CHAPTER 7  THE ROLE OF POLYSACCHARIDES DERIVED FROM MEDICINAL MUSHROOMS IN CANCER

Synopsis

This Chapter sets out the current information on the use of various mushroom polysaccharides in cancer treatment. Many human cancer cell-lines have been studied and in some cases direct cytotoxic effects have been demonstrated. Many of the mushroom polysaccharide compounds have proceeded through to Phases I, II and III clinical trials and several are used extensively in Asia to treat various cancer. It is anticipated that such proprietary mushroom compounds will mainly be used as complementary or adjunctive therapies to be used in addition to mainstream care.

INTRODUCTION

It has been generally recognised that in the treatment of cancer surgery with or without radiotherapy remains the modus operandi for most cancer cures. Radiotherapy is used quite successfully for many forms of cancer while chemotherapy has become an integral part of a multi-disciplinary treatment of cancers and has served also as a palliative measure in cases of advanced cancer.

However, in almost all cases, a major cause of treatment failure has been the development of distant metastases. While surgery and radiotherapy are all means of eradicating loco-regional disease they are of little value with distant metastases. For such distant metastases chemotherapy is the recommended approach but effectiveness is limited by toxic side-effects at high doses. Furthermore, within the holistic approach of clinical cancer therapy there is now increasing emphasis being given to patient quality of life (QOL) following these above classical treatments. Survival should not be the sole criterion for assessing the treatment results. Thus, it has increasingly become an accepted practice that the oncologist should combine all
available disciplines that could contribute to patient welfare after the main
treatment(s) has attempted to destroy the primary cancer site.

It is also well-recognised that both radiotherapy and chemotherapy invariably
damage or weaken the patient’s immunological defenses which may also have been
damaged by the cancer itself. From these observations there has now developed a
new awareness in cancer therapy, viz. is it possible to modify the host biological
response to malignant invasion? As discussed in the previous chapter, Biological
Response Modifiers have now evolved as the fourth method of cancer treatment in
addition to surgery, radiotherapy and chemotherapy. Such treatments with BRMs
are considered more biological than directly cytotoxic.

This chapter will set out the current information available on the use of various
mushroom polysaccharides in cancer treatment procedures. In all cases these
compounds have demonstrated pre-clinical efficacy, including direct cytotoxicity.
However, many drugs can be effective in the laboratory but fail in clinical practice
due either to inherent toxicity when used at effective dose rates or lack of efficacy.

While the vast majority of the published studies on the use of medicinal
mushroom polysaccharides in oncology have appeared in Oriental Journals, there
has been a major increase in publications in peer-reviewed Western Journals by
Asian scientists and a perceptible change in the attitude of Western medical doctors
and scientists towards the pharmaceutical developments derived from traditional
Chinese medicines (Kidd, 2000).

While all of the mushroom polysaccharides successfully used in animal and
human cancer treatments have been administered intravenously, several can also
be effective by oral (p.o.) administration. Delivering anticancer agents by oral
methods is becoming increasingly important in cost reduction of the regime for a
disease that requires protracted treatment and for the patient’s increasing preference and improved quality of life. Orally formulated chemotherapy is increasing in contemporary oncology practice driven not only by a preference for outpatient treatment but also by the potential for improved quality of life. Since cytostatic therapy often requires protracted drug administration, the use of a self-administered oral formulation is to be preferred (Demario and Rateim, 1998; Sulkes et al. 1998).

As discussed later in this section, two mushroom polysaccharides (Lentinan and Schizophyllan), both large molecules, are only effective by i.v. or i.p. administration. Furthermore, in this context, it is pertinent to note that a recent study with the antitumour β-1,6 glucan from Agaricus blazei with mice showed that i.v. administration gave highly satisfactory results while no effect was seen with oral administration. However, a simple acid treatment of the whole β-1,6 glucan produced molecular masses of c 10k Da which when administered orally to mice demonstrated activity (Fujimiya et al. 2000). This study could well have significant application with the other large β-glucans and so improving their oral bioavailability and increased use as immunonutriceuticals.

In a recent survey of the clinical testing of new oncology drugs, it was pertinent to note the large number of immunological research programmes as well as a number of studies examining drugs that stimulate apoptosis (aimed at inducing programmed cell death in cancer cells)(Pigache, 2001). In almost all the examples that will be discussed in this chapter the polysaccharides act mainly as immune-stimulants with little or no adverse drug reactions. Furthermore, several of these extracts have been shown to stimulate apoptosis in cancer cells (e.g. Fullerton et al., 2000).
Many of the mushroom polysaccharides have proceeded through Phase I, II and III clinical trials. With the exception of Lentinan (*L. edodes*), PSK and PSP (*T. versicolor*) where many hundreds of cancer patients have been subjected to clinical trials the other compounds have only been assessed in small numbers of patients. In Japan and China, Phase I clinical trials have little significance since no maximum tolerated dose was reached. Recently, the FDA in US has exempted Maitake-polysaccharides from Phase I study because of limited side-effects. While there are examples where the mushroom polysaccharides have shown efficacy against specific types of cancer as monotherapy the overwhelming successes have been demonstrated when they function together with proven and accepted chemotherapeutic agents. The degree to which medicinal mushrooms have been tested for *in vitro* and *in vivo* activity varies. In some cases, such as with Polysaccharopeptide (PSP) extensive *in vitro* activity has been demonstrated against a variety of cell lines (human leukemia cell line, S180/H238 sarcoma, P388 leukemia, etc.) and a number of xenografts (nasopharyngeal carcinoma, Lewis lung, etc.) (extensively reviewed in Xu, 1999). However, there still remains the need to carry out more systematic studies to complement the promising clinical data.

**Lentinus edodes**

There is an immense literature related to the anticancer effects of Lentinan on animals and humans and only the more relevant and recent medical studies will be presented here. Lentinan was first isolated and studied by Chihara *et al.* (1970) who demonstrated that its anti-tumour effects were greater than other mushroom polysaccharides and was active for some, but not all, types of tumours (Maeda *et al.*, 1974). The purified polysaccharide has been shown in numerous xenographs to cause tumour regression and in some cases even a complete response (for
extensive review of animal studies, see Hobbs, 1995, Wasser and Weis, 1999). The cytostatic effect of Lentinan is due to the activation of the host’s immune system. Also, pre-clinical and clinical toxicity with Lentinan is rarely noted. Accumulated information on anti-tumour activity, prevention of metastasis, and suppression of chemical and viral oncogenesis in animal models by Lentinan are summarised in Table 1 (Wasser and Weis, 1999).

While Lentinan is a pure polysaccharide composed only of atoms of carbon, oxygen and hydrogen, LEM and LAP, also present in mycelial extracts of *L. edodes*, are glycoproteins, and have demonstrated antitumour activity in xenograft models and clinical trials. Again, both LEM and LAP activate the host immune system (Mizuno, 1995). In Japan Lentinan is presently classified as a medicine whereas LEM and LAP are considered as food supplements (nutriceuticals).

There have been numerous clinical trials of Lentinan in Japan, though none have been placebo-controlled and double-blinded. However, Lentinan has been approved for clinical use in Japan for many years, and is manufactured by several pharmaceutical companies. Intraperitoneal Lentinan is widely used as an adjuvant treatment for certain cancers in Japan and China.

Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinoma (Furue *et al.*, 1981, Taguchi *et al.*, 1985a,b). In patients with inoperable or recurrent gastric cancer, tumour responses and prolonged median survival were also noted. In a randomised controlled study of patients treated with tegafur or a combination of Lentinan and tegafur overall survival was significantly prolonged in the Lentinan plus tegafur group. Of 145 patients, 68 received tegafur alone, and 77 received Lentinan plus tegafur. The respective 50% survival times for the two groups were 92 days
Table 1  Lentinan – pre-clinical animal models (Wasser and Weis, 1999)

<table>
<thead>
<tr>
<th>Model</th>
<th>Model</th>
<th>Dose of Lentinan (mg/kgxdays)</th>
<th>Tumour inhibition ratio (%)</th>
<th>Complete regression of tumour</th>
<th>Decreased tumour occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Allogeneic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>CD-1/ICR</td>
<td>0.2 x 10</td>
<td>78.1</td>
<td>6/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x 10</td>
<td>100.0</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 x 10</td>
<td>88.2</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 x 5</td>
<td>-8.5</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWM/Ms</td>
<td>1 x 10</td>
<td>100.0</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/J</td>
<td>4 x 5</td>
<td>96.5</td>
<td>9/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3H/He</td>
<td>4 x 5</td>
<td>36.2</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C57/Bl/6</td>
<td>4 x 5</td>
<td>51.8</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>Syngeneic</td>
<td>A/Ph.MC.S1</td>
<td>1 x 10</td>
<td>100.0</td>
<td>18/18</td>
<td></td>
</tr>
<tr>
<td>DBA/2.MC.CS1</td>
<td>DBA/2</td>
<td>1 x 10</td>
<td>76.5</td>
<td>2/7</td>
<td></td>
</tr>
<tr>
<td>P-815</td>
<td>DBA/2</td>
<td>5 x 4</td>
<td>89.0</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td>L-5178Y</td>
<td>DB/2</td>
<td>10 x 3</td>
<td>84.0</td>
<td>3/9</td>
<td></td>
</tr>
<tr>
<td>MM-46</td>
<td>C3H/He</td>
<td>5 x 2</td>
<td>100.0</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>Autochthonous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC-induced primary</td>
<td>DBA/2</td>
<td>1 x 10</td>
<td>80.5</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>Inhibition of metastasis</td>
<td>DBA/2.MC.CS-T</td>
<td>1 x 10</td>
<td>94.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MH-134</td>
<td>C3H/He</td>
<td>1 x 14</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Madison-109</td>
<td>25 x 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevention of onco genesis</td>
<td>SWM/Ms</td>
<td>1 x 10</td>
<td></td>
<td>83→31%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DBA/2</td>
<td>1 x 10</td>
<td></td>
<td>78→37%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3H/He</td>
<td>10 x 3</td>
<td></td>
<td>79→40%</td>
<td></td>
</tr>
</tbody>
</table>

Note: all tumours were solid, transplanted s.c. Route of Lentinan injection was i.p., except i.v. for P-815, L-5178Y, and MM-46. Tumor inhibition ratio = (C-T)C x 100, where C = average tumour weight of control mice and T = that of Lentinan-treated mice.

(tegafur alone) and 173 days (Lentinan plus tegafur). Sub-group analysis was also carried out by: (1) tumour extension, (2) histology; and (3) Borrman classification.

With each prognostic factor the addition of Lentinan significantly prolonged 50% survival (Table 2).

Overall more patients with the combined therapy appeared to survive longer: 19.5% survived more than one year, 10.4% more than two years and 6.5% more than three years. Using the criteria of the Japan Society for Cancer Therapy for Evaluation of Clinical Effects of Cancer Chemotherapy on Solid Tumors patients treated with
Lentinan had a significantly higher response rate (14.9%) than patients in the control arm (2.0%).

Table 2  Prolongation of life by various prognosis factors (Ajinomoto Co. 1984)

<table>
<thead>
<tr>
<th>Background</th>
<th>Treatment</th>
<th>No. of cases</th>
<th>50% survival (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal localisation</td>
<td>Tegafur group</td>
<td>13</td>
<td>166 days</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>19</td>
<td>237 days</td>
</tr>
<tr>
<td>Hepatic or peritoneal metastasis</td>
<td>Tegafur group</td>
<td>47</td>
<td>68 days</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>48</td>
<td>169 days $P &lt; 0.01$</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>Tegafur group</td>
<td>8</td>
<td>170 days</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>9</td>
<td>133 days</td>
</tr>
<tr>
<td>Well-differentiated adenocarcinoma</td>
<td>Tegafur group</td>
<td>31</td>
<td>105 days $P &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>34</td>
<td>223 days $P &lt; 0.01$</td>
</tr>
<tr>
<td>Poorly-differentiated adenocarcinoma</td>
<td>Tegafur group</td>
<td>34</td>
<td>91 days $P &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>40</td>
<td>169 days $P &lt; 0.01$</td>
</tr>
<tr>
<td>Bormann types 1 and 2</td>
<td>Tegafur group</td>
<td>7</td>
<td>119 days</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>7</td>
<td>391 days</td>
</tr>
<tr>
<td>Bormann types 3 and 4</td>
<td>Tegafur group</td>
<td>32</td>
<td>100 days $P &lt; 0.01$</td>
</tr>
<tr>
<td></td>
<td>LENTINAN + tegafur group</td>
<td>41</td>
<td>163 days</td>
</tr>
</tbody>
</table>

Lentinan combined with other chemotherapeutic agents appears to have efficacy in a variety of settings (Matsuoka et al., 1995). Furthermore when patients responded well to Lentinan treatment there was a significantly larger response (2.5 x ) in their killer T cell/suppressor T cell ratio (CD11–CD8+CD11+CD8+) in peripheral blood. The ratio of NK cells with higher activity to NK with moderate activity (CD57–
CD16+/CD57+ CD16+) was higher in the responders than in the non-responders and correlated well with survival times. However, these results remain controversial as a later study suggested that lymphocyte subset changes in peripheral blood did not necessarily correlate with the lymphocyte subset changes that were taking place in the tumour (Matsuoka et al., 1997).

Few adverse reactions to Lentinan have been noted. In a detailed study of 469 patients, 32 (6.8%) experienced an adverse reaction – none serious; the total number of episodes was 46 (9.8%) (Table 3). Only 2 patients required discontinuation of treatment due to unacceptable tolerance. Perhaps the most intriguing aspect of Lentinan use in conjunction with chemotherapy is its apparent ability to greatly reduce the debilitating effects of the chemotherapy, e.g. nausea, pain, hair loss and lowered immune status. Although there have been few formal quality of life studies this anecdotal evidence has been noted as a feature of many of the mushroom polysaccharides.

**Table 3 Adverse reactions attributable to Lentinan** (Ajinomoto Co., 1984)

<table>
<thead>
<tr>
<th>Type of adverse reaction (with an incidence greater than 0.5%)</th>
</tr>
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<tbody>
<tr>
<td>Rash/redness</td>
</tr>
<tr>
<td>Chest pressure sensation of oppression</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
</tr>
<tr>
<td>Headache/headache dull</td>
</tr>
<tr>
<td>Feeling of warmth</td>
</tr>
<tr>
<td>Diaphoresis</td>
</tr>
</tbody>
</table>

Other adverse reactions:

Fever, transient hot flushes of face, anorexia, leukopenia, 2 episodes each (0.5%); dizziness, decreased PBC, decreased haemoglobin, pharynx strangled sensation of pharyngitis, 1 episode each (0.2%) (from Ajinomoto Technical Document).
Schizophyllum commune

The polysaccharide derived from this mushroom is a $\beta(1,3)D$ glucan with $\beta$-$\beta(1,6)D$ glucan side-chains and is called Schizophyllan (or Sonifilan, Sizofiran, Sizofilan). As with all glucan preparations they are never homologous in terms of molecular weight but consist of molecules with a wide range of MWs. In the case of Schizophyllan the molecules are large and are normally administered in the clinical setting by the intramuscular or intraperitoneal route.

Schizophyllan has been shown to be cytostatic in Sarcoma 180 tumours xenographs. The survival of Sarcoma 180 xenographs was not affected by pre-treatment with Schizophyllan, while combined pre- and post-treatment and post-treatment alone resulted in increased survival. Schizophyllan had no effect on the survival of Sarcoma 37, Ehrlich carcinoma – or Yoshida sarcoma ascites tumours (Wasser and Weis, 1999).

Various clinical trials have been carried out in Japan, although many are not blinded. Despite this Schizophyllan has been approved for clinical use in Japan. Early clinical studies with Schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomised controlled study of 367 patients with recurrent and inoperable gastric cancer resulted found a significant increase in median survival (Furne, 1985). However, a similar study was unable to confirm this apparent success with Schizophyllan (Fugimoto et al., 1984). Recently Schizophyllan has also been shown to increase overall survival of patients with head and neck cancers (Kimura et al., 1994).

In a randomised controlled study of Schizophyllan in combination with radiotherapy, Schizophyllan significantly prolonged the overall survival of Stage II cervical cancer patients but not Stage III (Okamura et al., 1986, 1989). In a
prospective, randomised clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil) and Schizophyllan in various combinations, patients treated with Schizophyllan had a better overall survival than patients who had not received the polysaccharide (Miyazaki et al., 1995). However, the variety of treatment regimes significantly reduced the value of these results. However, separate analyses of patients with 10% or more activated CD4+ cells out of their total CD4+ population and with more than 25% activated CD8+ cells before the beginning of treatment showed that in this group the Schizophyllan-induced increase in survival was highly significant. Furthermore when Schizophyllan is injected intratumorally to cervical cancers there is a significant infiltration of Langerhans cells and T-cells (Nakano et al., 1996). Schizophyllan is currently produced commercially by several Japanese pharmaceutical companies.

**Grifola frondosa extracts**

Several studies have shown that β-D-glucan and glycoprotein complexes derived from this mushroom (also known as Maitake) have strong antitumour activity in xenographs (Kurashiga et al., 1997) and there have also been limited number of clinical trials. More recently, a highly purified extract, β-glucan (β-1,6 glucan branched with a β-1,3-linkage) (Grifron-D® GD) has become available. GD has considerable immunomodulating and antitumour activities in animal models, and is orally bioavailable (Nishida et al., 1988). Maitake D-fraction and crude Maitake powder have demonstrated remarkable inhibition of metastasis in an immuno-competent mouse model, especially in the prevention of hepatic metastases which in one series of experiments was reduced by 81% (Maitake powder) to 91% (D-fraction) (Namba, 1995). GD has been shown to have a cytotoxic effect on human
prostate cancer cells (PC9) *in vitro*, possibly acting through oxidative stress, and causing 95% cell death by apoptosis (Fullerton *et al.*, 2000). Vitamin C addition reduced the effective level of GD required. Simultaneous use with various anticancer drugs showed little potentiation of their efficacy except for the carmustine/GD combination (90% reduction in cell viability). This potentiation of GD action by vitamin C and the chemosensitising effect of GD on carmustine may well have significant clinical implications. Unpublished studies by the same authors (manuscript in preparation) again using prostate cancer cells *in vitro* have shown that the cytotoxic effects of the anticancer drug was significantly potentiated or enhanced with GD, possibly mediated through the inactivation of glyoxalase I, a vital detoxifying enzyme responsible for detoxification of cytotoxic metabolites / substances. This study suggests that GD may be useful with some anticancer drugs to improve the efficacy of ongoing clinical chemotherapy. The Maitake D-fraction is a relatively new compound and there are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers underway in the US and Japan. Most of these are at an early clinical stage (phase I / II).

Early pilot studies from China published in abstract form involving 63 cancer patients reported a response rate (partial and complete) against solid tumours at 95% and for leukaemia (type not specified) 90% (Jones, 1998). A recent Japanese non-randomised clinical study using the D-fraction has been carried out in a variety of advanced cancer patients (n=165). Patients took either oral D-fraction plus crude Maitake powdered tablets, or D-fraction plus placebo tablets in addition to chemotherapy (Nanba 1997a). Tumour regression or significant symptomatic improvement were observed in 11 out of 15 advanced hepatocellular carcinomas with D-fraction plus Maitake. When D-fraction plus Maitake was combined with
chemotherapy, the overall response rates were increased by 12-28% when results from all cancer types were combined.

As the authors of this study observed chemotherapy itself could also significantly lower the immune system of patients. They reported that many of the patients recovered from the severe side-effects caused by chemotherapy when D-fraction was given, although this conclusion appears to be an anecdotal observation. In a similar manner to Lentinan, there are now increasing examples of synergism between Maitake D-fraction and crude Maitake powder and conventional chemotherapy.
The US Food and Drug Administration has approved Grifron-D© (GD) for trial
under an Investigational New Drug Application (IND) for patients with advanced
cancer and some US-based clinical trials are currently underway at various
Institutions (Nanba 1997b). No details are available as yet. In conclusion, GD has
few side effects and anecdotal clinical reports appear to suggest that it might
alleviate some of the side-effects of chemotherapy. The apparent success of crude
Maitake powder by oral administration in cancer therapy and immune stimulation
would also support its suitability as a nutriceutical.

**Phellinus linteus**

*Phellinus linteus* has long been used in traditional Chinese medicine in the
form of hot water extracts from the fruit-bodies – ‘song gen’ in Chinese and
‘mishimakobsu’ in Japanese. In the last decade the effects of these extracts for
improving symptoms of digestive system cancers such as oesophageal duodenal,
colorectal, as well as hepatocellular, have been reported by practioneers of TCM.
As with most of these mushroom polysaccharide extracts tumour responses and / or
symptotomatic improvement (enhanced quality of life) have mainly been reported in
combination with conventional chemotherapy in an adjuvant or neo-adjuvant setting
(Mizuno, 2000). In Korea there has been a major National project involving industry,
government and academic laboratories using fermenter-cultivated mycelium from
several *P. linteus* strains (Aizawa, 1998). The major polysaccharide product has
been approved as a medicine and has been manufactured by the Korean New
Pharmaceutical Co. since 1997. Similar studies are also taking place in Japan by
the Applied Microbiology Laboratory, Obiken Co. Ltd. Meshima, the hot water
extracted polysaccharide product now manufactured by the Korean Company, has

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become available in Japan for sale as a functional food (an immunity activation substance).

Although there have been only a few phase II trials there have been reported tumour responses to the combination of Meshima with conventional chemotherapy. There are a considerable number of Korean and Japanese patents now in place and further trials with the Meshima polysaccharide product (oral formulation) are ongoing.

**Active Hexose Correlated Compound (AHCC)**

The components of this proprietary extract have been considered elsewhere in this Report, and the full details of preparation and content are not available. In contrast to the other anticancer glucans, the glucans of AHCC are low molecular weight, alpha-1,3 structures. As such, they should have low-immunopotentiating activity but still retain their tumouro-static activity.

Initial studies have evaluated AHCC in a chemo-prevention role by assessing its ability to prevent or delay recurrence of hepatocellular carcinoma after surgical resections (Kamiyama, 1999). In this non-randomised phase II trial 44 patients after partial heptatectomies were given oral AHCC at 3g per day. After one year the AHCC group had a significantly higher 1 year survival and lower recurrence rate than the control group as well as a significant lowering of a number tumour markers (CEA, αFP). However, this study has only appeared in abstract form while a second report, again in abstract form (Matsui et al., 1999) stated that recurrence was not lower in the AHCC group although the 1 year survival rate was higher.

The AHCC Research Association was formed in 1996 to advance the awareness of AHCC as an anticancer therapy. They state that of 300 cancer patients administered AHCC, 58 patients experienced same effect, 46 showing complete or partial responses. The participants in these studies had cancers of the
lung, breast, stomach, oesophagus, colon, liver etc. To date, the published evidence of the efficacy of this complex preparation must be treated with some scepticism until more detailed controlled studies are forthcoming.

**Ganoderma lucidum**

Over the last ten years there have been numerous reports of pre-clinical anti-tumour activity of *G. lucidum* extracts in a variety of tumours (Lee *et al.*, 1995; Wang *et al.*, 1997). Such extracts effectively inhibited metastasis in animal (mouse) models and increase survival when administered as monotherapy or in combination with conventional chemotherapy (Hwang *et al.*, 1989; Furusawa *et al.*, 1992; Lee *et al.*, 1995). Some preclinical studies have suggested that the anti-tumour action of *G. lucidum* polysaccharides could be a result of its biological response modifying effects (Chang, 1996). Ganopoly (an aqueous extract of *G. lucidum*) has been shown in *in vitro* systems and in xenographs to have immunomodulating effects, through the activation of macrophages, T-lymphocytes, and natural killer cells (Gao, 2000).

Within the realms of traditional herbal medicine in China and in several Asian countries many cancer patients use *G. lucidum* proprietary extracts as adjunct to conventional treatment or as the sole therapy. What then can be said of the effectiveness of such products on human cancers? Relatively few clinical studies have so far been published in Chinese while no clinical trials with *G. lucidum* extracts against various human cancers have been published in English peer-reviewed journals (Gao, 2000). However, an extensive open, non-randomised clinical trial has recently been carried out on of patients with advanced cancers using a proprietary aqueous extract of *G. lucidum* – Ganopoly (Zhou *et al.*, 2001). This compound is marketed as an over-the-counter product in Hong Kong, New Zealand, and Australia.
The clinical trial was carried out to evaluate the efficacy and safety of Ganopoly in 143 patients with advanced cancers of the lung, breast, liver, colorectum, prostate, bladder, brain and non-Hodgkin’s lymphoma that had already been treated with conventional chemotherapy. This trial explicitly follows many of the rules and conventions that define Western oncology trials. Eligibility criteria included confirmation of diagnosis, objective measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, life expectancy of 12 weeks or greater, no recent or concomitant anti-cancer therapy, and informed consent. All patients underwent evaluation of the extent of the disease, quality of life, hematologic, biochemical and selected immune function studies at baseline and after 6 and 12 weeks of Ganopoly therapy. Standard criteria were used to evaluate adverse events and responses. The extracts were given orally at 1800 mg three times daily. Patients were entered into the study from January 1997 through September 1998 if they met certain eligibility criteria and did not meet any of the recognised exclusion criteria. Eligibility and exclusion criteria were according to internationally accepted rules for clinical trials. WHO (1979) criteria were used to evaluate efficacy and toxicities were graded according to the Common Toxicity Criteria (Green and Weiss, 1992). A complete response (CR) was defined as the complete disappearance of all tumour masses without the appearance of any new lesions and normalisation of all clinical and laboratory signs and symptoms of active disease. A partial response (PR) was defined as a 50% or greater reduction of the products of the longest perpendicular diameters of the measured sentinel lesions without demonstrable new lesions elsewhere. Stable disease (SD) occurred when no new lesions appeared and no measurable lesions increased more than 25% in a cross-directional area. Progressive disease (PD) was defined as the appearance of
new lesions and/or an increase in the cross-sectional area of any previously known
lesions by greater than 25%. Quality of life was quantified with the previously
validated Functional Assessment of Cancer Therapy-General (FACT-G) scale (Cella
et al., 1993).

The trial was designed to enrol 15 assessable patients for each of the 8
tumour types with an initial entry of 120 patients. Further accrual would be halted if
no CR or PR were observed in each specific tumour type and the therapy would then
be judged to be inactive. Should one or more CRs or PRs occur among the 15
patients, another 25 patients would be brought in. Tumour types with four or more
CRs or PRs were to be targeted for further study. The planned accrual was
designed to provide a 90% likelihood of rejecting treatment with the true response
rate of 5% or less and a 90% probability of accepting treatment with a true response
rate of 20% or more. Data compiled at baseline, 6 weeks and 12 weeks were
analysed by two-way analysis of variance by ranks. Median values of quantities,
such as age and days since diagnosis, were compiled using Wilcoxon’s rank-sum
test, and categorical values (of quantities such as primary tumour site and off-study
reason) were compared using chi-squared tests and for 2X2 table, Fisher’s exact
test.

A total of 83 men and 60 women were enrolled and the median age of all
patients was 61 years. Ninety three percent of the patients had stage IV disease.
Twenty seven patients were not assessable for response and toxicity because they
were lost to follow-up or refused further therapy before 12 weeks of treatment.

Of the 100 fully assessable patients, 46 patients (32.2%) had progressive
disease (PD) before or at the 6 week evaluation point (range, 5 days – 6 weeks).
Sixteen patients (11.2%) developed PD between 6 and 12 weeks of therapy. No
objective (partial or complete) responses were observed, but 38 of 143 patients
(26.6%) had stable disease (SD) for 12 weeks or more (range 12 – 50 weeks).
There was no significant changes in the FACT-G scores in 85 assessable patients.
However, palliative effects on cancer-related symptoms, such as sweating and
insomnia were observed in many patients. In the group of patients with SD, FACT-G
scores improved in 23 patients, unchanged in 5 patients and declined in 1 patient.
Within this group, the median change for the baseline score to the 6- and 12-week
score was +7.6 and +10.3 score, both statistically significant (P < 0.05). No
significant change of the selected immune function parameters were observed in 75
assessable patients. However, in the group of 32 patients with SD for 12 weeks or
more, Ganopoly significantly increased lymphocyte mitogenic reactivity to
concanavalin A and phytohemagglutinin by 48-52% (P < 0.05) and significantly
enhanced natural killer cell activity by 75% (P < 0.05). Five adverse events (grade I)
were recorded, 3 of which were gastrointestinal (nausea 2; diarrhoea, 1).

While objective responses were not observed with this study the results do
indicate that this *Ganoderma* extract, Ganopoly, could well have an adjuvant role in
the treatment of patients with advanced cancer (Cassileth, 2000; Jacobson *et al*.,
2000).

Recently there has been a Phase II clinical trial with a herbal supplement PC
SPES which includes, with other components, extracts of *G. lucidum*, of patients
suffering from prostate cancer (Small *et al*., 2000). The treatment significantly
reduced the serum prostate-specific androgen (PSA) levels in all 33 androgen-
dependent prostate cancer patients with a duration of > 57 weeks. Further details are
not yet available.
Trametes versicolor

*Trametes versicolor* is not an edible mushroom but since ancient times extracts has been used in traditional Chinese medicine for therapeutic effects including the treatment of cancer. TCM used the extracts that were derived from whole fruit-bodies. Today two compounds, PSK (polysaccharide-K) and PSP (polysaccharide-peptide) are purified from this fungus by deep tank fermentation of the mycelium using a variety of strains. PSK (Krestin) was first isolated in Japan in the late 1960s while PSP was isolated about 1983 in China. Each compound has shown remarkable anticancer properties with few side-effects. Remarkably by 1987 PSK accounted for more than 25% of total national expenditure for anti-cancer agents in Japan. Numerous clinical trials have been carried out over the years and are briefly summarised below:

**PSK:**

There have been several decades of successful clinical trials using PSK to treat head and neck, upper GI, colo-rectal and lung cancers with some reported success in treating breast cancer as well. Clinical trials with PSK have recently been extensively reviewed by Kidd (2000) and will be briefly summarised here. Almost exclusively, clinical trials have been carried out in Japan.

**PSK and gastric cancer:**

PSK has been used as a form of immunotherapy for more gastric cancer patients than any other cancer type. In early 1970s Kaibara’s group began trialing PSK with their existing chemotherapy regimens for stage IV disease (Kaibara *et al.*, 1976). After surgical resection (partial or full gastrectomies), PSK at 3g per day was
added to a chemotherapy regimen of Mitomycin C and 5-fluorouracil (5-FU) (n=66).
When compared with a historical control group, the 2 year survival rate was more
than double, a finding that was later confirmed by Fujimoto et al. (1979) in a larger
prospective study (n= 230). Further studies by Hattori et al. (1979) (n=110) and
Kodama et al. (1982) (n =450) suggested that PSK gave some protection against the
immunosuppression that normally is associated with surgery and long-term
chemotherapy.

One of the few double-blind randomised controlled trials (n=144) examining
the role of single agent PSK found a significant increase in disease-free and overall
survival. PSK had significant effects on these patients immune systems as
measured by increased delayed-type hypersensitivity on skin tests and enhanced
chemotactic migration of neutrophils (Kondo and Torisu, 1985). All these studies
suggest that individuals with very low immunity are less likely to benefit from PSK
therapy than individuals with a reasonably competent immune system. Other non-
randomised trials in Japan have supported these findings (Mitomi and Ogoshi, 1986;
Niimoto et al., 1988; Maehara et al., 1990; Nakazato et al., 1994). Tsujitani et al.
(1992) had previously observed that dendritic cells could infiltrate gastric cancers in
some patients and biopsy examination correlated this dendritic infiltration of their
tumours with an increase in disease-free and overall survival post-surgery. It was
concluded that patients with gastric cancer with limited dendritic cell infiltration prior
to surgery when given PSK immunotherapy were more likely to have significant
response. The most recent phase III 2 arm trial of PSK in the treatment of gastric
cancer carried out by the “Study Group of Immunochemotherapy with PSK for
Gastric Cancer of Japan” showed that combining PSK with conventional
chemotherapy significantly improved disease-free and overall survival (Nakazato et al., 1994).

PSK and other cancers

In a non-controlled, retrospective analysis of combined radiation, chemotherapy and immunotherapy (using PSK or OK-32, another immuno-potentiatior) with 133 patients with oesophageal cancer, there were improvements in one-year and two-year survival (Okudaira et al., 1982). In another more recent study PSK improved overall survival in oesophageal cancer in patients with levels of pre-operative high α1-anti-chymotrypsin or sialic acid (Ogoshi et al., 1995). In a small scale trial in Taiwan for nasopharyngeal carcinoma PSK adjunct therapy had a small but significant impact on five-year survival (Go and Chung, 1989).

In a study of 185 patients with epidermoid carcinoma, adenocarcinoma or large-cell carcinoma (≤ IIIb) given PSK as an immune system potentiator following radiotherapy, almost four times more patients who were treated with PSK had significant improvements in disease-free survival than those not given PSK (Hayakawa et al., 1993). PSK was clinically significant with more advanced patients with Stage III disease than Stage I and II patients. PSK had greater activity for older patients (> 70 years) and patients with small primary tumours.

Early studies with breast cancer patients seemed to imply that long-term PSK immunotherapy in conjunction with chemotherapy could have beneficial results (Sugininachi et al., 1984). In a later much larger trial (914 patients) in-depth analysis implied that PSK significantly extended survival in ER-negative, Stage IIA patients without lymph node involvement (Toi et al., 1992). However, in a further large trial, Morimoto et al. (1996) could find no statistical evidence of any benefit from PSK. These contradictory studies may have been clarified by Yokoe et al. (1997) who
compared HLA B40 antigen positive patients treated with PSK against B40 negatives. It was found that B40-positive patients treated with PSK (3g/daily, two month course each year) in addition to chemotherapy had an improved 10 year overall survival rate compared to B-40 negative patients. Thus, HLA B40 may be a predictive factor for PSK response.

The foregoing studies give strong indications of the potential benefits of incorporating PSK into some cancer treatments as an adjunct to radio- or chemotherapy. Furthermore, PSK can improve immune status secondary to the side effects associated with traditional therapies. As stated by Kidd (2000) “after a quarter century of trials indicating PSK can improve cancer survival, the cumulative human findings amount to a recommendation for its inclusion in standard anticancer protocols. With its risk for adverse effects virtually nonexistent, PSK’s contribution to the benefit-risk profiles of these protocols can only be positive”.

PSP and clinical trials

While PSK has been almost exclusively developed and tested within Japan, PSP in contrast is a product of China and continues to be assessed for efficacy safety by their scientists and oncologists. There is a close similarity between PSK and PSP polypeptides although PSP lacks fucose and instead contains arabinose and rhamnose. Since the first development of PSP in 1983 there has been rapid progress through human clinical trials. Phase I clinical trials were carried out by Xu (1993) and it was shown that an oral dose of up to 6g/day was well talented and lacking in side-effects. Patients showed improvement in appetite and general condition, together with a stabilisation of haematopoietic parameters.
The Phase II study by the Shanghai PSP Research Group with 8 hospitals in Shanghai was carried out using patients with cancers of the stomach, lung and oesophagus. The dosage was 1g three times daily to a total of 190g. Results confirmed the role of PSP as a biological response modifer improving the immunological status of the patients after surgery, radiotherapy and/or chemotherapy (Liu and Zhou, 1993). Following the success of the Phase II clinical trials, a Phase III trial was conducted in a large cohort of patients (650) in Shanghai hospitals. 189 were randomised to taking PSP and placebo; 461 patients were unblinded to their therapy (Liu et al., 1999). These trials showed that PSP improved disease-free survival of gastric, oesophageal and non-small-cell lung cancers while again substantially reducing the normal unpleasant side-effects of conventional treatments (Sun and Zhu, 1999; Sun et al., 1999). PSP had a protective effect on the immunological functions of conventionally-treated patients, thus demonstrating that PSP can be classified as a clinical biological response modifier. Other BRMs such as LAK cells, IL-2, \( \alpha \gamma \) IFN or TNF are also being used in the treatment of advanced cancer cases (Liu, 1999). Yet, these drugs at effective doses, in many cases, produce quite severe side-effects such as fevers, chills, rashes, arthralgia, hypotension, oliguria, pulmonary oedema, congestive heart failure and CNS toxicities. Mao et al. (1998) have shown dramatic anti-tumour effects when PSP was combined with IL-2. As side-effects of IL-2 are dosage and schedule dependent, it is reasonable to expect that with PSP, a lower dose of IL-2 could be used clinically with subsequent decrease in the severity of the side-effects (McCune and Chang, 1993). A further observation noted that PSP in combination with radiotherapy induced a significant increase in the percentage of apoptotic cells at 24h, compared with radiation alone, and it has been surmised that the antitumour mechanism of PSP.
action may also involve the induction of DNA damage by apoptosis in the target
cancer cells (Stephens et al., 1991).

A common adverse reaction of radiotherapy and chemotherapy is
haematopoietic toxicity. Several studies have shown a strong amelioration of these
toxic effects by PSP (Shiu et al., 1992; Sun et al., 1999).

In a double-blind Phase II trial in Shanghai hospitals almost 300 patients
suffering from gastric, oesophageal or lung cancer were treated with conventional
radiotherapy and/or chemotherapy together with PSP or shark liver oil (batyl
alcohol). Quality of life was assessed by marked improvement of clinical symptoms
as well as improvements in blood profiles and/or immune indices and significant
improvement in Karnovsky performance status or body weight. PSP improved
overall clinical symptoms, together with most symptoms associated with cancer
therapy. PSP was found to be effective for 82% of the patients compared with 48%
for batyl alcohol (Liu and Zhou, 1993).

Many Phase III clinical trials of PSP combined with conventional therapies
have demonstrated significant benefits against cancers of the stomach, oesophagus
and lung (Jong and Yang, 1999; Yang, 1999). Most studies with PSP have not fully
explored the long-term survival benefit although in an open-label, randomised trial in
oesophageal cancer has shown that PSP did significantly improve one-year and
three-year survival (Yao, 1999). Liu (1999) has commented on the favourable action
of PSP in patients receiving bone autologous marrow transplants.

The corpus of laboratory and clinical evidence that PSP offers considerable
benefits to patients suffering from cancers of the stomach, oesophagus and lung
have led to the Chinese Ministry of Public Health granting it a regulatory license.
Despite the use of PSK and PSP in humans for many years, bioavailability and the pharmacokinetics has received little detailed study. More work in this area, as well as blind RCT’s, are required.

Safety data

Pre-clinical

Lentinan

Toxicity tests using Lentinan have been carried out in a variety of species with dosing ranges of 0.0001-30 mg/kg for 5-6 week by iv administration. Some swellings and proliferation of reticuloendothelial cells were at dosages >25mg/Kg. Some species in the \( \geq 2 \) mg/kg groups also developed gastrointestinal or urinary bladder haemorrhages with dermatological changes. All lesions occurred in high dose groups and tended to regress after discontinuing Lentinan administration.

Fertility of males was not affected at 0.1-1.0 mg/kg. No abnormalities were detected with doses 5.0-10 mg/kg during fetal organogenesis in rats and no abnormality at maximum dose of 5.0 ug/kg during perinatal and lactation period. There was little or no penetration into the foetus and no excretion into maternal milk (Ajinomoto Technical Document , 1988).

In antigenicity studies there were no anaphylactic reactions and no effect on allergic reactions. Lentinan had no effect in a mutagenicity test, haemolysis test, blood coagulation, ability to induce arthritis and no effect on adjuvant-induced arthritis.
The manufacturers of the other β-glucan products now being used in clinical work have carried out tests comparable to those with Lentinan and have obtained similar results.

**PSP**

PSP produced no teratogenic effects in mice or rats and exerted analgesic action in mice (Jiang et al., 1999; Jin, 1999). It has been shown that some compounds with proven antitumour and immunomodulatory activities inhibit ovulation and ovarian steroidogenesis, increase the incidence of oocyte degeneration and demonstrate aborti-facient and embryotoxic effects. The lack of deleterious effects of PSP on ovarian follicular development, steroidogenesis, ovulation, quality of ovulated oocytes, pregnancy and embryo development in mice would suggest it does not affect female reproduction (Ng and Chan, 1997).

Mutagenicity testing can now be viewed against an impressive background of basic scientific knowledge of genetic mechanisms and also the development of a wide range of experimental procedures that can be used as test systems. Recently, Zhong et al. (1999) have carried out an extensive series of experiments on possible genetic toxicity of the PSP polysaccharopeptide:

1. Mutagenicity tests to assess genotoxicity of PSP using a special strain of *Salmonella typhimurium* – no evidence of mutagenic activity.
2. Cytotoxicity tests of PSP with V79 Chinese hamster cells *in vitro* – no toxic effects against the V79 cell line.
3. *In vivo* micronucleus tests to assess the cytogeno-toxicity on mammalian somatic cells – PSP showed no evidence of mutagenic potential when administered in this *in vivo* test.
4. Chromosome observation tests, metaphase analysis of bone marrow cells in mice – the results of cytogenic lesions in mice showed that the number of chromosomes had not changed in PSP treated groups even at the high dose rate 126 mg/kg.

Subchronic toxicity tests have been performed with various concentrations of PSP on rats by p.o. administration. PSP was administered at dosage rates of 1.5, 3.0 and 6.0g/kg body weight every day for up to 62 days. At the time of the final administration of PSP and 2 weeks after the last administration, the general conditions, i.e. blood indexes, serum biochemistry indexes and patho-histology indexes of the PSP groups were compared to the control group and no obvious differences were observed (Jiang et al., 1999). A further study with mice demonstrated that acute, chronic, genetic, reproductive and two-generation teratogenic toxicity were very low at 50-100 times the oral clinical dose (Jin, 1999 – contains many relevant references on PSP safety tests).

**Other Medicinal Mushrooms**

Recent studies have shown that crude extracts of *Ganoderma lucidum* and *G. lipsiense* do not exhibit genotoxic properties (clastogenicity and/or aneugenicity), at any dose level tested. Using the Cytokinensis – Blocked Micronucleus Assay (CBMA) on cultured human lymphocytes there was no evidence that the extracts contained clastogens (the micronuclei containing one or more acentric chromosome fragments) and anangens (the micronuclei containing one or more whole chromosomes) (Steinmetz et al., 2001). Similar studies should be performed for the other important medicinal mushrooms. Hot aqueous extracts of wild *Ganoderma* fruit-bodies were assessed for cytotoxicity and in vivo genotoxicity by both acute and subchronic oral exposure of mice (dose equivalent of 220 g fresh *Ganoderma* fruit-
body/kg body weight). No evidence was found for genotoxic chromosomal breakage nor cytotoxic effects by the extracts (Chiu et al. 2000). Previous suggestions that Ganoderma extracts had anti-mutagenic properties were not substantiated using Comet Assays.

A recent study by Badalian et al. (2001) examined certain pharmacological activities of Flammulina velutipes (an edible medicinal mushroom) and Paxillus involutus (poisonous mushroom) and Tricholoma tigrinum on mice, using methanol-soluble and water-soluble residues separated from methanol extracts of fruit-bodies. The fungal extracts did not show any particular analgesic effects while algogen activity and significant spasmolytic papavertine-like activities were observed for P. involutus. Both P. involutus and T. tigrinum showed effects on the central nervous system with increased dynamic activity and curiosity of the mice. F. velutipes showed little evidence of any of these pharmaceutical disturbances.

Clinical

In the clinical setting tens of thousands of patients have been treated with PSP. Many patients have been successfully taking PSP for over 10 years with no serious adverse effects (Yang, 1999).

The highly purified β-glucan (Grifron-D®) from the Maitake mushroom Grifola frondosa has also been approved by the FDA for trial under an Investigational New Drug Application (IND) for patients with advanced cancer and some clinical trials are now underway. Due to the absence of adverse reactions in previous trials and with no significant pre-clinical toxicity the FDA has exempted this polysaccharide from a Phase I study (Fullerton et al., 2000). Furthermore, a number of large phase III

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