β-glucan vaccine adjuvant approach for cancer treatment through immune enhancement (B-VACCIEN) in specific immunocompromised populations (Review)

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Abstract. The incidence of cancer, which is the second leading cause of mortality globally, continues to increase, although continued efforts are being made to identify effective treatments with fewer side-effects. Previous studies have reported that chronic microinflammation, which occurs in diseases, including diabetes, along with weakened immune systems, may ultimately lead to cancer development. Chemotherapy, radiotherapy and surgery are the mainstream approaches to treatment; however, they all lead to immune system weakness, which in turn increases the metastatic spread. The aim of the present review was to provide evidence of a biological response modifier β-glucan [β-glucan vaccine adjuvant approach to treating cancer via immune enhancement (B-VACCIEN)] and its beneficial effects, including vaccine-adjuvant potential, balancing metabolic parameters (including blood glucose and lipid levels), increasing peripheral blood cell cytotoxicity against cancer and alleviating chemotherapy side effects in animal models. This suggests its value as a potential strategy to provide long-term prophylaxis in immunocompromised individuals or genetically prone to cancer.

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1. Introduction

Cancer is a lethal disease, responsible for ~9.6 million deaths annually, thus rendering it the second leading cause

of mortality globally (1). The most common cancer types include lung, breast, colorectal, prostate, skin (melanoma) and stomach cancer. The treatment approach to cancer is multifactorial, with chemotherapy, radiotherapy and surgery being the main methods of treatment (1). The immune system plays a major role in all aspects of cancer, including its origin, development, metastasis, therapy and prevention. Cancer cells and the immune system are in a constant crosstalk, wherein cancer cells undergo three phases: i) Elimination; ii) equilibrium; and iii) escape. In the elimination phase, immune cells, particularly innate immune cells, are in constant surveillance, eliminating cells that are abnormal. The elimination process induces cancer cells to undergo immune-editing or sculpting, causing an increase in the number of cells that have decreased immunogenicity and become resistant to the immune surveillance process; this part is the equilibrium phase. The cells that become resistant can escape the immune system and develop into advanced-stage cancer (2).

2. Vulnerable populations require a continuous implementation approach for cancer prevention

It is possible to identify subsets of vulnerable populations who are at high risk of either developing cancer or who have cancer, but require intervention for preventing cancer progression. These populations include:

i) Aged individuals with 'inflammaging'. There is sufficient evidence regarding how age-related pathologies, including cancer, cardiovascular diseases and type 2 diabetes, have a common inflammatory background, involving the process termed as 'inflammaging'. In inflammaging, there is a constant systemic proinflammatory state with increased levels of circulating ILs, including IL-6 and IL-1, as well as TNF-α and other inflammatory markers. A chronic antigen load caused by infections, cellular senescence, a dysregulated DNA damage response, altered gut microbiota, meta-inflammation and some microRNAs (miRNAs/miRs) that are associated with aging also influence the causative factors for cancer, simultaneously influencing and aiding inflammaging, thereby leading to cancer formation and progression (3). Along with increasing age, studies have revealed that there is an enhancement in the number of natural killer (NK) cell subpopulations, as well as a redistribution. The re-distribution is characterized by an increase of $CD56^{bright}$ cell populations, which are more immature, and of $CD56^{dim}$ mature cells, with intrinsic, reduced cytotoxic activity at single-cell level. Moreover, NK cells from elderly populations produce less IFN-γ upon IL-2 stimulation (4). This immunocompromising nature may also influence the elderly population in becoming prone to cancer development.

ii) Individuals with genetic risk variants, which are prone to cancer development either caused by the variants themselves or due to negative influences on the immune system (5). The association between genes and cancer is well known. For instance, the most commonly mutated gene in all cancer types is p53. Moreover, inherited mutations in the BRCA1 and BRCA2 genes are associated with hereditary breast and ovarian cancer syndromes (5). The study by Imai *et al* (6) examined immune system weakness and cancer development. Between 1986 and 1990, these authors assessed the natural cytotoxic activity of peripheral blood mononuclear cells using an isotope-release assay in 3,625 residents of a Japanese population who were mostly aged >40 years. These authors also conducted an 11-year follow-up survey for the cohort members, aiming to examine cancer incidence and mortality. The follow-up results indicated that medium and high cytotoxic activity of peripheral-blood lymphocytes was associated with reduced cancer risk, whereas low activity was associated with increased cancer risk (6).

iii) Individuals with lifestyle and metabolic disorders: For >70 years, the association diabetes and cancer has been hypothesized (7). Epidemiological data have demonstrated that patients with diabetes are at an increased risk of developing various types of cancer, with an increased mortality rate. Several pathways have been proposed for the association between diabetes and cancer, including: a) Hyperglycaemia leading to increased cancer risk via augmented oxidative stress and DNA damage; b) hyperinsulinemia, due to exogenous insulin or insulin analogues [this view has been challenged by a previously published study (7)]; and c) chronic microinflammation with cytokine dysregulation (7). Hyperglycaemia in diabetes generally favours malignant cell proliferation by providing energy to the cancer cells. Increased levels of chronic inflammatory markers, including IL-1β, IL-6 and TNF-α, have been observed in diabetic patients, which may indicate the activation of the immune response in the progression and development of cancer cells. The uncontrolled proinflammatory response environment in diabetes, which is caused by chronic accumulation of glycated biomolecules and advanced glycation end products, leads to a chronic inflammatory state induced by the activation of the transcription factor NF-κB and the generation of reactive oxygen species (ROS) in cells. These factors promote a tumour-favourable microenvironment and potentially trigger immune system overactivation, ultimately leading to cancer growth (8,9). With regards to metabolic syndromes, chronic inflammation and cancer, a chronic and stable background inflammation has been proposed, referred to as a 'hypothalamic microinflammation' (9). This is caused by the hypothalamus atypically undergoing proinflammatory signalling activation, occurring alongside increases in age and the development of metabolic syndrome. This hypothalamic microinflammation has also been reported to programmatically control whole-body aging. Since aging is also associated with a chronic inflammatory state, negatively associated with longevity but positively with neurodegenerative diseases, an association between the hypothalamus and a microinflammatory state leading to cancer has become increasingly evident (10).

iv) Individuals with immune system weaknesses due to a) aged individuals with inflammaging; b) individuals with genetic risk variants; and c) individuals with lifestyle and metabolic disorders.

v) Patients with cancer undergoing chemotherapy, radiotherapy or surgery, which lead to therapy-induced immune dysfunction (11-13). Chemotherapy or a chemo- and radiotherapy combination have been reported to significantly delay the immune recovery to pre-treatment baseline levels. Similarly, surgery leads to a window of opportunity that allows the residual cancer cells, including those that have undergone distant metastases, to gain a foothold in the absence of NK cell surveillance (12).

Since conventional therapies have an associated risk of therapy-induced immune dysfunction, it is important to identify an approach that is effective in the long term as an adjunct to the other interventions, which would help maintain the normal function of the immune system, thereby enhancing its immune surveillance and antitumour properties, and ultimately playing a potential role in cancer prevention.

3. A vaccine therapy approach for cancer treatment

According to the Centers for Disease Control and Prevention in the USA, a vaccine is a product that stimulates the immune system of an individual to produce immunity against a specific disease (14). Vaccines in cancer may be therapeutic or preventive. Preventive cancer vaccines include proteins, peptides, DNA or RNA that can elicit or boost pre-existing antitumour immunity, leading to cancer elimination and the production of long-term memory to prevent tumour recurrence (15). The purpose of a therapeutic cancer vaccine is to control the cancer burden. Such vaccines include autologous patient-derived immune cell vaccines, tumour antigen-expressing recombinant virus vaccines, peptide vaccines, DNA vaccines and heterologous whole-cell vaccines derived from established human tumour cell lines (16). The personalized dendritic cell vaccine sipuleucel-T (Provenge) and recombinant viral prostate cancer vaccine PSA-TRICOM (Prostvac-VF) are widely known vaccines in the pre-approval/authorized approval/late clinical trial stages (17). Vaccines are often administered with adjuvants, which help to improve poorly immunogenic vaccines (18). Different types of novel adjuvants have been identified and applied with cancer vaccines, which include inorganic nanoparticles, organic molecules and polymers (19). Pathogens stimulate a 'danger sensing' signal via pathogen-associated molecular patterns (PAMPs). Inorganic nanoparticle-based adjuvants function in a similar manner to PAMPs, thereby stimulating antitumour immunity. Organic molecule-based adjuvants include small molecule-based factors, such as modified PAMPs, and are novel ligands for pattern recognition receptors (PRRs). Agonists of the Toll-like receptor family, which are type I transmembrane proteins that regulate the innate and adaptive immune responses (19), and agonists of stimulator of IFN genes (20) are examples of organic adjuvants. Polymer-based adjuvants concurrently help in drug delivery and act as PAMPs for immune system activation. At present, alum (a mixture of diverse aluminium salts) has been the only approved adjuvant in humans and remains one of the most common adjuvants in human vaccines. In addition to aluminium salts, oil-in-water emulsions containing squalene (e.g., MF59 and AS03), *in vitro*-assembled influenza-virus-like particles (e.g., virosomes) and the liposome-based adjuvant system AS01, are other licensed adjuvants in human vaccines (21).

However, it remains unknown whether there is a nutrition-based supplementation that can act as a potential vaccine adjuvant to facilitate cancer treatment, which can be both preventive and therapeutic.

4. β-glucan vaccine adjuvant approach for cancer treatment through immune enhancement (B-VACCIEN)

β-glucans, as a result of their high biocompatibility and tolerability and satisfactory safety profile, possess numerous beneficial properties, establishing them as promising adjuvant candidates (21-23). Dietary phytochemical carbohydrates have been considered as effective cancer-preventing and therapeutic adjuvants, which can be supplemented continuously for a longer time period (24). Among such phytochemicals, saponins and β-glucans are widely distributed in the plant kingdom, and in their purified extract form, represent two of the most potent immunological adjuvants when injected as a mixture with antigen, or immunomodulators when orally ingested (25).

β-glucans are naturally occurring polysaccharides that are constituents of the cell walls of yeast, fungi (including mushrooms), some bacteria, seaweed and cereals (oat and barley) (26). β-glucans are functional bioactive compounds possessing hypocholesterolaemic, hypoglycaemic, immunomodulatory, antitumor, antioxidant and anti-inflammatory activities. Moreover, their macromolecular structure and functionality vary, depending on the source (27). Yeast-derived 1,3-1,6 β-glucans have been reported to exert more prominent biological response modifier (BRM) effects compared with β-glucans from other sources, including oats or barley (28). An immunomodulator includes any molecule or substance capable of interacting with the immune system, resulting in the up- or downregulation of specific components of the immune response (29). Immunomodulators comprise of an array of synthetic, natural and recombinant molecules. Natural molecules, including those found in curcumin, thyme, bay leaf, resveratrol, ginseng, echinacea, aloe vera, astragalus, goldenseal, flavonoids and essential oils, have been studied for their immunomodulation properties as nutritional supplements. However, direct comparison studies of individual immunomodulators are limited. Vetvicka *et al* (26) indicated that, amongst >20,000 published studies, compared with other immunomodulators, glucan was the most prominent one.

Glucans are BRMs that exert significant effects on various components of the immune system. Glucans are recognized by PRRs present on the membranes of immune cells, such as macrophages, monocytes, dendritic cells and NK cells, with their key receptors being Dectin-1 and complement receptor 3 (CR3; CD11b/CD18). Additional receptors include Toll-2, lactosylceramides and the scavenger receptor family (26).

In terms of cancer immunity, β-glucans have been demonstrated to possess various functions, including: i) the increase of infection resistance (which is of particular importance in virus-associated cancer types); ii) exerting antitumour effects by activating the adaptive and innate arms of the immune system; iii) stimulating immune cells, including leukocytes, T helper (Th) and NK cells; and v) exerting anticoagulant effects (30). β-glucans activate early innate reactions by acting as PAMPs. Glucan-activated B cells have been revealed to secrete proinflammatory lymphokines, including IL-8, through the involvement of several molecules including Dectin-1 receptors, MAPK and NF-κB and activator protein-1 transcription factors. β-glucans have also been demonstrated to be potent cellular immunity activators. The effects of β-glucan against various types of infection have been demonstrated, such as for example *Leishmania* (L.) *major*, *Leishmania donovani*, *Candida albicans*, *Toxoplasma gondii*, *Streptococcus suis*, *Plasmodium berghei*, *Staphylococcus aureus*, *Escherichia coli*, *Mesocestoides corti*, *Trypanosoma cruzi*, *Eimeria vermiformis* and *Bacillus anthracis* (26).

The antitumour effects of β-glucans against a wide variety of tumour types (26). The mechanism of the antitumor activity induced by β-glucans is proposed to occur via the enhancement of the immune system against tumour cells, as well as by inhibiting tumour invasion and progression via a complex modulation of the apoptotic and angiogenic mechanisms. The antitumour mechanisms enhanced by β-glucans involve several pathways. For instance, β-glucans bind with specific receptors, such as Dectin-1, expressed on myeloid cells, converting them into antigen presenting cells. This binding also activates $CD4^+$ and $CD8^+$ T-cells, which are stimulated to produce the proinflammatory cytokine, TNF-α, the antitumor cytokine, IFN-γ, granzyme B and perforins, all of them cytotoxic against cancer cells (31). The tumoricidal effects are also induced by switching suppressive M2 macrophages into inflammatory M1 macrophages and, in turn, activating Th1-type T cells, thereby enabling cancer cell destruction via the secretion of proinflammatory cytokines through these T-cells. Interactions between β-glucan and polymorphonuclear cells induce the release of ROS in the microenvironment, ultimately leading to tumour cell death. β-glucans also activate NK cell cytotoxicity via the production and release of proinflammatory cytokines and via complement activation (31). β-glucans have been demonstrated to effectively modify the tumour microenvironment, resulting in significant reduction of primary tumour growth and distant metastases (31). β-glucans have a strong synergy with antibodies (Abs) that naturally occur in cancer (26).

β-glucans as adjuvants. Japan is a forerunner on the use of β-glucans, and β-glucans from the Shiitake mushroom (lentinan) and *Coriolus versicolor* (polysaccharide-K) have been licensed drugs since 1983. As of 2019, 177 clinical trials valuating β-glucans have been listed in the United States database, ClinicalTrials.gov, for cancer, cholesterol-lowering effects and immune-modulation (26). Clinical trials on β-glucans in combination with the other cancer therapies, including monoclonal Abs (mAbs), have been reported mentioned in the review article by Vetvicka *et al* (26), revealing significant tumour regression, a favourable Ab response, elevated immune cell number and function, fewer side-effects, decreased cancer-related fatigue and an improved nutritional state (26). Thus, β-glucans can serve as potential adjuvants with other cancer treatments.

β-glucans are potential adjuvants that aid immunomodulation, in combination as well as when administered alone (32). Since glucan receptors, including Dectin-1, CR3, lactosylceramide, natural cytotoxicity receptor p30 and scavenger receptors, are expressed on different types of immune cells, including macrophages, NK cells and neutrophils, β-glucans have a distinct affinity with these receptors, according to their different chemical structure. Thus, they are capable of triggering different host responses, making them potential immune adjuvants. β-glucan particles derived from *Saccharomyces cerevisiae* cell walls have also been suggested to be used as vaccine adjuvant carriers for protein antigen delivery, and for the targeted delivery of compounds to macrophages and dendritic cells (33). β-glucans have been reported as trained immunity-based adjuvants for rabies vaccines and have been demonstrated to elicit B-cell and T-cell specific responses in a study on canines (34).

With regards to adjuvant immunotherapy for cancer, since both dendritic cell priming and check-point inhibitor blockades have been revealed to be required for immunotherapy (23), β-glucans serve as an ideal candidate, as they induce dendritic cell priming and potentiate Abs against immune checkpoint molecules (26). β-glucans have been used as adjuvants in association with chemotherapy in different types of cancer, including oestrogen receptor-negative human breast, gastric, colorectal and non-small-cell lung cancer, as well as haematological diseases. The advantages of β-glucans as effective anticancer therapy adjuvants include the following: i) They are non-immunogenic due to the absence of the protein and peptide components; ii) they are non-toxic, as even doses up to 10 mg/kg have been reported to be well-tolerated *in vivo,* with no adverse effects; and iii) they provide the opportunity for beneficial structural modifications, due to the presence of multiple aldehyde and hydroxyl groups (35).

β-glucans can serve as effective adjuvants with latest therapies, including mAb-based and immune checkpoint targeted therapies for cancer. Combination therapy using β-glucan and mAbs targeting immune checkpoint molecules, including programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1), has been investigated in preclinical models with promising antitumour efficacy, as reviewed by Vetvicka *et al* (26). Several clinical trials have evaluated the interaction of glucans with mAbs in humans. β-glucan has been examined in association with pembrolizumab in head and neck cancers, metastatic melanoma and breast cancer with positive outcomes in terms of safety, tolerability and increased survival. β-glucans function via the iC3b-receptor CR3 (CD11b/CD18), thereby enhancing leukocyte-mediated killing of tumour cells that are coated with iC3b via naturally occurring antitumor Abs (36).

Trained innate immunity (TRIM) is the innate immune system memory induced by modulation of mature myeloid cells or their bone marrow progenitors. This process helps mediating the sustained increased responsiveness to secondary challenges (37,38). It is important to note that β-glucans are effective inducers of TRIM, specifically via epigenetic reprogramming of the innate immune cells at the level of bone marrow (central TRIM), as well as peripheral TRIM (37,38). Vetvicka and Vetvickova (39) reported that highly purified and active glucans exert significant pleiotropic effects against cancer.

Cancer cell resistance is an important hurdle in anticancer therapies. β-glucans are potential candidates for overcoming treatment resistance in cancer. This effect has been reported in treatment-resistant Lewis lung carcinoma (LL/2) cells, in which *Candida* cell wall-derived-β-glucan exerted a significant cytotoxic effect on both the parent cell line and cancer stem cells derived from the parent cell line (40).

5. Chronic microinflammation, cancer and β-glucans

Accumulating evidence has indicated that chronic inflammation may lead to cancer development. Underlying infection or inflammation have been linked to 25% of all cancer cases (41). Any unresolved inflammation on account of the failure in the precise control of the immune response can continue to disrupt the cellular microenvironment, leading to alterations

in cancer-related genes and post-translational modifications in key cell signalling proteins involved in the cell cycle, DNA repair and apoptosis (41). The identification of mononuclear inflammatory cells in close association with areas of hyperplasia and cellular atypia has been demonstrated even at very early stages of tumour development, further supporting the concept that inflammation is a major driving force that contributes to tumour initiation and/or initial tumour progression. The upregulation of non-specific proinflammatory cytokines (IFN-γ, TNF, IL-1α/β or IL-6) by immune cells, such as macrophages, mast cells and neutrophils, has been shown to promote tumour development (41). The inflammatory processes elicited by cancer itself are likely to be involved in their progression. Inflammation is also the common mechanism of action for numerous cancer risk factors, including infection, obesity, tobacco smoking, alcohol consumption, exposure to microparticles, dysbiosis and chronic inflammatory diseases, including pancreatitis and colitis. The administration of certain anti-inflammatory drugs, including aspirin, has also been reported to significantly reduce cancer risk. Thus, preventing or reversing inflammation has been suggested as a promising approach to cancer control (42).

Chronic-microinflammation culminating in cancer likewise requires focusing on metabolic disorders, including diabetes and cancer development. Several pathways have been proposed for the association between diabetes and cancer, including: i) hyperglycaemia leading to increased cancer risk via augmented oxidative stress and DNA damage; and ii) hyperinsulinemia, involving chronic microinflammation with cytokine dysregulation, which both require further attention. The uncontrolled proinflammatory response environment in diabetes caused by the chronic accumulation of glycated biomolecules and advanced glycation end products creates a chronic inflammatory state, via the activation of the transcription factor NF-κB and ROS generation in cells. Thus, a tumour-favourable microenvironment is promoted and immune system overactivation is potentially triggered, thereby leading to cancer growth. Moreover, with regards to chronic inflammation and cancer, a state of chronic and stable background inflammation has been proposed, known as 'hypothalamic microinflammation' (10), which occurs when the hypothalamus atypically undergoes proinflammatory signalling activation, and is associated with age increase and the development of metabolic syndrome.

β-glucans, particularly those that are yeast-derived, aid in combatting chronic microinflammation, thereby contributing to a cancer-preventive response, alongside their metabolic balancing activities (43,44), further adding to their effects in cancer prevention. A previous study on a yeast-derived β-glucan identified its antioxidant activity via H_2O_2 scavenging, as well as its *in vivo* anti-inflammatory potential in terms of myeloperoxidase activity and malondialdehyde and nitric oxide level reduction (45). In another study, the regular intake of β-glucan was demonstrated to exert an anti-inflammatory effect, which occurred by acting on IL-6, a pleiotropic cytokine that plays a pivotal role in acute phase responses in the balancing of the pro- and anti-inflammatory pathways (46).

Use of a β-glucan vaccine adjuvant approach for cancer treatment. In the majority of the clinical trials on β-glucans as a cancer treatment adjuvant an oral route of administration has been used and also across different age groups, as reviewed by Vetvicka *et al* (26), indicating that β-glucan can be applied universally as an effective treatment adjuvant (26,39). Following oral administration, β-glucans directly interact with gastrointestinal mucosa cells and are transferred into the general circulation. Vetvicka *et al* (26) proposed a process of β-glucan internalization after which it rapidly enters into the systemic circulation. The solubility of β-glucan is a critical factor for oral administration and the speed of transfer across the gut is dependent on the physicochemical characteristics of glucan (26). In this regard, an AFO-202 BRM glucan (BRMG) derived from a black yeast (*Aureobasidium pullulans* AFO-202 strain) demonstrates high purity and functionality. AFO-202 glucan is a water-soluble β-glucan which has been used for human consumption for several decades (47) and, as a result of these characteristics, it can serve as a potential β-glucan vaccine adjuvant approach to treating cancer.

AFO-202 β-glucan has been revealed to be beneficial in maintaining blood glucose levels and lipid levels in the normal range in human studies (43,44), assisting in the prevention of the metabolic-micro and chronic inflammation axis that may ultimately lead to cancer. AFO-202 β-glucan has been proven to stimulate the production of IL-8 or soluble Fas (sFas), although not that of IL-1β, IL-6, IFN-γ, TNF-α or sFas ligand (sFasL) (47). IL-8 exerts anti-inflammatory activity and helps in T-cell recruitment, as well as ROS metabolism enhancement. Moreover, IL-8 serves as a barrier against invading microorganisms, with airway epithelial release of IL-8 contributing to the immune defence of the host by promoting neutrophil chemotaxis (48). Tumours have been demonstrated to express FasL and downregulate Fas to escape from host immune surveillance. Elevated sFasL serum levels are associated with cancer progression (49).

Cytokines, including IL-1, IL-4 and IL-6, secreted by immune cells in the tumour microenvironment are observed in a wide range of solid tumour types, with the expression of their receptors by cancer cells aiding in immune evasion (50). IL-6 promotes tumour growth, with its elevated serum levels and expression in tumours being negative prognostic markers for cancer patient survival (51).

While IFN-γ has been long considered as a central player in antitumor immunity, it also has pro-tumorigenic roles. For instance, IFN-γ-mediated activation of the nonclassical major histocompatibility complex class Ia genes has been shown to aid in melanoma cell evasion from cytotoxic T-lymphocyte (CTL)-mediated cytolysis, in turn leading to clinical failure of melanoma peptide vaccines (52). IFN-γ is also associated with the influx of monocytic and granulocytic myeloid-derived suppressor cells to the tumour microenvironment, leading to the suppression of the anticancer T-cell response. Furthermore, IFN-γ-induced PD-L1/2 ligands on cancer cells causes their binding to their immune inhibitory receptor PD-1, finally suppressing T and NK cell immune effector activities, thereby promoting cancer progression (52).

TNF-α, primarily secreted by tumour-associated macrophages, initiates chronic inflammation. TNF-α has a dual role: wherein it causes tumour cell apoptosis when administered in high doses, however, long-term low dose administration has been revealed to accelerate tumour metastasis in a lung

cancer cell line (40). TNF-α also induces the expression of angiogenic factors, thereby promoting tumour angiogenesis and accelerating tumour metastasis via the upregulation of tumour-associated calcium signal transduction protein-2 via the ERK1/2 signalling pathway (40,53).

AFO-202 β-glucan, could play a key role in preventing the cytokine imbalance-induced inflammation caused by chemotherapy or other cancer therapies through the balancing of anticancer cytokines activation and pro-tumorigenic cytokines suppression, thus offering benefits in anticancer prevention and therapeutics (11-13). It has been reported that the Dectin-1-based recognition of tumour cells orchestrates innate immune cell antitumour responses (54). The key receptor via which AFO-202 β-glucan exerts its biological response and modifying effects is Dectin-1 (54), thereby suggesting its potential use as a β-glucan vaccine adjuvant. AFO-202 β-glucan has been shown to aid against infections. For example, AFO-202 β-glucan may enhance immunity against *Leishmania amazonensis* and malaria through the increase of NK cell activity and cellular immunity, extending its application potential for the suppression of infection, apart from its anticancer effects (55). At present, the metabolic balancing effects of this AFO-202 β-glucan (43,44) and its vaccine adjuvant effects as a potential effector in enhancing the immune response to the avian influenza A H5N1 and H5N2 vaccines have been reported (56). The AFO-202 β-glucan has been demonstrated to enhance the immune system in animal (57) and human clinical studies (58), increasing the eosinophil and monocyte counts and decreasing the neutrophil-to-lymphocyte ratio (NLR), through the increase in the lymphocyte-to-CRP (LCR) and leukocyte-to-CRP (LeCR) ratios. Another strain of the *Aureobasidium pullulans*, N-163 derived β-glucan has been demonstrated to attenuate lipotoxicity (decrease in non-esterified fatty acids (NEFA) (59) with anti-inflammatory effects of significant control of IL6, D-Dimer and NLR apart from anti-fibrotic effects in animal (60) and human clinical studies (58).

In tumour animal models, the comparative antitumour effect of *Aureobasidium pullulans*-derived β-glucan has been shown to be significantly higher than those of other glucan types (61). The administration of the AFO-202 β-glucan, lead to an increase in the anti-tumour immune response and its maintenance at normal levels, similar to the levels of control groups without chemotherapy administration (62). The percentage of tumour size decrease has been reported to be higher when *A. pullulans* β-glucan was administered (63) along with chemotherapy than with chemotherapy (64) alone. In another study, 11 healthy human volunteers consumed 15 g AFO-202 β-glucan orally three times per day for 1 month. NK cell cytotoxic activity was assessed using peripheral blood provided by the volunteers before and after the intake. NK cell activity was evaluated using the ^{51}Cr release test with an effector to target (E/T) ratio of 50/1, using peripheral blood mononuclear cells as functional cells and K562 cells as target cells. The rate of increase of the cytotoxic activity was 90.9% (62).

Fungal β-glucans have been shown to increase NK cell activity in cancer patients of different age groups (65) and this further attests to the significance of its application in aged individuals and in those who are immunocompromised due to cancer.

The evolution of the immune system includes a curve upwards during cancer, with contributions from viruses and chronic inflammation. With lifestyle and metabolic disorders having become major healthcare-related issues in the latter half of the past century, and with microinflammation serving as the underlying mechanism leading to cancer in such individuals, senile immune system weakness or inflammaging are unavoidable. These changes may occur in any individual, even though they may not present with chronic inflammation. All the aforementioned factors adversely affect the immune system. Addressing this issue requires a holistic approach that can potentially act against viral and other infections, inflammation and metabolic disorders, in addition to acting as a continuous supportive mechanism for the prevention of the immune surveillance system weakening. Apart from these factors, genetic components of the immune system or genetically prone cancer types may further lead to immune system weakness. Genetics should be also considered, as in these individuals vulnerable to cancer, the time at which immune system weakness develops or the deterioration of the cancer aggressiveness may occur remains unknown. A continuous vaccine adjuvant approach could include the use of food supplements, including β-glucan. Although it remains unknown whether immunoenhancement will completely achieve treating any cancer already formed, it is suggested as a potential strategy to address the periodic or intermittent jeopardy to the immune system. The duration of immune system weakness after surgery, as well as the immune system weakness induced by chemo- or radiotherapy, requires definite examination; immunosuppression is considered a major reason for treatment failure in cancer (66). Treatment strategies to overcome immune system weakness after cancer therapies require large-scale translational and clinical research. It is hoped that this kind of research will yield further insights into how chemotherapy, surgery or radiotherapy-related cancer treatments can be supplemented by B-VACCIEN (Fig. 1), to alleviate side-effects. This goal can be achieved by effectively engaging the immune system, in order to reduce cancer-adverse reaction-related morbidity and mortality.

β-glucans are effective chemotherapy adjuvants, due to their protective antioxidant effects against chemotherapy-induced cytotoxicity (67). It is significant to underline that a randomized phase I/II trial studying the side effects and optimal dose of OPT-821 (a saponin-based immunoadjuvant OBI-821) with vaccine therapy when given together with β-glucan, as well as the examination of the effectiveness of this regimen in the treatment of younger patients with neuroblastoma is currently ongoing (68).

While several animal studies have demonstrated promising results for the use of a β-glucan vaccine adjuvant approach for the treatment of cancer, human studies fall short of the expected outcome (69). This may be attributed to the source of the β-glucan; careful selection of the source and the process involved in the extraction-purification is essential for an efficient antitumour response. A comparative study on the various types of β-glucans in different tumours and stages will be also essential for the development of additional, more effective β-glucan vaccine adjuvant therapeutics. Moreover, tumour microenvironment is extremely complex and is a challenge for the successful combination of β-glucan and various cancer therapies, including immune-modulating Abs. The overexpression

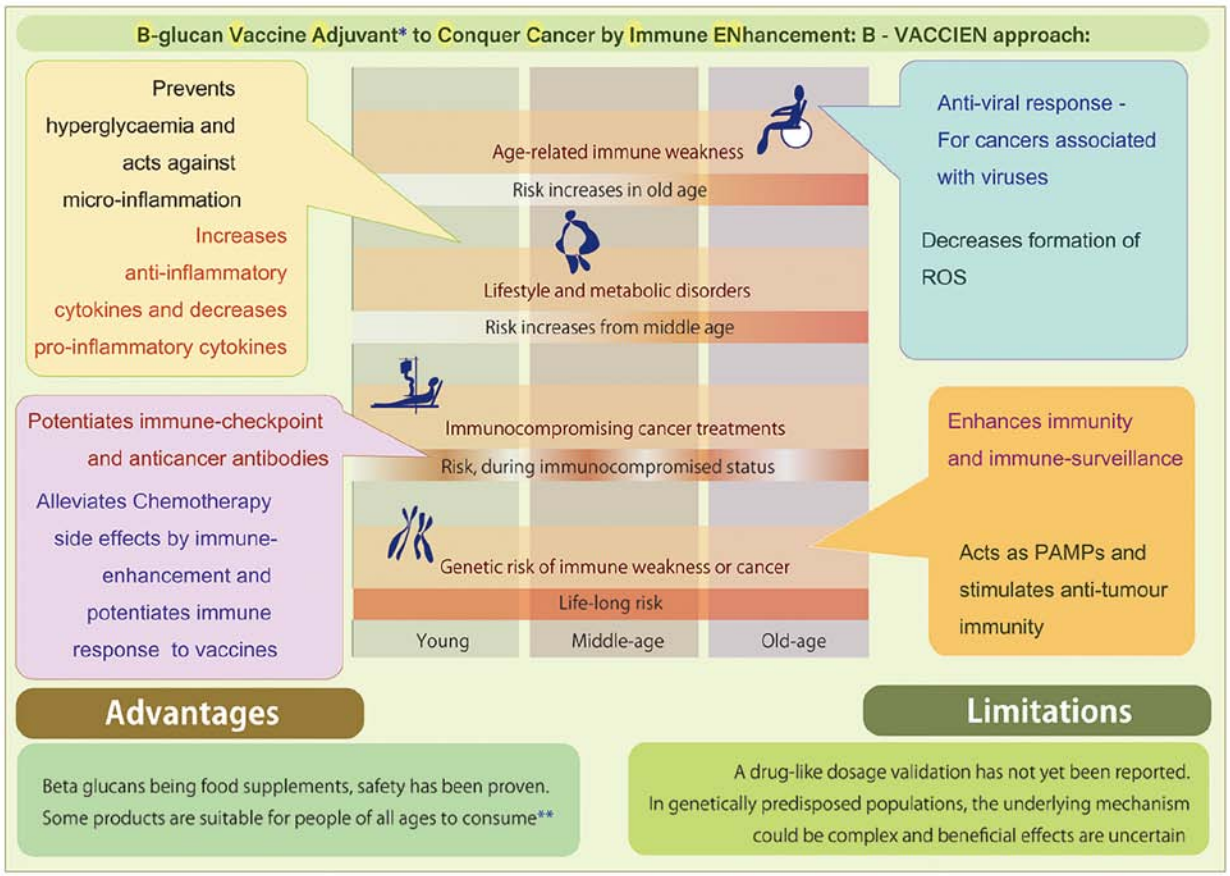

Figure 1. Illustration summarizing the β-glucan vaccine adjuvant approach to treating cancer via immune enhancement (B-VACCIEN). ROS, reactive oxygen species; PAMP, pathogen-associated molecular pattern. *https://doi.org/10.1080/21645515.2021.1880210; **safety data of the AFO-202 strain of black yeast *Aureobasidium pullulans* produce: Nichi Glucan.

of some membrane complement regulatory proteins can limit the β-glucan-primed immune cell infiltration into tumours. Additional strategies of modifying the tumour microenvironment are required to overcome these challenges (35).

6. Conclusion

Several factors and pathogenic processes have been identified, that can predispose an individual to a high risk of developing cancer and/or enable the progression of cancer, including: i) Chronic and microinflammation caused by infections, aging or metabolic disorders, including diabetes; ii) genetic causes; and iii) immune system weakness, either due to cancer or cancer therapy. Therefore, the prevention of cancer in the general population and in patients undergoing surgical or chemotherapeutic treatments is practically feasible, only if a consistent and simple approach can be followed, as for example a nutritional supplement to combat the compromise of the immune system and chronic microinflammation. The current review presented evidence of a BRMG, with regards to its potential function as a β-glucan vaccine adjuvant approach for the treatment of cancer through immunoenhancement. This approach may aid in the treatment of cancer in specific immunocompromised populations, as it induces a wide variety of biological response modifications. For example, the BRMG application may balance metabolic parameters, including blood glucose and lipid levels, increase peripheral blood cell cytotoxicity against cancer and alleviate chemotherapy-induced side effects in animal models. Thus, the use of a β-glucan vaccine adjuvant approach was suggested for the treatment of cancer via immunoenhancement as a potential strategy for a long-term prophylaxis in immunocompromised individuals or genetically prone to cancer.

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Authors' contributions

NI, VDD, KR and SJKA contributed to the conception and design of the study. RS performed the literature search and

data analysis. SJKA and SP confirm the authenticity of all the raw data. SJKA and SP drafted the manuscript. KSR, SV, HO, TK, GK, SS, SRBK and MI performed the critical revision of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

SJKA is a shareholder in GN Corporation, Japan which in turn is a shareholder in the manufacturing company of the AFO 202 β-glucan.

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