages degraded the large beta-1,3-glucans into smaller soluble beta-1,3-glucan fragments that were taken up by the CR3 of marginated granulocytes. These granulocytes with CR3-bound beta-1,3-glucan-fluorescein were shown to kill iC3b-opsonized tumor cells following their recruitment to a site of complement activation resembling a tumor coated with mAb.

Murphy EA, Davis JM, Brown AS, Carmichael MD, Mayer EP, Ghaffar A.

**Effects of moderate exercise and oat beta-glucan on lung tumor metastases and macrophage antitumor cytotoxicity.** J Appl Physiol. 2004 Sep;97(3):955-9.

Department of Exercise Science, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina 29208, USA.



Both moderate exercise and the soluble fiber beta-glucan can have beneficial effects on the initiation and growth of tumors, but the data are limited, and there is no information on their combined effects. This study tested the independent and combined effects of short-term moderate-exercise training and the soluble oat fiber beta-glucan (ObetaG) on the metastatic spread of injected tumor cells and macrophage antitumor cytotoxicity. Male C57BL/6 mice were assigned to one of four groups: exercise (Ex)-H<sub>2</sub>O, Ex-ObetaG, control (Con)-H<sub>2</sub>O, or Con-ObetaG. ObetaG was fed in the drinking water for 10 days before tumor administration and death. Exercise consisted of treadmill running (1 h/day) for 6 days. After rest or exercise on the last day of training, syngeneic B16 melanoma cells ( $2 \times 10^5$ ) were administered via intravenous injection (n = 8-11 per group). Lungs were removed 14 days later, and tumor foci were counted. Additional mice (n = 8 per group) were killed, and peritoneal macrophages were assayed for cytotoxicity against the same mouse tumor cell line at various effector-to-target ratios. Both moderate exercise and ObetaG decreased lung tumor foci and increased macrophage cytotoxicity. However, there were no differences in lung tumor foci and macrophage cytotoxicity between Ex-ObetaG and either Ex-H<sub>2</sub>O or Con-ObetaG. These data suggest that, although not additive in their effects, both short-term moderate-exercise training and consumption of the soluble ObetaG can decrease the metastatic spread of injected B16 melanoma cells, and these effects may be mediated in part by an increase in macrophage cytotoxicity to B16 melanoma.

Sier CF, Gelderman KA, Prins FA, Gorter A. **Beta-glucan enhanced killing of renal cell carcinoma micrometastases by monoclonal antibody G250 directed complement activation.**

Int J Cancer. 2004 May 10;109(6):900-8.

Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands.

Metastases from renal cell carcinomas (RCC) are resistant to radiation and chemotherapy but are relatively immunogenic. We have investigated the possibility to eliminate human RCC micrometastases using MAb G250. G250 penetrates human micrometastases completely in a spheroid model and induces complement deposition rapidly on the outmost cell layers. However, complement dependent cytotoxicity (CDC) was barely detected using either (51)chromium release assays or confocal microscopy, due to relatively low expression of the G250 antigen and the effect of membrane bound complement regulatory proteins. Addition of blocking anti-CD59 MAbs enhanced formation of C5b-9 and consequently complement mediated lysis (13%). Complement assisted cellular cytotoxicity (CACC) was not detectable, although the iC3b ligand and CR3 receptor were present on respectively target and effector cells. Addition of soluble beta-glucan induced the killing of MAb and iC3b opsonized spheroids by effector cells (6-21%). Despite a lower affinity for G250 antigen, a bispecific anti-G250\*anti-CD55 MAb enhanced cell killing in spheroids comparable to the parental G250 MAb. Our results suggest that complement-activating G250 in combination with anti-mCRP MAbs is able to kill human RCC cells in micrometastasis in vitro. For CACC the presence of CR3-priming beta-glucan seems to be obligatory. In vivo, bi-MAb may be more effective as therapeutic agent due to its increased C5a generating properties. Copyright 2004 Wiley-Liss, Inc.

Hong F, Hansen RD, Yan J, Allendorf DJ, Baran JT, Ostroff GR, Ross GD.

**Beta-glucan functions as an adjuvant for monoclonal antibody immunotherapy by recruiting tumoricidal granulocytes as killer cells.** Cancer Res. 2003 Dec 15;63(24):9023-31.

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The tumor-killing mechanisms available to monoclonal antibodies (mAbs; e.g., antagonism of growth factor receptors, antibody-dependent cell-mediated cytotoxicity) limit efficacy. Previous studies suggested that i.v. beta-glucan might function as an adjuvant for antitumor mAbs. beta-Glucan had been shown to function via the iC3b-receptor complement receptor 3 (CR3; CD11b/CD18) thereby enhancing leukocyte killing of tumor cells coated with iC3b via naturally occurring antitumor antibodies. Therapy with beta-glucans was limited by levels of natural antibodies and by tumor escape through elimination of antigen-positive cells. Accordingly, it was hypothesized that beta-glucan responses could be improved by combined administration with antitumor mAbs. Five tumor models were explored in BALB/c or C57Bl/6 mice using tumors that expressed either high levels of naturally occurring antigens (e.g., G(D2) ganglioside) or recombinant human MUC1. In comparison with antitumor mAb or beta-glucan alone, combined treatment with mAb plus beta-glucan produced significantly greater tumor regression in all models that included mammary, s.c., and hepatic tumors. Tumor-free survival only occurred in models that incorporated stable expression of the target antigen. beta-Glucan enhancement of the mAb tumoricidal response did not occur in mice deficient in either leukocyte CR3 (CD11b(-/-)) or serum C3, confirming the requirement for CR3 on leukocytes and iC3b on tumors. Granulocytes appeared to be primarily responsible for tumoricidal activity, because beta-glucan therapeutic responses did not occur in granulocyte-depleted mice. These data suggest that the therapeutic efficacy of mAbs known to activate complement (e.g., Herceptin, Rituxan, and Erbitux) could be significantly enhanced if they were combined with beta-glucan.

Fullerton SA, Samadi AA, Tortorelis DG, Choudhury MS, Mallouh C, Tazaki H, Konno S.

**Induction of apoptosis in human prostatic cancer cells with beta-glucan (Maitake mushroom polysaccharide).** Mol Urol. 2000 Spring;4(1):7-13.

Department of Urology, New York Medical College, Valhalla, New York 10595, USA.

**PURPOSE:** To explore more effective treatment for hormone-refractory prostate cancer, we investigated the potential antitumor effect of beta-glucan, a polysaccharide of the Maitake mushroom, on prostatic cancer cells in vitro. **MATERIALS AND METHODS:** Human prostate cancer PC-3 cells were treated with various concentrations of the highly purified beta-glucan preparation Grifon-D(R) (GD), and viability was determined at 24 h. Lipid peroxidation (LPO) assay and in situ hybridization (ISH) were performed to unravel the antitumor mechanism of GD. **RESULTS:** A dose-response study showed that almost complete (>95%) cell death was attained in 24 h with GD  $\geq$  480 microg/mL. Combinations of GD in a concentration as low as 30 to 60 microg/mL with 200 microM vitamin C were as effective as GD alone at 480 microg/mL,

inducing >90% cytotoxic cell death. Simultaneous use with various anticancer drugs showed little potentiation of their efficacy except for the carmustine/GD combination (approximately 90% reduction in cell viability). The significantly (twofold) elevated LPO level and positive ISH staining of GD-treated cells indicated oxidative membrane damage resulting in apoptotic cell death. **CONCLUSION:** A bioactive beta-glucan from the Maitake mushroom has a cytotoxic effect, presumably through oxidative stress, on prostatic cancer cells in vitro, leading to apoptosis. Potentiation of GD action by vitamin C and the chemosensitizing effect of GD on carmustine may also have clinical implications. Therefore, this unique mushroom polysaccharide may have great a potential as an alternative therapeutic modality for prostate cancer.

Nagahashi S, Suzuki H, Nishiwaki M, Okuda K, Kurosawa Y, Terada S, Sugihara T, Andou K, Hibi T. **TS-1/CDDP/Lentinan combination chemotherapy for inoperable advanced gastric cancer** Gan To Kagaku Ryoho. 2004 Nov;31(12):1999-2003. Japanese.

It is reported that TS-1 administered orally shows a significant anti-neoplasm effect on advanced gastric cancer, and, furthermore, approximately 70% or greater effectiveness is reported for combination chemotherapy with cisplatin (CDDP). Lentinan is reported to extend the survival period in advanced cancer, and in combination with Tegafur. In the present study, combination chemotherapy with TS-1/CDDP/Lentinan was conducted for patients with inoperable advanced gastric cancer, and the validity, safety and resultant QOL of the treatment were evaluated. TS-1 was administered for 3 weeks at 80 mg/m<sup>2</sup>, followed by withdrawal for 2 weeks, and CDDP was prescribed once for patients at 70 mg/m<sup>2</sup> on the 8th day after starting TS-1 administration. For patients aged 80 or above, however, the dose was reduced, and given separately to the patients. Lentinan was administered at 2 mg/week. The rate of effectiveness for the 9 registered patients was 100%. This high rate was obtained regardless of changes in the histopathological findings. Critical side effects (grade three or above) were anemia and pigmentation, in one case each. An improvement in QOL was also observed for combination therapy including Lentinan. In cases of inoperable advanced gastric cancer, TS-1/CDDP combination chemotherapy showed higher efficacy regardless of the pathological alterations, and higher and sustained improvement of QOL was also observed with the addition of Lentinan to the protocol

Hamuro J. **Anticancer immunotherapy with perorally effective lentinan**

Gan To Kagaku Ryoho. 2005 Aug;32(8):1209-15. Review. Japanese.

Lentinan is a beta(1-3) glucan clarified to have a life prolonging effect in non-operable, recurrent gastric cancer patients in combination with chemotherapy. The long lasting issue remaining to be resolved has been the ineffectiveness of Lentinan when administered per-orally. Beta(1-3) glucans possess the particulate size around 100-200 microm in aqueous solution which dampered the absorption through abdominal mucosa. Subsequently the particulate size of Lentinan impaired the immunostimulating potency, to induce reductive form of antigen presenting cells, macrophages and dendritic cells relevant for the polarization of Th1/Th2 balance to Th1. The situation is also the case for the clinical benefit of lentinan to reduce the side effect of chemotherapeutic agents such as TS-1, Gemzar, CDDP, known to be a critical dose limiting factor of these agents and to improve quality of life of the patients. Using the modern nano-technology procedures, Mitherapist, containing 15 mg/dl Lentinan, with a particulate size of 0.2 microm able to pass through mucosal barrier was provided. It was found in randomized double blind clinical testing that Mitherapist is effective against allergy by reducing an antigen specific IgE level through polarization to Th1 biased immune response even by per-oral administration. Per oral administration also exhibited the reduced side effect of chemotherapeutic agents such as TS-1, Gemzar, CDDP and greatly improved quality of life of the cancer patients. The role of hypoxia in local neoplastic tissues will be also discussed.

Ueda Y, Naito K, Iwamoto A, Tagi T, Shimizu K, Shiozaki A, Tamai H, Ochiai T, Sonoyama T, Yamagishi H. **Two case reports of complete regression of liver metastases from colorectal cancer after locoregional immunochemotherapy** Gan To Kagaku Ryoho. 2004 Oct;31(11):1671-3. Japanese.

Hepatic arterial infusion (HAI) with pharmacokinetic modulating chemotherapy (PMC) has been well known to be one of the most effective protocols for unresectable liver metastases from colorectal cancer. PMC is a combination of oral UFT and continuous hepatic arterial 5-FU infusion. We present herein the cases of two patients with multiple liver metastases from colorectal cancer in whom complete regression (CR) was achieved by HAI with PMC in combination with Lentinan (immunostimulator). These patients received HAI via an implantable port system with a 4-24-hour continuous perfusion of 5-FU at 1,000 mg/m<sup>2</sup> plus Lentinan at 2 mg/body once a week, and oral administration of UFT at 200-300 mg/m<sup>2</sup>/day everyday. CR of all metastatic lesions in the liver was achieved 4 months after the initiation of the treatment in both patients. One patient maintained CR for 3 months, but he died due to a recurrence of liver metastases and peritoneal dissemination 19 months after the initiation of the treatment. The other patient has been well without recurrence for 21 months. Because the liver is the largest immunologic organ, Lentinan could have activated lymphocytes and macrophages in the liver. Judging from the clinical experience of these two cases, HAI with PMC in combination with Lentinan could be one of the most promising treatment strategies for unresectable liver metastases from colorectal cancer.

Kawaoka T, Yoshino S, Kondo H, Yamamoto K, Hazama S, Oka M. **Clinical evaluation of intrapleural or peritoneal repetitive administration of Lentinan and OK-432 for malignant effusion** Gan To Kagaku Ryoho. 2005 Oct;32(11):1565-7. Japanese

LTN and OK-432 combined therapy is effective for controlling Th1/Th2 balance. We tried a repetitive administration of LTN and OK-432 in the pleural or peritoneal cavity for patients with malignant effusion. Of all 11 lesions of the 10 cases, 7 revealed complete remission and 1 revealed partial response. The level of IL-12 (p70) and IFN-gamma in ascites of two gastric cancer patients after the second administration of LTN and OK-432 was much higher than those after the first administration, whereas the level of IL-10 was not suppressed strongly. In 8 lesions that we could confirm complete

remission or partial response, 7 lesions were improved after two or three administrations of LTN and OK-432. In conclusion, a repetitive intracavitary administration of LTN and OK-432 is effective for malignant effusion.

Kimura Y, Iijima S, Kato T, Tsujie M, Naoi Y, Hayashi T, Tanigawa T, Yamamoto H, Kurokawa E, Matsuura N, Kikkawa N. **TS-1 and lentinan combination immunochemotherapy for advanced or recurrent gastric cancer: a preliminary report** Gan To Kagaku Ryoho. 2003 Oct;30(11):1791-3. Japanese

The immunocompetence and nutritional state of patients with advanced or recurrent gastric cancer is low, making it important to conduct chemotherapy while at the same time improving or maintaining their immunocompetence and nutritional state. To reduce the side effects but not the antitumor effect of TS-1, a 2-week regime of TS-1, and 1-week drug-free interval, in combination with the immunotherapeutic agent lentinan (LNT) was started in 5 patients with advanced or recurrent gastric cancer. Toxicity, efficacy, immunocompetence and nutritional state were investigated preliminarily to examine whether or not usefulness of lentinan could be evaluated. The IAP tended to decrease. TS-1 and lentinan combination immunochemotherapy was able to be carried out safely in patients with advanced recurrent gastric cancer. In order to examine the usefulness of combined LNT, it is thought to be necessary to perform a randomized trial using toxicity and not only efficacy but QOL and immunological and nutritional parameters as indicators.

Nakayama H, Aoki N, Hayashi S, Wakabayashi K, Karube H, Ogame H, Aoki H, Sakamoto N, Masuda H, Hemmi A. **A case of long survival with UFT and lentinan treatment in a patient with peritoneal metastasis of gastric carcinoma** Gan To Kagaku Ryoho. 2004 Feb;31(2):241-3. Japanese.

The patient was a 50-year-old female with peritoneal metastasis of Type 4 gastric cancer. She underwent a relative curative resection with total gastrectomy and peritonectomy. Postoperative chemotherapy with 5'-DFUR following 5-FU and CDDP was performed. Thirteen months after surgery, cancer recurrence was suspected due to elevated levels of the serum tumor markers carcinoembryonic antigen (8.9 ng/ml) and alpha fetoprotein (85.8 ng/ml). She was additionally treated with UFT 300 mg/day and Lentinan 2 mg/week. The serum tumor markers decreased gradually returned to normal levels. At 5 years and 8 months after surgery, she is alive without any sign of recurrence.

Nimura H, Mitsumori N, Tsukagoshi S, Nakajima M, Atomi Y, Suzuki S, Kusano M, Yoshiyuki T, Tokunaga A. **Pilot study of TS-1 combined with lentinan in patients with unresectable or recurrent advanced gastric cancer** Gan To Kagaku Ryoho. 2003 Sep;30(9):1289-96. Japanese.

TS-1, an anticancer, antimetabolite agent, has shown clinically superior antitumor activity against unresectable advanced or recurrent gastric cancer (UARG). A biological response modifier, lentinan (LNT) prolonged the survival period of patients with UARG when combined with tegafur (FT). To assess the efficacy, the safety and prognostic factors of chemo-immunotherapy using TS-1, a FT derivative, and LNT, we conducted a multi-institutional pilot study in patients with UARG. Patients were treated with TS-1 at 80 mg/m<sup>2</sup>/day (bid) for 4-weeks, and LNT was given at 2 mg/body (i.v.) in a week, followed by a 2-week rest for 4 cycles. Twenty-two patients were entered from 4 institutes and 19 patients were eligible. The median survival time in eligible patients was 400 days. The incidence of hematological toxicity (grade 2 leukopenia), and non-hematological toxicity (grade 3 nausea or fatigue) was 5.3% (1/19) and no grade 4 toxicity was observed. The response ratio was 37.5% in 8 patients who had been administered the planned dose of TS-1. In subset analyses, the survival period of the patients with normal (< 500 micrograms/ml) serum immunosuppressive acidic protein level was significantly ( $p < 0.0001$ ) better than that of the higher one. The survival period for those patients whose granulocytes/lymphocytes ratio was not more than 2 tended to be better. From the prolonged survival periods, chemo-immunotherapy using TS-1 combined with LNT would seem to have a benefit against UARG, and reduced toxicity. Future clinical trials are warranted to confirm its potency.

Nakano H, Namatame K, Nemoto H, Motohashi H, Nishiyama K, Kumada K.

**A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unresectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group.** Hepatogastroenterology. 1999 Jul-Aug;46(28):2662-8.

BACKGROUND/AIMS: Lentinan is one of the host-mediated anti-cancer drugs which has been shown to affect host defense immune systems. Although the mechanisms involved in the antitumor effects of lentinan have been reported experimentally, the clinical outcome on prolongation of survival and improvement of quality of life in gastric cancer patients with unresectable or recurrent diseases has yet to be clarified. The aim of the present study was to investigate whether administration of lentinan prolonged survival or improved quality of life in these patients. METHODOLOGY: A multi-institutional randomized prospective protocol, consisting of patients administered tegafur and cisplatin (control group), and patients administered lentinan, tegafur and cisplatin (lentinan group), was performed. Quality of life was investigated using a questionnaire survey. RESULTS: Median survival was significantly longer in the lentinan group than in the control group (297 days vs. 199 days,  $p = 0.028$ ). One-year survival rate was greater in the lentinan group than in the control group (49.1% vs. 0%). Total QOL score, especially appetite and sleep quality, was significantly improved with the administration of lentinan. CONCLUSIONS: Lentinan is considered to prolong survival and improved quality of life when gastric cancer patients with unresectable or recurrent diseases are treated in combination with other chemotherapeutic agents