

β1,3-GLUCAN ANTICANCER EFFICACIES AND SYNERGIES: A REVIEW

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ABSTRACT

β1,3-glucans from fungi, cereals, seaweeds and bacteria have been shown to possess favourable biological and anti-carcinogenic activities including upregulation of phagocytosis, cytokine production enhancement, superoxide and nitrite production; antibody secretion and stimulation of signalling pathways associated with proto-oncogene expression. However, human dietary supplements containing β1,3-glucans vary in efficacy due to glucan source, the lifecycle stage of the source at extraction, extraction methods, purity, concentration and combination with other immunomodulators. A review of efficacy of some commercially available β1,3-glucan products is presented. Three apparently efficacious products in which β1,3-glucan was the only immunomodulator were identified: Glucan #300®, Maitake Gold 404® (diluted Yukiguni Maitake MD Fraction®) and Betamune®. A trial of Maitake Gold 404® produced evidence of standardisation problems. It is recommended that Yukiguni Maitake MD Fraction® (a more standardised alternative), Glucan #300® and Betamune® be comparatively trialled at optimal doses across immunological measures and tumor reduction. β1,3-glucans have been shown to be synergistic with conventional cancer therapies and monoclonal antibodies, as well as immunomodulators including vitamin C, transresveratrol, humic acids and Ashwagandha (*Withania somnifera*). Trialled commercially available products containing immunomodulator combinations have been shown to be inefficacious, apart from RVB300®, a β1,3-glucan/transresveratrol/vitamin C combination. The efficacies of various combinations of β1,3-glucans with other immunomodulators and the details of specific β1,3-glucan/monoclonal antibody synergies in treating particular cancer cell lines, require systematic elucidation.

Keywords: Cancer, Chemotherapy, Glucan, Immunomodulators, Monoclonal Antibodies

1. INTRODUCTION

The estimated number of Americans to receive a new cancer diagnosis in 2014 is 1,665,540, with almost 1,600 deaths per day predicted (ACS, 2014). Cancer is the cause of one in every four American deaths (ACS, 2014). Marked morbidities are associated with both cancer and chemotherapy (Nanba, 1997). The identification of novel pharmaceuticals containing effective treatment agents, is essential for evidence based pharmacy practice. Possible agents include β-glucans: Complex, structurally diverse β-glucose

homopolymers isolated from the cell walls of grains, fungi, seaweeds and bacteria (Jamois *et al.*, 2005; Novak and Vetvicka, 2008; 2009; Vetvicka and Vetvickova, 2010; Vetvicka *et al.*, 2002; Vetvicka *et al.*, 2007a; Vetvicka and Yvin, 2004). Nomenclature designates glycosidic bond positions, position β(1,3) with side chains bound by 1,6 glycosidic linkages predominant in studies, due to their allegedly superior immunological and anti-carcinogenic activity (Novak and Vetvicka, 2009). Degree of branching, detailed structure and solubility and hence biological activity of β1,3-glucans differ according to source and isolation method (CMEC,

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2006; Liu *et al.*, 2009; Lowman *et al.*, 2003; Novak and Vetvicka, 2008; 2009; Tada *et al.*, 2007; 2009; Vetvicka *et al.*, 2002; Zhang *et al.*, 2007).

1.1. β 1,3-Glucan Anti-Cancer Immunological Effects and Applications

Tumorcidal properties of polysaccharides have been researched since the 1940s (Novak and Vetvicka, 2008; Vetvicka and Vetvickova, 2011a) and by 2010 the active immunomodulatory properties of β 1,3-glucans, including anti-bacterial and anti-tumoractivity, had been demonstrated in over 6,000 papers and across all tested species (Novak and Vetvicka, 2009; Vetvicka and Vetvickova, 2010; Vetvicka *et al.*, 2007a). Anti-tumor activities have been shown in cancer models including colorectal (Vetvicka *et al.*, 2002), lung (Vetvicka *et al.*, 2007a), mammary (Vetvicka and Yvin, 2004) and gastric cancer (Shomori *et al.*, 2009). Japanese studies have repeatedly asserted the anti-cancer effects of Maitake β -glucan MD fraction, from the mushroom *Grifola frondosa*, also termed "D fraction" (Hishida *et al.*, 1988; Inoue *et al.*, 2002; Kodama *et al.*, 2002a; 2002b; Masuda *et al.*, 2008; Nanba, 1997; Nanba *et al.*, 1987), notably in human subjects with breast, liver or lung cancers (Kodama *et al.*, 2002a; Nanba, 1997).

Unlike other natural immunomodulators, purified β 1,3 glucans retain their bioactivity for a long time after administration, facilitating characterisation of their anti-cancer mechanisms of action at molecular and cellular levels (Hong *et al.*, 2004; Kodama *et al.*, 2002b; Vetvicka and Vetvickova, 2007a). β 1,3-Glucans are treated as pathogens by pattern recognition receptors on macrophages, neutrophils, monocytes and Natural Killer cells (NK cells) (Hong *et al.*, 2004; Kodama *et al.*, 2002a; Liu *et al.*, 2009; Novak and Vetvicka, 2009; Vetvicka and Vetvickova, 2005). β 1,3-Glucans from different sources possess differential receptor affinities (Liu *et al.*, 2009), most importantly for Complement Receptor 3 (CR3) on NK cells, macrophages and neutrophils and Dectin-1 receptor on macrophages (Hong *et al.*, 2004; Li *et al.*, 2006; Liu *et al.*, 2009; Masuda *et al.*, 2012; Novak and Vetvicka, 2008; 2009; Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2011a; Vetvicka and Yvin, 2004; Vetvicka *et al.*, 2007a; Vetvicka *et al.*, 2013; Wu *et al.*, 2006).

After β 1,3-glucan-CR3 binding, granulocytes specifically attack iC3b-opsonized tumor cells and pathogens (Li *et al.*, 2006). Targeting leads to enhanced phagocytosis by macrophages, monocytes and

neutrophils (Vetvicka and Vetvickova, 2007b; Vetvicka and Yvin, 2004; Vetvicka *et al.*, 2007a; Wu *et al.*, 2006), followed by superoxide and nitrite production (Nanba, 1997; Novak and Vetvicka, 2009; Vetvicka and Vetvickova, 2010), then pro-and anti-inflammatory cytokine secretion enhancement (Deng *et al.*, 2009; Jamois *et al.*, 2005; Kodama *et al.*, 2002a; Nanba, 1997; Novak and Vetvicka, 2008; 2009; Shomori *et al.*, 2009; Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2007b; Vetvicka and Yvin, 2004; Vetvicka *et al.*, 2002; Wu *et al.*, 2006; Xu *et al.*, 2010), tumor regression and enhanced host survival (Hong *et al.*, 2004; Kodama *et al.*, 2002a; Vetvicka and Yvin, 2004; Zhang *et al.*, 2007). The relevant cytokines can function either as growth factors or cancer growth inhibitors and include interleukins such as IL-1, IL-2, IL-6, IL-10, IL-12, IFN- γ and TNF- α (Deng *et al.*, 2009; Jamois *et al.*, 2005; Kodama *et al.*, 2002a; 2002b; Nanba, 1997; Tada *et al.*, 2007; Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2007a; Vetvicka and Yvin, 2004; Wu *et al.*, 2006; Xu *et al.*, 2010). Also via CR3 binding, β 1,3-glucan administration inhibits the adhesion molecules favouring metastasis (Masuda *et al.*, 2008; Novak and Vetvicka, 2009).

Additional interrelated immunological events following receptor binding include NK cell activity (Hamano *et al.*, 1999; Kodama *et al.*, 2002a; 2002b; Nanba, 1997; Vetvicka and Vetvickova, 2005; Vetvicka and Yvin, 2004; Wu *et al.*, 2006); granulocyte (Ito *et al.*, 2009; Lin *et al.*, 2007) and CD4⁺, CD8⁺ T and B lymphocyte migration (Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2007b; Vetvicka *et al.*, 2002; Xu *et al.*, 2010); and increase of antibody production (Vetvicka and Vetvickova, 2007b; Vetvicka and Vetvickova, 2010; Vetvicka and Vetvickova, 2012a; Vetvicka *et al.*, 2007a; Xu *et al.*, 2010). β 1,3-glucan stimulates the Syk-phosphatidylinositol 3-kinase and phosphorylated extracellular signal-regulated kinase signalling pathways (Li *et al.*, 2006; Masuda *et al.*, 2012), affecting expression of genes involved in cancer cell proliferation, invasion and apoptosis (Novak and Vetvicka, 2009; Vetvicka *et al.*, 2007b; Vetvicka *et al.*, 2008; Xu *et al.*, 2010; Zhang *et al.*, 2007). The question of the genetic landscape underlying cancers is complex: The simultaneous over-or under-expression of specific combinations of genes is closely associated with carcinogenesis. Further, relevant genes may possess a range of isoforms which differentially promote or suppress cell growth and which are differentially expressed by normal or cancerous cells (Novak and Vetvicka, 2009; Otake *et al.*, 2007).

1.2. Potential Sources of Variability in β 1,3-Glucan Product Efficacy

The concentration of effective β -glucan in a product and the dose bear a strong relationship to immunological effects, as evidenced by (Deng *et al.*, 2009), who measured the human oral dose response curves for Maitake Gold 404® (containing Yukiguni Maitake MD Fraction®) across a wide range of immunological parameters. Optimal dose for many parameters was around 5-7 mg/kg/day, with highest dose causing reductions in some immunological reactions. The effective dose of β -glucan within a product is affected by source, growth stage of the source, purity and extraction method.

Unfortunately, a significant part of research has been conducted with only partially purified β 1,3-glucans, making assessment of efficacies of specific glucans problematic (Vetvicka and Vetvickova, 2007a; Xu *et al.*, 2010). Individual fungal species incorporated in products may contain multiple ingredients which can contribute to immunological and biological activity (Vetvicka and Vetvickova, 2007a), including many types of β -glucans: Numerous different β 1,3-glucans have been identified in Maitake mushrooms and *Saccharomyces cerevisiae* or *Candida albicans* yeasts alone (Cui *et al.*, 2007; Ito *et al.*, 2009; Lowman *et al.*, 2003; Ohno *et al.*, 1986; Tada *et al.*, 2009; Vetvicka and Vetvickova, 2005; Zhang *et al.*, 2007). There is significant growth condition-related variation in β 1,3-glucan branching and molecular weight within individual sources and hence variable potentially anti-tumor properties (Jamois *et al.*, 2005; Lowman *et al.*, 2003; Novak and Vetvicka, 2009).

Further heterogeneity of immunological efficacy between commercially available β 1,3-glucan products relates to isolation methods (CMEC, 2006; Vetvicka and Vetvickova, 2005). For example, two different methods of preparation of the β -glucan Maitake MD fraction produce β -glucans which are protein bound, or otherwise: Maitake Gold 404® and Grifron Pro Maitake D Fraction® respectively (Vetvicka and Vetvickova, 2005).

The increased heat and alkalic solvents commonly used in extraction may destroy triple helical structures underlying some mechanisms of action in highly branched β 1,3-glucans (Novak and Vetvicka, 2008; 2009; Ohno *et al.*, 1986; Vetvicka and Vetvickova, 2010; Zhang *et al.*, 2007), such as Maitake MD fraction. Opinions vary as to whether, following hydrolytic removal of Maitake MD fraction side branches, the fraction lacks immunostimulatory activity (Lin *et al.*, 2009; Miyake *et al.*, 2005). Therefore, hot water extraction is recommended above the less costly alcohol

extraction, which removes side branches and may destroy some efficacy (Zhang *et al.*, 2007).

Poorly isolated β 1,3-glucans have comparatively small biological and immunological effects and in some cases may be even toxic (CMEC, 2006; Jamois *et al.*, 2005; Novak and Vetvicka, 2008; Vetvicka and Vetvickova, 2010). However, soluble glucans can be further fractionated (precipitated with various concentrations of ethanol) so as to obtain a product with specific chemical composition and enhanced purity and efficacies (Nanba, 1997; Wu *et al.*, 2006; Zhang *et al.*, 2007). Yukiguni MD Fraction® from Maitake mushrooms is hot water extracted and fractionated (Kodama *et al.*, 2002a; Nanba and Kubo, 1997).

The burgeoning of β 1,3-glucan products commercially available as human dietary supplements (in 2014 over 400 on the U.S. market alone) raises the importance of identification of those with superior anti-carcinogenic properties. Proprietary extracts from yeast alone vary in manufacturer, solubility and purity (Vetvicka and Vetvickova, 2010; Vetvicka *et al.*, 2008). With the notable exception of the work of Vetvicka's group, direct comparisons of immunological and biological actions of β 1,3-glucan products are almost non-existent (Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2007a; 2007b; 2009; 2010; Vetvicka *et al.*, 2008). From the perspective of cancer patients, evidence-based use of such products is critical.

Furthermore, β 1,3-glucan products for human consumption containing additions of any number of other alleged immunomodulators, including *Astragalus*, *Echinacea*, *Aloe vera* or *Hydrastis Canadensis* (Goldenseal) are increasingly available (Vetvicka and Vetvickova, 2012c). The immunological effects and toxicities of such combinations are generally unknown. It is crucial to patient interests that combinations of commercially available β 1,3-glucans with anti-cancer medications or other immunomodulators be evidence based.

2. COMPARATIVE STUDIES OF POTENTIALLY ANTI-CANCER IMMUNOMODULATION BY β 1,3-GLUCAN PRODUCTS

In 2005 Vetvicka and Vetvickova compared the immunostimulating properties of lentinan and two different preparations of Maitake D or MD fraction: Maitake Gold 404® containing diluted patented MD fraction from Yukiguni biofarm, a β 1,3-glucan-protein complex (Nanba and Kubo, 1997) and Grifron-Pro

Maitake D Fraction®, a β 1,3-glucan: Agents 1, 2 and 3 on **Table 1** (Vetvicka and Vetvickova, 2005). Following oral administration to mice, all three preparations stimulated phagocytosis to the same extent. However, Maitake Gold 404® was the only product effective at low doses. It produced large between-sample phagocytosis stimulation variation specifically of neutrophils, suggesting a lack of standardisation of effective MD fraction concentration.

Unlike lentinan, both Maitake extracts stimulated production of CD4⁺ T cells. Lentinan took longer to stimulate IL-1 β and only Maitake Gold 404® stimulated TNF- α 60 min after administration. Lentinan and Grifron-Pro Maitake D Fraction® were most effective at stimulating IL-2 production, whereas Maitake Gold 404® scored slightly under control, an anomalous result again suggesting effective concentration issues. It is to be noted that, in general, almost all β 1,3-glucans stimulate IL-2 production (Deng *et al.*, 2009; Nanba, 1997; Vetvicka and Vetvickova, 2007a; Vetvicka and Vetvickova, 2007b; Vetvicka and Vetvickova, 2010).

All glucans enhanced NK activity, but Maitake Gold 404® had the strongest effect. The researchers concluded that β 1,3-glucans from Maitake possess similar types of immunomodulatory properties to those from yeasts and that in some ways they are more immunologically active.

Vetvicka and Vetvickova later conducted a review of the magnitude of some immunological effects of a range of β 1,3-glucan human dietary supplements (**Table 2**), in which β 1,3-glucan was the only potentially immunomodulatory agent (**Table 1** agents 2,4-9), (Vetvicka and Vetvickova, 2007b). In this study, Now Beta®, Immutol®, Immune Builder®, Glucan #300® and Maitake Gold 404® stimulated the greatest rates of phagocytosis.

The three agents stimulating the greatest migration of CD4⁺ and CD8⁺ T lymphocytes were Now Beta®, Glucan #300® and Maitake Gold 404®. Glucan #300® and Maitake Gold404® stimulated the highest IL-2 production. Glucagel T® and Glucan #300® stimulated the highest antibody response. Although it was possible for an agent to score highly on one measure of immunomodulation and negligibly on others (for example, Glucagel T® stimulated well antibody formation, but on no other activity), clearly the most active agents across immunological measures were Glucan #300®, Now Beta® and Maitake Gold 404®. Senseiro®'s apparently low immunological activity was contrary to a study asserting the anti-tumour properties of *Agaricus blazei* fractions (Kawagishi *et al.*, 1989). It might be possible that *A. blazei* contains components lacking in Senseiro® and responsible for its anti-tumor activity.

Table 1. A partial list of β 1,3-glucan products commercially available as human food supplements

β 1,3-glucan product	Source
1) Lentinan	Fruit body of <i>Lentinula edodes</i> (Shiitake) mushroom
2) Maitake Gold 404®	<i>Grifola frondosa</i> (Maitake) mushroom ^{a)}
3) Grifron-Pro Maitake®	<i>Grifola frondosa</i> (Maitake) mushroom ^{a)}
4) Glucan # 300®	Yeast: Highly purified Whole Glucan Particle (WGP)
5) Now Beta® (also called "now Glucan"®)	Yeast, mushrooms
6) Glucagel T®	Barley
7) Immutol®	Yeast
8) Immune Builder®	5 mushrooms: <i>Agaricus blazei</i> , <i>Coriolus versicolor</i> , <i>Ganoderma lucidum</i> (Reishi), <i>Lentinula edodes</i> , <i>Grifola frondosa</i>
9) Senseiro®	<i>Agaricus blazei</i> Murill Kyowa mushroom
10) Krestin ("PSK")	<i>Coriolus versicolor</i> (<i>Trametes versicolor</i>) mushroom
11) ImmunoFibre	Barley
12) Wellmune®	<i>Saccharomyces cerevisiae</i> WGP
13) Betamune®	<i>Saccharomyces cerevisiae</i>
14) Epicor®	<i>Saccharomyces cerevisiae</i>
15) Aktival®	<i>Saccharomyces cerevisiae</i>
16) Sweet Beta Glucan®	<i>Saccharomyces cerevisiae</i>
17) Beta Right 101®	<i>Saccharomyces cerevisiae</i>
18) NSC®	<i>Saccharomyces cerevisiae</i>
19) Better Immunity®	<i>Saccharomyces cerevisiae</i>
20) Macroforce®	<i>Saccharomyces cerevisiae</i>
21) Solgar®	<i>Saccharomyces cerevisiae</i>
22) Swanson®	<i>Saccharomyces cerevisiae</i>

a) Described as β 1,6 glucans in Japanese studies (Kodama *et al.*, 2002a; Nanba, 1997; Nanba and Kubo, 1997)

Table 2. β 1,3-glucan products found to be most efficacious immunomodulators in comparative studies

Most effective product/s	Immunological measure	Study
Maitake Gold404®	Phagocytosis	Vetvicka and Vetvickova (2005)
Now Beta®	Phagocytosis	Vetvicka and Vetvickova (2007b)
Immutol®		
Immune Builder®		
Glucan #300®		
Maitake Gold 404®		
Now Beta ®	CD4+ and CD8+ T lymphocyte migration	Vetvicka and Vetvickova (2007b)
Glucan #300®		
Maitake Gold 404®	IL-2 production	Vetvicka and Vetvickova (2007b)
Glucan #300®		
Maitake Gold 404®	Antibody formation	Vetvicka and Vetvickova (2007b)
Glucagel T®		
Glucan #300®		
Glucan #300®	Most active across measures	Vetvicka and Vetvickova (2007b)
Now Beta®		
Maitake Gold 404®		
Glucan #300®	Greatest phagocytosis	Vetvicka and Vetvickova (2007a)
Glucan #300®	Longest lasting phagocytosis	Vetvicka and Vetvickova (2007a)
Krestin		
Krestin	IL-2 production	Vetvicka and Vetvickova (2007a)
Glucan #300®	IL-2 production, first 2 days	Vetvicka and Vetvickova (2007a)
Betamune®	Most active at lower doses	Vetvicka <i>et al.</i> (2008)
	Phagocytosis in widest effector cell range	
	Generally strongest cytokine response	
	Greatest tumor weight reduction	
	Increased expression of the widest range of proto-oncogenes	
Glucan #300®	Recovery of phagocytosis	Vetvicka and Vetvickova (2009)
	Cytokine production.	
	T and B cell proliferation and NK cell activity after mercury instigated immunosuppression.	
Glucan #300®	Phagocytosis	Vetvicka and Vetvickova (2010)
Glucan #300®	Superoxide and nitrite production	Vetvicka and Vetvickova (2010)
Wellmune®		
Maitake Gold 404®, Krestin, Glucagel T®		
Glucan #300®	IL-2 production	Vetvicka and Vetvickova (2010)
Wellmune®		
Maitake Gold 404®, Krestin		
Beta Right®		
Glucan #300®	Antibody production	Vetvicka and Vetvickova (2010)
Wellmune®		
Beta Right®		
Glucagel T®		

In an additional study (Vetvicka and Vetvickova, 2007a), the same group examined murine cholesterol and blood glucose level changes, as well as immunological changes (phagocytosis by peripheral leukocytes, IL-2 production) following administration of Glucan #300®, Now Beta®, Krestin and ImmunoFiber® (agents 4, 5, 10 and 11 respectively, **Table 1**). Glucan #300® stimulated greatest

phagocytosis and, along with Krestin, longest lasting increases in that measure. IL-2 production was most lastingly stimulated by Krestin, although the highest level of stimulation in the first two days after β 1,3-glucan administration was by Glucan #300®. Glucan #300® was again the most active sample.

Vetvicka *et al.* (2008) examined changes in the release of seven cytokines and levels of phagocytosis in

a murine and *in vitro* model following administration of different doses of β 1,3-glucans WGP, Lentinan and Betamune® (agents 1, 12 and 13, **Table 1**). *In vivo* tumor inhibition and breast cancer related gene expression were also examined. WGP and Lentinan were only active at the highest dose. Betamune® was more active at lower doses than the other agents, stimulated phagocytosis in a wider effector cell range, in general stimulated the strongest cytokine response and resulted in the greatest tumor weight reduction. It also stimulated increased expression of the widest range of proto-oncogenes, potentially associated with upregulation of the adhesion molecules and angiogenesis contributing to tumor metastasis and growth, a finding similar to that in other studies on glucans (Vetvicka *et al.*, 2007b). This result should be considered in light of the complexities of the genetic landscapes underlying cancers (Novak and Vetvicka, 2009; Otake *et al.*, 2007).

Another study compared the β 1,3-glucan-mediated recovery of phagocytosis, production of three cytokines, T and B cell proliferation and NK activity after mercury-instigated immunosuppression (Vetvicka and Vetvickova, 2009). Following administration of β 1,3-glucans 4-6 and 13 (**Table 1**), Glucan #300® was the only agent active on all measures and was consistently most active.

An additional comparative study evaluated sixteen commercially available β 1,3-glucan products in terms of immunological activity (**Table 1**, agents 2, 4-7, 10, 12 and 14-22) and tested phagocytosis, antibody formation, phagocyte superoxide and nitrite production and cytokine production (Vetvicka and Vetvickova, 2010). Only Glucan #300® stimulated phagocytosis across dose ranges. The glucans most active in stimulating superoxide and nitrite production were Glucan #300®, Wellmune®, Maitake Gold 404®, Krestin and Glucagel T®. Glucans stimulating the highest IL-2 production included Glucan #300®, Wellmune®, Maitake Gold 404®, Krestin and Beta Right®. The highest antibody production was produced by Glucan #300®, Wellmune®, Beta Right® and Glucagel T®.

As in previous comparative study, many of the glucans tested (e.g., Swanson®), showed little immunological activity and some agents produced a high level of activity on some measures only. Glucan #300® showed the greatest activity across all measures (**Table 2**). The authors have repeatedly hypothesised that this was partly because Glucan #300® had a high degree of purity compared to other agents: For example, Glucan #300® is 85% pure and Swanson® 70% (Vetvicka and Vetvickova, 2010).

3. β 1,3-GLUCAN SYNERGIES WITH ANTI-CANCER MEDICATIONS AND IMMUNOMODULATORS

Clinical and experimental evidence has clearly established the anti-cancer synergy of a range of β 1,3-glucans with conventional cancer therapies including cyclophosphamide (Tada *et al.*, 2007; Vetvicka *et al.*, 2007a), interferon- α (Louie *et al.*, 2010), mitomycin C (Nanba, 1997), dendritic cell-based immunotherapy (Masuda *et al.*, 2010) and radiotherapy (Gu *et al.*, 2005). Kodama *et al.* (2002a) published a non-randomised clinical trial in which Yukiguni Maitake MD Fraction® plus whole Maitake powder, taken with various chemotherapies, enhanced immunocompetent activities by a factor of 1.2-1.4 compared with chemotherapy alone. A previous limited, non-controlled study published by Nanba found 12-28% improved efficacy of the whole Maitake MD fraction and chemotherapy above that of chemotherapy (Nanba, 1997). Both studies found tumor regression or complete remission in some patients. However, the MD fraction doses administered were significantly under the optimal ranges determined by (Deng *et al.*, 2009) and the greater efficacy achieved with addition of whole Maitake powder may have been the result of additional MD fraction related β -glucans in the powder rather than any true synergy of fraction and powder. A commercially available MD Fraction-whole Maitake powder product derived from Yukiguni Maitake and combined approximately in the ratio used in the Nanba study is Maitake QOL Extract® (Muir, 2012; Nanba, 1997).

Diverse β 1,3-glucans ameliorate the neutropenia, bone marrow hematopoietic cell depression and secondary infections prevalent with conventional treatments including methotrexate (Sener *et al.*, 2006), cyclophosphamide (Vetvicka *et al.*, 2007a), 5-fluorouracil (Vetvicka *et al.*, 2007a), doxorubicin (Lin *et al.*, 2004), paclitaxel (Lin *et al.*, 2010), cisplatin (Masuda *et al.*, 2009), mitomycin C (Nanba, 1997) and X-ray and radiation therapy (Gu *et al.*, 2005). The evidence for Maitake MD fraction in this regard is notable in terms of numbers of studies (Kodama *et al.*, 2002a; Lin *et al.*, 2004; 2007; 2009; 2010; Louie *et al.*, 2010; Masuda *et al.*, 2009; Nanba, 1997). Human subjects administered MD fraction also experience reduced chemotherapy side effects, including pain, lost appetite, vomiting, nausea and hair loss (Nanba, 1997).

3.1. Monoclonal Antibodies

Combination of anti-tumor monoclonal antibodies (mAbs) with standard chemotherapy increases mAb efficacy, but adverse effect incidence and severity increase to an extent that limits utilisation (Liu *et al.*, 2009). Enhanced effectiveness of immune responses to a range of cancers (including breast, lung and lymphoma), tumor shrinkage and prolongation of survival have repeatedly been demonstrated in murine and human xenograft models with co-administration of complement activating mAbs to tumor antigens, plus β 1,3-glucans. The latter include the commercially available Glucan #300 and WGP: Agents 4 and 12 on **Table 1**, as well as a related yeast β 1,3-glucan, PGG and a barley derived β 1,3-glucan (Hong *et al.*, 2004; Liu *et al.*, 2009; Modak *et al.*, 2013; Salvador *et al.*, 2008; Yan *et al.*, 2005).

PGG has the advantage of being the only tested β 1,3-glucan product which does not amplify inflammatory cytokine responses, a desirable attribute in mAb therapy (Liu *et al.*, 2009). Unfortunately it is only available to patients via trials. Bevacizumab, an anti-vascular endothelial growth factor (VEGF) mAb, inhibits tumor microvasculature and triggers iC3b deposition on surface vasculature of a human ovarian carcinoma cell line, thus making such cells a target for PGG β -glucan primed neutrophil CR-3 related toxicity (Liu *et al.*, 2009; Salvador *et al.*, 2008). In one study, combination therapy prevented tumors from growing and led to long-term survival in 86% of murine subjects with tumors (Salvador *et al.*, 2008). However, the combination provided no benefit above mAb alone in Colo38 tumors, since they do not express membrane bound VEGF (Salvador *et al.*, 2008). Therefore the identification of cancer types possessing membrane-bound VEGF is crucial for the selection of patients to undergo combination PGG β -glucan bevacizumab therapy.

Similarly, cetuximab, a humanised anti-Epidermal Growth Factor Receptor (EGFR) mAb, when combined with PGG reduced tumor burden and greatly increased the rate of murine long-term survival (Liu *et al.*, 2009). EGFR is highly expressed in very aggressive colorectal, lung, breast, pancreatic and gastrointestinal tumors and melanoma.

Anti-GD2 immunotherapy using mAb 3F8 has been shown to exert a small anti-tumor effect in chemoresistant neuroblastoma. The addition of a barley β 1,3-glucan to 3F8 therapy in one study did not lead to increased CR3 mediated cytotoxicity (Modak *et al.*, 2013). However, some patients developed anti-mouse antibody reaction. This was previously unobserved in

3F8 administration and associated with increased survival. Further investigation of the immunological effects of the combination is merited.

β -glucan binding of neutrophil CRs enhances specific humoral response targeting of tumor cells opsonised with complement iC3b and antibodies (Li *et al.*, 2006; Novak and Vetvicka, 2009), constituting a mechanism of action for the synergy of mAbs and β -glucans in tumor shrinkage and enhanced host survival (Gelderman *et al.*, 2004; Hong *et al.*, 2004; Liu *et al.*, 2009; Yan *et al.*, 2005). Monoclonal antibodies with no inherent tumor regression activity may mediate regression which can be complete (Hong *et al.*, 2004). For example, Herceptin and Erbitux are mAbs against Her2 and Her1 growth factors respectively which do not stimulate murine tumor regression unless administered with β 1,3 glucans (Hong *et al.*, 2004). Rituxan performs similarly (Hong *et al.*, 2004). Vaccines which elicit anti-tumor antibodies may have increased efficacy if β 1,3 glucan co-administration occurs (Hong *et al.*, 2004; Liu *et al.*, 2009; Yan *et al.*, 2005), representing an area for future investigation.

3.2. Vitamin C

Vitamin C and β 1,3-glucans increase some of the same classes of immune responses: NK cell and macrophage activity amplification, specific antibody formation and cytokine levels (Schwager and Schulz, 1998; Vetvicka and Vetvickova, 2012a). When combined, vitamin C and β 1,3-glucans produce synergistic increases in adaptive biological and immunological measures (Ditteova *et al.*, 2003; Verlhac *et al.*, 1998), most notably when the glucan is in liposomised form.

3.3. Transresveratrol (Trans-3,4',5-Trihydroxystilbene)

Transresveratrol is a nonflavonoid polyphenol with independent cancer chemopreventive-immunomodulatory properties, typically isolated from grape skin or Japanese knotweed (Vetvicka *et al.*, 2007b). It has been found to ameliorate the side effects of administration of doxorubicin and cisplatin chemotherapy (Do Amaral *et al.*, 2007; Vetvicka and Vetvickova, 2012a). Vetvicka *et al.* (2007b) combined transresveratrol with WGP and found synergistic potentiation of phagocytosis, proliferation of CD4⁺ T lymphocytes, production of potentially lymphocyte stimulating cytokines and splenic recovery from administration of 5-fluorouracil. Synergistic upregulation of genes encoding for anti-apoptosis, cell growth and adhesion pathways (such as Cdc42 and NF- κ B2) occurred.

3.4. Vitamin C Plus Transresveratrol

A recent study used a combination of vitamin C, transresveratrol and Glucan#300 against murine lung cancer and breast cancer in order to evaluate the treatment potential of the combination (Vetvicka and Vetvickova, 2012a). The triple combination was found to be the strongest enhancer of granulocyte numbers, phagocytosis and antibody formation, achieving the greatest reduction in metastasis (86%).

In a subsequent study, mercury or perfluorinated hydrocarbon poisoning were used to depress a range of immunological parameters in mice, including phagocytosis, NK cell activity, mitogen-induced proliferation and expression of CD markers, antibody formation and secretion of cytokines IL-6, IL-12 and IFN- γ (Vetvicka and Vetvickova, 2012b). A vitamin C/transresveratrol/Glucan #300 combination was shown to ameliorate immunosuppression.

In an additional study the same researchers compared five commercially available preparations, most of which combined β 1,3-glucans with other immunomodulators (Table 3), with the commercially available vitamin C/transresveratrol/Glucan #300 combination “RVB 300®,” in terms of levels of phagocytosis, respiratory burst (nitrite and superoxide production), IL-2

production, antibody response to ovalbumin and NK cell cytotoxicity (Vetvicka and Vetvickova, 2012c).

Only RVB 300® showed immunological activity across measures. Except for nitrite and superoxide anion formation, the other samples showed no such activity, despite their inclusion of β 1,3-glucans with some evidence base. This may be due to low doses of active, poor isolation techniques or component immunomodulators negating each other’s efficacy.

3.5. Humic Acids

Humic acids are high molecular weight aromatic polymers found mainly in decomposing peat, some of which have anti-viral and lymphocyte-proliferative effects (Vetvicka *et al.*, 2010). Vetvicka *et al.* (2010) used Glucan #300® and β 1,3-glucan laminarin in combination with humic acids in testing. Two types of humic acids independently demonstrated synergy when combined in specific ratios with the β -glucans, in terms of production of specific cytokines (IL-2, IL-4, IL-5, IL-6 and TNF- α) and of MPC-1 and phagocytosis functions of neutrophils and macrophages. The most strongly aromatic humic acid in combination with glucan achieved the greatest reduction of mouse breast cancer tumor weight in all tested samples: 92% (Vetvicka *et al.*, 2010).

Table 3. Products containing combined immunomodulators

Product	Immunomodulatory components
Qivana®	Reishi mushroom (<i>Ganoderma lucidum</i>) Cordyceps mushroom (<i>Cordyceps sinensis</i>) Coriolus mushroom [Krestin] (<i>Coriolus versicolor</i>) Maitake mushroom (<i>Grifola frondosa</i>) Zhu Ling mushroom (<i>Polyporus umbellatus</i>)
BioBran®	MGN-3 arabinoxylan compound from partially hydrolysed rice bran
Transfer factor Plus®	Zinc (as zinc methione) Transfer factor E-XF™ (extract of egg yolk and cow colostrum) Nano Factor™ (nano-filtered cow colostrum) Inositol hexaphosphate B-Sitosterol and other polysterols Aloe leaf gel extract Olive leaf extract Oat seed extract Baker’s yeast extract Cordyceps sinensis mushroom extract Maitake (<i>Grifola frondosa</i>) mushroom extract Shiitake (<i>Lentinus edodes</i>) extract
Manapol Plus®	Aloe gel powder
Immunizen®	β -glucan from Maitake mushroom (<i>Grifola frondosa</i>) Colostrum Arabinogalactan Lactoferrin
RVB 300®	β 1,3 glucan from baker’s yeast Glucan #300 (<i>Saccharomyces cerevisiae</i>) Resveratrol, Vitamin C

3.6. Ashwagandha (*Withania Somnifera*)

Ashwagandha is an herbal extract used for stress reduction in traditional Indian medicine. When administered with Yukiguni Maitake extract (MD fraction) a synergistic inhibition of stress-related corticosterone increase and cytokine reduction occurred (Vetvicka and Vetvickova, 2011b), with implications for maintenance of a healthy immune function during stress related to a cancer diagnosis for a human patient.

3.7. Whole Maitake (*Grifola Frondosa*) Powder

In a previously referred to non-randomised clinical trial (Kodama *et al.*, 2002a) administered Yukiguni Maitake MD Fraction® plus Yukiguni whole Maitake powder to liver, lung and breast cancer patients. They found 58.3-68.8% cancer regression or rigorously defined symptomatic improvement. Nanba (1997) had previously reported similar findings. In each study the combination of products achieved superior results to those produced with MD Fraction alone, although it is unclear as to whether this may be due to β -glucan dose increase due to addition of glucan in the whole powder, or synergy of MD fraction with a component or components of the whole powder.

4. DISCUSSION

Studies concerned with β 1,3-glucan product efficacies and synergies are usually confounded by the issues of unknown optimal dose and unknown degree of product standardisation. Although (Deng *et al.*, 2009) established the human oral dose response curves for Maitake Gold 404® across a wide range of immunological parameters, in general it is unknown as to whether murine and human studies used optimal doses of the range of β 1,3-glucans tested, (and whether the optimal dose for one β 1,3 glucan is the same as that for another). Nevertheless, β 1,3-glucan products found to be efficacious as tumoricidal agents or carcinogenesis immunological mechanism inhibitors consistently include Betamune® (Vetvicka *et al.*, 2008), Glucan #300® and Maitake Gold 404® (Vetvicka and Vetvickova, 2005; Vetvicka and Vetvickova, 2007a; 2007b; Vetvicka and Vetvickova, 2009; Vetvicka and Vetvickova, 2010).

Maitake Gold 404® products are dilutions of Yukiguni Maitake MD fraction® and are produced by a range of US manufacturers (iHerb, 2014). Administration of a Maitake Gold 404® product in one study resulted in between sample variation in neutrophil

stimulation and anomalous low IL-2 stimulation (Vetvicka and Vetvickova, 2005), suggesting product standardisation problems. Many additional marketed products contain non-patented substances claimed to be MD or D fraction, hence unknown doses of intact or authentic molecules of active (iHerb, 2014). Yukiguni Maitake MD Fraction® is extracted from bio farmed high β 1,3-glucan content specimens, under uniform optimal growing conditions, with rigorous quality control, resulting in standardisation (Deng *et al.*, 2009; Kodama *et al.*, 2002a). Glucan extraction involves fractionation so as to obtain a relatively pure product, which is patented and proprietary (Nanba and Kubo, 1997). So that studies use a known dose of effective MD fraction, it is recommended that only MD fraction obtained directly from Yukiguni be used in future trials, rather than manufacturer diluted or look-alike products. To date, apart from one known study (Vetvicka and Vetvickova, 2005), only Japanese studies have used the original product, generally with very efficacious results (Inoue *et al.*, 2002; Kodama *et al.*, 2002a; 2002b; Masuda *et al.*, 2012; Miyake *et al.*, 2005). In addition, it is contrary to patient interests that numerous diluted products on the US market contain low levels of patented active compared with the original product and that recommended doses are subtherapeutic (Deng *et al.*, 2009; iHerb, 2014).

Systematic identification of optimal dose for each β 1,3-glucan product must be determined (preferably in human subjects), before meaningful comparisons of product efficacies may be made. After optimal doses are identified, it is suggested that Yukiguni MD fraction®, Glucan #300® and Betamune® effects be trialled across all immunological measures and tumor weight, so that conclusions on comparative immunological and anti-cancer effects may be derived.

Agents containing β 1,3-glucans, have shown synergy with conventional cancer treatments and produced amelioration of their adverse effects (Gu *et al.*, 2005; Kodama *et al.*, 2002a; Lin *et al.*, 2004; 2010; 2009; Louie *et al.*, 2010; Masuda *et al.*, 2009; 2010; Nanba, 1997; Novak and Vetvicka, 2008; Sener *et al.*, 2006; Vetvicka *et al.*, 2007a). Although combinations of mAbs and β 1,3-glucans have been shown to produce synergistic tumoricidal activity (Hong *et al.*, 2004; Liu *et al.*, 2009; Modak *et al.*, 2013; Salvador *et al.*, 2008; Yan *et al.*, 2005), further research is needed to elucidate the specific cancer cell lines for which particular mAb- β 1,3-glucan combinations possess efficacy.

RVB300® (β -glucan/transresveratrol/vitamin C) is a β -glucan-based combination immunomodulator product

tested which demonstrated favourable immunological effects (Vetvicka and Vetvickova, 2012c). Maitake QOL Extract® is composed of Yukiguni whole Maitake powder and Yukiguni Maitake MD Fraction®, combined in approximately the ratio shown by Nanbato produce cancer regression in human patients above that produced by MD Fraction alone (Kodama *et al.*, 2002a). After the accurate determination of the MD fraction within the whole Maitake in QOL®, it is recommended that Maitake QOL Extract® and RVB300® be comparatively trialled at optimal dose across immunological measures and tumor weight. Systematic elucidation of synergies between β 1,3-glucans and other immunomodulator combinations is a promising area in product development.

5. CONCLUSION

Accumulated evidence shows that products containing β 1,3-glucans can be anti-carcinogenic immunomodulators which possess utility as adjunctive therapy with chemotherapy, other cancer therapies and other immunomodulators. Unfortunately, many studies suffer from experimental design issues including non-randomisation and incomplete control of dose of specific glucan. It is recommended that these issues be addressed in further research.

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