Professor Solomon P. Wasser and Medicinal Mushroom Science, with Special Attention to the Problems of Mycotherapy in Oncology

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ABSTRACT: This article is dedicated to the 75th anniversary of Solomon P. Wasser and discusses the challenges within the research direction he founded with Professors T. Mizuno, S.T. Chang, and other colleagues, known as medicinal mushroom science. This research organically grows out of taxonomic studies, since understanding of the classification system leads to greater ability to make forecasts and predictions in the practical field as well as to increase knowledge of close relationships between organisms, allowing greater eeconomic organization within the search and screening for new practically significant organisms. Through the efforts of Professors Wasser, Mizuno, and Chang, the *International Journal of Medicinal Mushrooms* (*IJMM*) was created, combining the efforts of physicians using mushroom raw materials as an auxiliary tool and specialists in fungal biotechnology. In this work, the basic prerequisites for cancer mycotherapy are described, based on data on the mechanism of action of fungal metabolites on cancer targets. A large section of this report is devoted to a review of evidence-based medicine tools and an overview of their use among teams of Chinese researchers. It has been shown that the main recipients of mycotherapy, as well as other types of immunotherapies, are patients with stage 3 cancer who have undergone surgery to remove the primary tumor node and are undergoing chemotherapy; for these patients, their immune status must be increased, and the immune system requires a periodic rebooting. In this respect, Dectin stimulation using fungal glucans is comparable to cytokine therapy and can be characterized as an "endogenous cytokine therapy." The results of the combined treatment at this stage are to be quantified using evidence-based medicine tools, for which we recommend including the consumption of mushroom extracts in the patient questionnaire. This article also discusses challenges in the pharmacokinetics of β-glucans and triterpenoids.

KEY WORDS: evidence-based medicine, fungal biotechnology, *Ganoderma lucidum*, lingzhi, immunotherapy, mycotherapy, medicinal mushrooms, pharmacokinetics, β-glucans, triterpenoids, prognostic taxonomical system, target cancer therapy, *Trametes versicolor*

ABBREVIATIONS: HR, hazard ratio; **IFα**, interferon-alpha; **NKC**, natural killer cell; **TNF**, tumor necrosis factor

I. INTRODUCTION

On August 26, 2021, the mycological community celebrated the 75th anniversary of Solomon P. Wasser, Dr Sci (Biology), PhD, a world-renowned scientist, Corresponding Member of the National Academy of Sciences of Ukraine, and emeritus professor of the Institute of Evolution at the University of Haifa in Israel. As an authoritative specialist and biologist with a wide profile, Wasser is a member of the editorial board of several scientific journals. In addition, he is the Editor-in-Chief of the journals *Algology*, *International Journal of Algae*, and *International Journal of Medicinal Mushrooms (IJMM)*. He has also authored > 600 scientific papers, including 60 monographs. A range of fungal and algal taxa described in his honor are recognized for the merits of Wasser as a taxonomist: *Agaricus wasseri* Bon et Courtec.,[1] *Lepiota wasseri* Bon,[2] *Lichenochora wasseri* S. Kondr.,[3] *Pachykytospora wasseri* Zmitr., Malysheva et Spirin,[4] *Placogeia wasseri* L.N. Bukhtiyarova et G.V. Pomazkina,[5] and *Crepidotus wasseri* Kapitonov, Biketova, Zmitr. et Á. Kovács.[6]

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Wasser began as a taxonomist and theorist in mycology, and then became a cofounder of an important applied field, which he called medicinal mushroom science. In this work, we will consider the theoretical heritage of Wasser and his contribution to applied science in the relationship between these two layers.

II. PROFESSOR WASSER AS A MONOGRAPHIC TAXONOMIST AND THEORIST OF MYCOLOGY

The creation of the classification system to reflect genealogical relations between taxa, (traditionally called the natural system) is declared by taxonomists as the main goal of their work, which not only has huge fundamental value but also has led to an increase in global understanding of this field. In practice, the heuristic power of such an approach to the natural system is in demand due to an increase in its prognostic ability—that is, the possibility of predicting certain properties in groups that have not yet been studied but are genealogically related to groups studied in one or another way.[7]

The prognostic capabilities of the phylogenetic system are associated with the idea that the degree of divergence of groups is inversely related to the preservation of their phenotypic features, including those that are in demand in practical terms.[8–10] At the level of large groups, in the process of evolution, systems of prohibitions are developed for the morphophysiological convergence of organisms. The labeling of such groups creates opportunities for predictive assessments of varying degrees of certainty concerning both the evolutionary tendencies of organisms and some of their essential properties from the point of view of applied research.[11]

The confirmation of a close relationship of taxa makes it possible to develop several recommendations for the selection of strains and isolation from nature of several closely related organisms, saving time for the deployment of exploratory studies across the entire taxonomic spectrum. This is applicable to biotechnologically important groups such as the Basidiomycetes.

After graduating with honors from Uzhgorod University, Wasser began his postgraduate study at the Mycology Department of the Institute of Botany Ukrainian Academy of Sciences in Kiev, in 1969. Professor M.Ya. Zerova became head of the department, and in her student years saw the makings of a thoughtful and hardworking researcher.

Wasser's PhD thesis, "Agaricales s. l. of the Steppe Zone of Ukraine," represents a great contribution to the knowledge of the mycobiota of the steppe zone of Eastern Europe. The task of identifying the diversity of fungi in the arid zone objectively does not belong to the category of the lungs, but Wasser wonderfully solved it: > 700 species were identified (about 55% of this number consisted of the agaricoid Basidiomycetes), among which were extremely rare species in the USSR, including *Agaricus cupreobrunneus*, *A. porphyrocephalus*, *Galeropsis desertorum*, and *Leucoagaricus macrorhizus* (now *L. barssii*). Most notably, and for the first time for science, three new species (*Leucocoprinus bohusi* Wasser, *A. amanitaeformis* Wasser, and *Paxillus zerovae* Wasser) were proposed; two new combinations as well as several taxa of intraspecific rank have since been proposed. In 1973, Wasser and his postgraduate colleague, I.M. Soldatova, who studied aphyllophoralean fungi, began work on the monograph *Higher Basidiomycetes of the Steppe Zone of Ukraine*, which was published in 1977.[12]

In 1973–1980, Wasser devoted himself entirely to the work of a monographic agaricologist. He went on numerous expeditions to Ukraine and other regions of the USSR, including the Baltic states, the Caucasus, Central Asia, South, Western and Eastern Siberia, and the Far East, becoming acquainted with the herbarium of the Komarov Botanical Institute of the USSR Academy of Sciences. He ordered the specimens for study from the herbaria of Canada, the United States, Sweden, Denmark, Holland, Great Britain, France, Czechoslovakia, Hungary, and several USSR republics. The result of this painstaking work is described in his monograph, *The Fungal Flora of Ukraine: Agaricaceae*,[13] and his doctoral dissertation, "Agaricaceae (Fr.) Cohn of the Soviet Union." Three new species and some

nomenclature combinations have been described in this cycle: *A. longicaudus* Wasser, *A. moelleri* Wasser, *A. romagnesii* Wasser, *L. moseri* (Wasser) Wasser, and *Lepiota subgracilis* Kühner ex Wasser. The main provisions of the doctoral dissertation were published in the monograph by Wasser, *Agaricales Fungi of the USSR*.[14]

After he graduated with a doctor of science degree, Wasser began a critical study of Amanitales and published the monograph, *The Fungal Flora of Ukraine: Amanitales*, in 1992.[15]

For a series of works carried out during this period in the field of taxonomy of agaricoid Basidiomycetes in 1991, Wasser was awarded the Alexander and Helen Smith Prize of the Mycological Society of America.

In 1995, Wasser was invited to work at the Institute of Evolution at the University of Haifa (Israel), founded by the renowned evolutionary biologist Professor Eviatar Nevo. There, Wasser created the International Center for Biotechnology and Biodiversity of Fungi and Algae, which organized a few fundamental and applied research studies and, in parallel, trained young scientists. Thirty-five graduate students from Israel, Ukraine, Armenia, Georgia, Bulgaria, Romania, and other countries received invaluable research skills in the center headed by Wasser and now work in the world's leading laboratories. Among the numerous followers of Wasser, in the formation of which this school played the main role as scientists, it should be noted that M.Ya. Didukh, M. Asatiani, G. Songulashvili, S.O. Voytyuk, D. Tura, A. Kosakyan, G. Barsegyan, N. Mikiashvili, M. Stajic, R. Petrova, K.G. Savchenko, D.N. Gotman, A.Yu. Biketova, M. Krakhmalny, B.-Z. Zaidman, D. Lewinson, L. Sharvit, M. Yassin, J. Bar-Tov, and M. Rivchun have already received recognition in the world research community.

Overall, the activities of the team headed by Wasser at the Institute of Evolution have two (partly overlapping) directions. The first is associated with intensive research on the biodiversity of cryptogamic organisms in Israel, the main result of which is the creation of a multivolume "flora," which has the general name "Biodiversity of Cyanoprocaryotes, Algae and Fungi of Israel." Specialists from countries such as Ukraine, Germany, the United States, Mexico, Brazil, India, Russia, and Uzbekistan can also be involved in cooperation if required. Wasser and these specialists jointly investigated cyanophycean, desmidian, diatom, euglenophycean algae, lichenized and operculate Discomycetes, powdery fungi, soil micromycetes, aphyllophoroid and heterobasidial, gasteroid, rust, and smut fungi as well as yeast. As a monographist of the Agaricaceae, Wasser thoroughly studies this family within Israel. To date, 13 monographs related to this series have been published.

The inner workings of the scientific center, organized by the famous evolutionist E. Nevo, affect the activities of Wasser, several projects of which during these years have a general biological influence. He was the editor of three monographs entitled *Evolutionary Theory and Processes: Modern Perspectives*,[16] *Evolutionary Theory and Processes: Modern Horizons*,[17] and *New Horizons in Evolution*,[18] which were timed to coincide with the anniversaries of Nevo.

III. CONTOUR OF MEDICINAL MUSHROOM SCIENCE

It should be noted that Wasser began to show attention to the applied aspects of mycology as early as the late 1970s.[19] In 1999, through the efforts of Wasser, *IJMM*, a journal that publishes and summarizes modern experience in the field of pharmacology, biotechnology, and biomedical aspects of higher fungi usage, was created. The leading role in enhancing research on the medicinal properties of Basidiomycetes belongs to Wasser himself, who has been summarizing the results of studying the chemical properties and biomedical aspects of the use of medicinal mushrooms for two decades and taking part in experimental work in these directions.[20–30]

For the past 20 years, Wasser has regularly organized international conferences on medicinal mushrooms, sometimes yielding the honorary chair to his long-time Chinese colleague, Professor S.T. Chang.

The latest conference (the 10th International Medicinal Mushroom Conference) was held in Nantong, China, in September 2019.

IJMM was organized by Professors Wasser, Mizuno, Chang, and other colleagues from different countries. The journal acquired its face and formed a specific discourse—the mutual immersion of theoretical (cancer biology, immunology, glycobiology, fungal phylogeny) and practical (immunotherapy, *in vitro* experiments, biotechnology of Basidiomycetes) scientific directions. It began to accumulate data on the pharmacology and biotechnology of fungi, which until that time had appeared in various pharmacological journals. Demand for *IJMM* grew among biotechnologists dealing with fungi on a global scale. Currently, the journal enjoys the well-deserved respect of mycologists and biotechnologists.

At the designated scientific crossroads, a new field of knowledge began to grow, called medicinal mushroom science.[23,24,31–33] The field of medicinal mushroom science has been built by many scientists in the last 40 years. However, the following individuals have played a major role: Professors T. Mizuno, S.T. Chang, S.P. Wasser, T. Ikekawa, A.L. Weis, J.E. Smith, R. Beelman, V. Sasek, G. Zervakis, Sh. Zhou, K.K. Tan, G. Guzman, N. Ohno, Leo J.L.D. van Griensven, U. Lindequist, Yu Li, P.Ch.K. Cheung, J. Holliday, J. Buswell, S. Rapior, N.P. Denisova, A. Smania Jr., V. Elisashvili, S. Badalyan, M. Berovic, Ch. Hobbs, P. Stamets, A.S. Buchalo, G.T. Liu, Zh.-B. Lin, Ph.G. Miles, H.P. Molitoris, P.A. Volz, H. Kawagishi, M. Mizuno, O.S. Isikhuemnen, H.W. Kim, B.K. Kim, J. Lelley, H.-Ch. Lo, J.-L. Mau, N. Psurtseva, M. Rai, G. Venturella, M.L. Gorgano, V.K. Varshney, H.H. Aydin, V. Sabaratnam, I. Jakopovic, M. Stajic, M. Niksic, B. Strukelj, V.E.C. Ooi, C. Zhuang, B. Xu, I. Zmitrovich, and M. Asatiani. This accumulates our knowledge about the medicinal effect of mushrooms, including antitumor, immunomodulatory, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, hepatoprotective, and antidiabetic effects. Medicinal mushrooms have beneficial effects not only as medicines but also as a new class of products under different names, such as dietary supplements, functional foods, nutraceuticals, mycopharmaceuticals, and designer products that provide health benefits when used daily as part of a healthy diet. The increased interest in traditional remedies for various physiological disorders and the recognition of the numerous biological activities of mushroom products has led to the emergence of the term "mushroom nutraceuticals," which should not be confused with nutraceuticals, functional foods, and pharmaceuticals. A nutraceutical for mushrooms is a purified or partially purified extract or dried biomass from the mycelium or fruiting body of a fungus that is consumed in capsule or tablet form as a dietary supplement (not food) and potentially has therapeutic applications. Regular intake can enhance the immune response of the human body, thereby increasing resistance to disease and in some cases causing regression of the disease state. Thus, acting as immunopotentiators, medicinal mushroom preparations alter the biological responses of the host.

As the main directions of the science of medicinal mushrooms, Wasser has identified the following: 1) taxonomy and nomenclature of medicinal mushrooms, 2) study of culinary and medicinal mushrooms in pure culture, 3) challenges in the production of dietary supplements from medicinal mushrooms, and 4) technology for testing mushroom raw materials for medicinal properties and drug development. This is an actively developing area, which is the focus of the *IJMM* pages.

In 2010, Wasser and colleagues[23,24] described in detail the drug-discovery pathway for the development of mushroom pharmaceuticals. The drug-discovery pathway for mushroom bioactive metabolites includes the following basic steps: 1) mushroom cultivation and biomass production, 2) extraction of a pool of substances from biomass, 3) screening of extracts, 4) the effect of selected extracts on the molecular target of interest, 5) chemical fractioning of selected extracts, 6) identification of the mechanism of action and efficacy active fractions (compounds), 7) testing of substances on experimental animals, 8) drug preclinical testing, and 9) drug clinical trials. The latter steps are already actively carried out in China; however, in European medicine, they have not yet entered the standard protocols, which is associated with a certain gap in knowledge leading to speculation.

In describing the prospects for medicinal mushroom science in the 21st century, Wasser highlights the main directions for further application of efforts. The first is further study of the role of polysaccharide-protein or polysaccharide-peptide complexes in the pharmacological activity of medicinal mushrooms. Second, additional studies of extracts for the specification of their activity against viral infections, bacterial infections, metabolic syndromes, cancer, and cholesterolemia are needed. Third, regrading development of new methods and processes in the study of medicinal mushrooms (as an example, Wasser cites a new method for the extraction of water-soluble β-glucans from medicinal mushrooms with nanoparticles using insoluble tungsten carbide as a model for the nanoknife technology[34]). Fourth, high-quality, long-term, double-blind, placebo-controlled clinical trials of drugs with a large number of study groups are needed. Fifth, trials of bioactive medicinal mushroom complexes in farm animals are also needed. For example, on the one hand, there are research areas that could potentially be expanded from even the use of farm animals as biomedical models, including obesity, diabetes, aging, cardiovascular diseases, infectious diseases, neurobiology, cancer, nutrition, immunology, ophthalmology, and reproductive function. On the other hand, a revolution in veterinary medicine is possible by proposing new types of feed, antibiotic substitutes, and antiviral agents. Finally, intellectual property protection of medicinal mushroom genetic resources for inventions is another area for research.

Like any new field of knowledge, much about medicinal mushroom science remains unknown. Therefore, Wasser has singled out and grouped the main unresolved challenges in this field to focus the efforts of various specialists on their solutions in the future.

First, the worldwide standardization of dietary supplements from medicinal mushrooms is still in its infancy, including a lack of understanding of the bioactive effects of dietary supplements. Currently, we do not have internationally recognized standard dietary supplements and protocols for the production and testing of medicinal mushroom products. Only appropriate standard nutritional supplements and protocols can guarantee product quality. Without consistent quality of medicinal mushroom products, commercially available medicinal mushroom preparations will be very different and will vary greatly in composition and potency. So, it is not known whether the bioactive effects are caused by one component or are the result of the synergistic action of several ingredients. There are insufficient data to determine which components are better for the fruiting bodies of mushrooms or submerged mycelium powder compared to extracts. Many questions remain. Are simple dried fruit bodies and mycelium powders as effective as hot water, alcoholic, or hydroalcoholic extracts? Between crude extracts and isolated fractions, which one is more effective and has a higher safety profile? The role of low molecular weight complex nutritional supplements for medicinal mushroom extracts is still unclear. In addition, what is more effective—a combination of components containing biomass or extracts of 2–10 different types in one tablet or the presence of one species in one tablet? How can we evaluate the effectiveness of different mushroom products when mixing many types in one product (a "shotgun" approach)? Since mushroom foods can be cytokine stimulants, at what age are they safe for young children because their immune systems are not yet mature? What dosages are safe and effective during pregnancy and lactation? The lack of underdeveloped standard nutritional supplements for the recommended use of medicinal mushroom nutritional supplements, including precise dosages and durations of supplementation, requires a very serious study of nutritional supplements. Some studies show that too high a dose can lead to immune suppression, whereas too low a dose may not elicit an immune response. In addition, the main challenges associated with mushroom-based dietary supplements are related to their wide variety, the current lack of standard food additives for production, and the lack of test protocols required to ensure product quality. The active ingredients of many current commercial mushroom products are not listed.

Second, commercial counterfeiting of medicinal mushroom products with false species (e.g., various *Ganoderma* spp. for *G. lucidum*, *Stereum* spp. for *Trametes* spp., and various *Cordyceps* spp. for *C. sinensis*)

is very common. There are difficulties in producing pure β-glucans for sale (90–95% of β-glucans on the market are considered counterfeit and falsified).

Third, we still have not solved the challenges associated with the safety of several well-known medicinal mushroom products.

Fourth, what is the role of consuming fresh mushrooms? Consumption of fresh mushrooms has been found to increase, for example, antibodies against β-glucan in human serum.

Fifth, there is still no information on the use of dietary supplements with medicinal mushrooms and their crossbreeding or interaction with certain medications.

Sixth, we still do not know the role of molecular weights in the pharmaceutical activity of β-glucans. Challenges with the effectiveness of high molecular weight β-glucans versus low molecular weight β-glucans still exist. The most effective are high molecular weight preparations of scleroglucan. But, for example, only low molecular weight lentinan has a higher antitumor activity. We must consider the different reactivity of β-glucans in each person. For example, the titer of anti-β-glucan and the increase in titer with the introduction of β-glucan differ. The reactivity of peripheral blood leukocytes to β-glucan differs significantly in each person. Furthermore, reactivity to β-glucans, for example, varies considerably between different mouse lines.

Seventh, water solubility is one of the most important characteristics of β-glucans. We still do not know what the main factors are that affect the solubility and pharmaceutical activity of β-glucans: molecular weight, side chain length, number of side chains in the main chain, ratio bonding [(1,4), (1,6), and (1,3)], and acid ionization should be considered.[20] Soluble β-glucans appear to be more potent immune-modulators than insoluble β-glucans. However, the reasons for this are not entirely clear.

Eighth, we need to clarify the differences between plant β-glucans, yeast β-glucans, and β-glucans from medicinal mushrooms. What is the difference in structure, solubility, and biological activity? For example, the structure of β-glucan in cereals consists mainly of β-1,3- and β-1,4-bonds rather than β-1,6-bonds. In addition, plant β-glucans are linear rather than branched. Typically, the molecular weight of plant β-glucans is lower than that of medicinal fungi. The biological activity of plant β-glucans has not been fully studied. Usually, yeast β-glucans are only partially soluble in water, and β-glucans of many medicinal mushrooms are insoluble in water. Why do they have different biological activities? What are the main advantages of β-glucans of medicinal mushrooms compared to, for example, β-glucans of cereals or β-glucans of yeast?

Ninth, why do β-glucans have a triple-helix conformation, and is the triple-helix structure advantageous for single-strand medicinal mushrooms? Unfortunately, we do not understand which structural features best induce certain activities and, more importantly, what is the presence of hydrophilic groups located on the outer surface of the helix. In the literature, one can see conflicting data on the biological activity of three-helical and single-helical structures of the same β-glucan, such as schizophyllan. We still do not know which of them has the greatest biological activity—a closed triple helix or a partially open triple helix. Molecular docking techniques for glucan–receptor interactions will undoubtedly shed light on this range of questions.

Finally, Wasser proposes to devote to the promotion of this new field of knowledge, with which it is necessary to acquaint society and consumers. Interest and achievements in current research are not always visible or available to the public. Incredibly, today many people around the world are completely unaware of the health benefits of medicinal mushrooms. Specialists in this area should educate deans, presidents, chancellors, alumni and journalist associations, business leaders, and government officials. It is necessary to answer questions and concerns because the positive effect of this kind of research is not always adequately covered in the press. In this aspect, Wasser was a great prophet, since even a new niche emerged in the media, where young poseurs compete to subvert emerging trends.[35] But there is a real modern challenge to medicinal mushroom science connected with the circumstance, which we next discuss in further detail.

IV. NEW CHALLENGES

Considering the specificity of oncotherapy associated with strict adherence to treatment protocols developed for certain tumors, as well as a rather limited set of drugs that provide strong stimuli to neoplastic transformed cells, substances of fungal and plant origin, carrying obviously weaker stimuli, until now have not been used by oncologists in so-called first-line therapy.

Although the use of *G. lucidum* in cancer treatment is generally known, Jin et al.[36] carried out a systematic review of clinical trials of *G. lucidum* extract, both as an adjunct to the main chemotherapeutic treatment and as a first-line drug. Their results showed that patients who were given *Ganoderma* extracts along with chemotherapy/radiation therapy responded better to treatment compared to patients who received only chemotherapy/radiation therapy. However, the use of *Ganoderma* extracts as a first-line drug did not show the same rate of tumor regression as in combination therapy. An immunogram of the patients showed that the intake of *Ganoderma* extracts contributed to a slight (by 2–4%) increase in the fractions of CD3, CD4, and CD8 T lymphocytes and to an increase in the activity of granulocytes and natural killer cells (NKCs). In conclusion, the authors stated that they have not obtained reliable data justifying the use of *Ganoderma* extracts as a first-line drug, and the assessment of long-term results of treatment is beyond the scope of this study. At the same time, the authors recommended the use of *Ganoderma* extracts as an adjunct to traditional treatment, considering the enhancement of their combined effect on the tumor with chemotherapy and the stimulation of innate immunity. The authors identify the following as promising areas of research: 1) differential study of the activity of *Ganoderma* extracts on various types of cancer, 2) standardization of studies by stages of the tumor process, 3) analysis of long-term results of treatment using mushroom extracts, and 4) analysis of differentiation of treatment results by demographic groups.

Despite the multidimensional nature of the study by Jin et al.[36] as well as an earlier thesis, a lack of reliable data justifying the use of *Ganoderma* extracts as a first-line drug remains. The statement, "there is no scientific evidence confirming the effectiveness of these preparations,"[37] in the aspect of cancer therapy was supported in the text of a paper by a single reference to the work of Jin et al. discussed earlier; however, any inputs (*in vitro*, *in vivo* efficacy as a first-line drug, an auxiliary drug, an immunomodulator in long-term treatment?) were not specified.

To judge how justified this type of skepticism is, one can only have the results of evaluating the effectiveness of treatment with evidence-based medicine tools.

V. TREATMENT WITH MEDICINAL MUSHROOMS AND EVIDENCE-BASED MEDICINE

A. The Experience of Analyzing the Nearest Results of Combined (Using Mushroom Extracts) Adjuvant Treatment of Cancer

Surgical removal of the primary tumor node and macrometastases (if any) remains the primary task of oncotherapy, and the quality of treatment at this stage (e.g., the degree of surgical intervention, determination of the width of the resection margin) is the main prognostic factor. In the case of widespread tumor progression when several organs are affected or in the case of primary multiple lesions over a large area, physicians resort to chemotherapy, radiation therapy, or a combination thereof; sometimes (neoadjuvant therapy) before surgery, the tumor is reduced in size through chemotherapy.[38] The use of mushroom medicinal raw materials at this stage is not a serious alternative to the methods listed and, as far as we know from the literature, has not been proposed by anyone in this capacity.

After the removal of large tumor nodes, all of the standard treatment regimens involve adjuvant chemotherapy aimed at destroying micrometastases (cancer cells and microemboli), in which the primary tumor,

starting from stage 3, managed to spread through the lymphatic pathways. Adjuvant chemotherapy regimens take into account several factors (first, whether tumor elements are found in a "key" for a particular organ lymph node, along with the degree of their differentiation and the nature of their growth) and usually involve periodic intravenous administration of solutions with a combination of strong cytostatic agents; one such agent is directed on the disorganization of the cytoskeleton of a cancer cell, and the second exhibits high proapoptotic activity.[39] In some countries (primarily China), the combination of adjuvant chemotherapy (or regional radiation therapy) with oral capsules or aqueous extracts of mushrooms, most often lingzhi (*G. lucidum*) and *T. versicolor*, is allowed in hospitals.

In 2019, a team of Hong Kong researchers led by Zhong[40] carried out a systematic review and meta-analysis of randomized controlled trials of mushroom extracts [lingzhi (*G. lucidum*) and *T. versicolor*] in combination with traditional adjuvant therapy, from which the authors obtained information from publications in PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure, Wanfang Data, and Chinese Academic Journals database; data processing was carried out according to a completely modern protocol.[41] Two members of the cited team independently assessed the potential risks of bias for all included studies using the Cochrane Risk of Bias Assessment Tool.[42] During the course of the described study, three main indicators used in clinical oncology were analyzed: overall survival, relapse-free survival (in the short term), and overall efficacy as well as the immunological indicators of patients (overall survival was defined as the time from the start of therapy to the moment of patient death, regardless of reason). An important statistical tool demonstrating the effectiveness of combination treatment was the pooled hazard ratio (HR), which compares the rate of survival between treated and untreated patients (including those who received placebo). Different therapeutic situations associated with chemotherapy, radiation therapy, and their combination were analyzed separately, and a total of 23 trials involving 4246 patients with cancer were analyzed.

The results obtained by Zhong et al.[40] generally correlate with those obtained by Jin et al.[36] and demonstrate clear benefits of the combination treatment. A significantly lower risk of mortality (HR, 0.8) and a better overall survival rate (1.30) were associated with the intake of *T. versicolor* and *Ganoderma* extracts and capsules. The authors did not reveal a significant effect of combined treatment on relapse-free survival (in the short term). From the immunological parameters, the effect of the combined treatment on increased levels of cytotoxic T lymphocytes and T helper cells, which are an important link in antitumor protection, was reliably revealed.

We undertook such a detailed analysis of the work of Zhong et al. to draw the attention of European and American clinicians to the experience of their Chinese colleagues.

An even more important task seems to us to study the long-term (≥ 5 years) results of treatment with the use of bioactive complexes produced by medicinal mushrooms.

B. Improvement of Long-Term Results of Treatment Is the Main Goal of Immunotherapy

A good prognosis (70–95% 5-year survival rate) in the treatment of primary cancer refers to stages 1–2 of the disease, when the tumor does not go beyond the organ and does not meet the lymph nodes. The survival rate of patients who receive treatment for primary oncological disease at stage 3 is described mainly by exponential curves with a rather sharp decrease in the proportion of survivors in 1–3 years and a tendency toward stabilization of the proportion of patients with remission after a 5-year mark.[43] The causes of high mortality in the first 3 years may be metastases not detected during the initial diagnosis, active dissemination of the tumor during treatment, nonradical surgical treatment, and the absence of adjuvant therapy. Survival at stage 4 with active secondary tumor nodes is most often described by linear regression models, and even traditional drugs are ineffective and, in some cases,

even accelerate the process of tumor dissemination. Palliative treatment comes to the fore at stage 4 of the disease.

Thus, stage 3, at which most tumors are detected, represents the last of the stages of the disease at which it is possible to reach a stable remission, taking the metastatic process under immune control. Even in this case, persistent cancer cells will increase their adaptive potential[44] and tolerogenic activity,[45] but the maintenance of the patient's immune depots and a periodic reboot of the immune system can significantly slow this process, and the period of remission may exceed the 20-year mark.[46]

The period of adjuvant therapy at stage 3 does not exceed 12 months and most often is 2–6 months. After that, treatment with hard cytostatic agents is no longer carried out and the maintenance of a high immune status of the body is encouraged in every possible way, using both phytotherapeutic and special immunotherapeutic agents. The most effective way to continue the fight against tumor derivatives through mild means is so-called cytokine therapy—that is, an exogenous administration of hormone-like molecules normally produced by the cells of the immune system themselves, which can lead to a noticeable activation of the cytotoxic component of the immune system.[47]

Here we will demonstrate the effectiveness of cytokine therapy using two characteristic examples, although the number of reports on the effectiveness of this method grows every year as this practice spreads.

American oncologist Bukowski[48] evaluated the effectiveness of cytokine therapy with interferon-alpha (IFα) and interleukin-2 in the treatment of aggressive metastatic kidney cancer. The combined administration of these cytokines according to a specific scheme led to tumor regression in 10–15% of patients with metastatic lesions. Randomized trials have shown better survival rates in patients receiving IFα monotherapy.

A Russian team led by Ilyushin[49] evaluated the efficacy and tolerability of the drug tumor necrosis factor (TNF)-thymosin 1 alpha recombinant (Refnot) in the complex treatment of HER2/neu and estrogen receptor and progesterone receptor–negative (triple-negative) metastatic breast cancer, which is practically not amenable to treatment. The results of this experiment were positive: the median progression-free survival was significantly higher in the Refnot group and amounted to 8.4 months versus 5.8 months in the control group. Tumor regression was also higher in the group with Refnot included (72% versus 55% in the control group). Both treatment regimens had a similar safety profile. The manifestations of nonhematological toxicity were comparable in both groups.

Endogenous stimulation of the released cytokines is associated with the fact that the image-recognizing receptors of the immune system are tuned, among other things, to the elements of the fungal cell wall, which is typical for therapy using raw materials of medicinal mushrooms. This treatment tactic can rightfully be called "endogenous cytokine therapy."[50] So-called Dectin-1 stimulation of dendritic cells is well studied using experiments.[51] Dectin-1 is a type II transmembrane protein with a lectin-like carbohydrate recognition domain linked by a stem to the transmembrane region followed by a cytoplasmic domain containing an immunoreceptor tyrosinase-based activation motif. This receptor specifically binds to β-1,3-glucans and is phosphorylated by the nonreceptor tyrosine kinase Src, which activates spleen tyrosine kinase Syk and induces the CARD9-Bcl10-Malt1 complex. It is this complex that mediates the systemic response of the immune cell: the activation of nuclear factor-κB and the production of cytokines that intensify the signaling activity of all links of the immune system, leading in the final links to the release of the proapoptotic factor TNF-α (monocytes, macrophages) and having a cytolytic effect of granzymes and perforins (produced by populations of T lymphocytes and NKCs).[52–55]

In addition to T-cell stimulation, the activation of Dectin receptors prevents the development of tolerogenic phenotypes of dendritic cells and contributes to the maintenance of their immune phenotypes for a long time—that is, its "freezes" the process of micrometastatic dissemination.[56]

Most likely, in the coming years, Chinese clinicians will already have data on the long-term results of treatment using mushroom extracts after adjuvant therapy (patient of groups from studies conducted in approximately 2012–2018). For oncoimmunotherapists in other countries observing patients at the stage of stable remission, it would also be very useful, as follows from the aforementioned, to include in patient questionnaires an item related to the possible intake of medicinal mushrooms.

Thus, the tools of evidence-based medicine should be used wisely, calculating the effectiveness of the drug in relation to a specific link in cancer therapy and to a specific therapeutic situation. At the same time, the individual goal of the user of these tools should not be the destruction of the work of large biotechnological teams of the world for self-affirming purposes, but the quantitative determination of effectiveness of plant/fungal supplements for specific therapeutic situations.

C. What Do We Know About Beta-Glucan and Triterpenoid Pharmacokinetics?

The main groups of oncostatic substances contained in fungal raw materials are 1) polysaccharides (first, β-glucans), with which their immune-mediated effect on the body is associated, and 2) triterpenoids (e.g., ganoderic acids), the molecules of which are antagonists of growth factor receptors and are also able to inhibit cyclin-dependent kinase. Therefore, they have a direct effect on cancer cells, leading to the arrest of the cell cycle and disruption of proliferative signaling pathways.[57–61]

Some fungal glucans are water soluble and are used mainly in the form of aqueous extracts, while triterpenoids are used in alcohol extracts.

Two questions have arisen. First, to what degree are the extracts, which have proven themselves well *in vitro*, effective under the conditions of oral administration, considering that the direct introduction of these substances into the blood is impossible since it leads to inflammatory and hematotoxic reactions? Second, is it possible to use a nonfractionated fungal raw material in oncotherapy?

The pharmacokinetics of β-glucans was substantially studied by Chan et al.[62] on model animals. The most important result of this study was the fact that the linear 1→3 β-glycosidic chain of glucans does not break as it passes through the gastrointestinal tract. The absorption of β-glucans occurs mainly in the proximal part of the small intestine. In the liver, some of the molecules are captured by macrophages; in vacuoles, they are roughly fragmented and transferred by macrophages in this form to the reticuloendothelial system, where they are partially released by the macrophages that have captured them and stimulate several receptors of other macrophages, monocytes, and various populations of lymphocytes. Theoretically, β-glucans differing in branching characteristics may differ in the effectiveness of their action on the receptors of immune cells and their testing seems to be a promising task; but so far, the practice of fungal glucan usage does not imply their purification and fractionation, which does not detract from the significance of these works.[63]

A large review of the pharmacokinetics of triterpenes mainly of plant origin[64] indicates their limited absorption in the gastrointestinal tract, which creates difficulties in finding forms of their therapeutic use. Thus, derivatives of betulinic acid (accumulated by the mycelium of *Inonotus obliquus*) were tested on a noncellular model (the so-called parallel analysis of the permeability of an artificial membrane), which makes it possible to predict the passive transport of betulinic acid derivatives through intestinal cell membranes. However, it turned out that betulinic acid itself is able to overcome this barrier, and its lower molecular weight derivatives have this ability only in small amounts (from 5 to 33% for various substances), which is a poor indicator for drugs.

Thus, the only suitable dosage form for testing and using fungal triterpenoids is an alcoholic extract, and the requirement for the chemical purity of the active substance is many times higher in comparison with the situation of the therapeutic use of β-glucans. However, in practice, in most cases it should be easier to purify and to characterize a small chemical compound as a triterpene than a high molecular weight β-glucan.

VI. CONCLUSIONS

The new challenges described here should not deny the basic achievements of phytopharmacology and medicinal mushroom science; rather, they raise the question of goal setting in studies of the action of anticancer and other medicinal substances of medicinal mushrooms using evidence-based medicine, considering the stage of the disease and combined treatment with traditional first-line drugs. As recent studies show, the agenda is 1) differential study of the activity of fungal extracts on different cancer types, 2) standardization of studies by stages of the tumor process; and 3) analysis of long-term results of treatment using fungal extracts.

In general, the analysis of the array of works devoted to the use of fungal metabolites in medicine testifies in favor of the productivity of this direction. This is emphasized by the depth of the scientific insight of Wasser, who was able to correctly assess this aspect.

The scientific approach of Wasser, which began, it would seem, with purely academic studies of flora, taxonomy, and phylogeny of fungi, quite naturally for such a scale of personality subsequently led him to the solution of pressing practical issues, aimed at preserving the health and life of many people throughout the world. The successful promotion of ideas and projects in the field of medicinal mushroom science became possible due to the enormous scientific and human authority gained by Wasser over all these years and to his outstanding organizational skills, which also manifested themselves in a constellation of students and followers. Wasser has his own well-recognizable creative style. Its main brainchild is *IJMM*, in which data on the pharmacology and fungal biotechnology, previously scattered in various pharmacological journals, were highlighted and began to accumulate, so *IJMM* became in demand on a global scale by biotechnologists dealing with mushrooms.

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