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REVIEW ARTICLE

Potential of medicinal mushroom extracts as adjunctive agents to enhance conversion therapy in colorectal cancer with liver and peritoneal metastases

Agustín Sánchez-Cobos*

Independent Researcher, Elx/Elche, Alicante, Spain

Abstract

Colorectal cancer (CRC) with liver metastases (LM) and peritoneal metastases (PM) presents a formidable challenge with poor prognosis despite advances in systemic chemotherapy and combined surgical approaches, including cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Median overall survival (OS) for such unresectable cases ranges from 12 to 24 months with modern regimens (e.g., 5-fluorouracil [5-FU], leucovorin, and oxaliplatin/5-FU, leucovorin, and irinotecan + targeted therapies). However, conversion to resectable diseases remains limited by disease extent and treatment toxicity. This paper proposes that medicinal mushroom extracts, particularly polysaccharides (e.g., polysaccharide krestin and polysaccharopeptide) from *Trametes versicolor* and bioactive compounds from species, such as *Ganoderma lucidum*, could enhance conversion therapy outcomes in CRC with LM and PM. Evidence from pre-clinical and clinical studies demonstrates that these extracts exert anticancer effects through multiple mechanisms, including suppression of multidrug resistance, blockade of immune checkpoints (e.g., programmed cell death protein 1/programmed death-ligand 1), regulation of oncogenic signaling pathways (e.g., phosphoinositide 3-kinase/protein kinase B, mitogen-activated protein kinase, and Nuclear factor kappa-light-chain-enhancer of activated B cells), and robust immunomodulation. Meta-analyses of over 8,000 patients reported improved survival and tolerability when combining the extracts with chemotherapy post-surgery. Similarly, meta-analysis in the present study, involving 2,397 patients, reveals a 10% relative increase in the probability of survival at 5 years, with a survival risk ratio of 1.10 (95% confidence interval: 1.04–1.15), based on post-operative data without direct pre-operative validation. Integrating these extracts into neoadjuvant regimens could enhance chemotherapy efficacy, increase surgical eligibility, and mitigate toxicity, potentially extending OS. While direct evidence in unresectable CRC with dual metastases is lacking, the biological plausibility and safety profile of mushroom extracts warrant prospective trials to validate their role in conversion therapy.

Keywords: Colorectal cancer; Liver metastases; Peritoneal metastases; Conversion therapy; Medicinal mushrooms; *Trametes versicolor*; *Ganoderma lucidum*; Immunomodulation

***Corresponding author:**
Agustín Sánchez-Cobos (agustinsanchezcobos@protonmail.com)

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1. Background and disease context

Colorectal cancer (CRC) is a leading cause of cancer-related mortality, with metastases significantly impacting prognosis. The peritoneum and liver are common sites of metastasis, with peritoneal metastases (PM) occurring in 8–15% of patients during the first treatment cycle[1] and liver metastases (LM) in 15–25% of patients at diagnosis.[2] When both sites are involved, the disease is particularly challenging, traditionally associated with poor outcomes due to its extensive nature.

1.1. Assessment of the efficacy of systemic chemotherapy as a standalone treatment

Systemic chemotherapy, typically administered intravenously, aims to control cancer spread and prolong survival. For patients with peritoneal carcinomatosis, studies reported a median survival of 6–9 months without treatment,[3] extending to 12–24 months with systemic chemotherapy alone, depending on the regimen and patient characteristics.[1,4-7] Earlier studies using 5-fluorouracil (5-FU) and leucovorin showed disappointing results, with survival rarely exceeding 8 months.[3] For patients with both PM and LM, the prognosis is likely worse, with median survival potentially under 12 months at the lower end, though specific data for this subgroup are limited. Modern regimens, such as 5-FU, leucovorin, and oxaliplatin (FOLFOX) and 5-FU, leucovorin, and irinotecan (FOLFIRI), combined with targeted therapies, including bevacizumab and cetuximab, have improved outcomes, with a lower response in PM compared to LM.[8]

A pooled analysis from the Analysis and Research in Cancers of the Digestive CRC database, involving 17,924 patients, showed that compared to patients with LM (including those with additional sites, such as peritoneum) in the chemotherapy alone and chemotherapy + anti-vascular endothelial growth factor (VEGF) subgroups, non-LM patients had better overall survival (OS) and progression-free survival (PFS) within first-line and second-line settings.[9] However, among patients with *RAS* wild-type status in the chemotherapy + anti-epidermal growth factor receptor (EGFR) first-line and second-line subgroups, there was no significant difference in OS and PFS between non-LM and LM patients.

These findings suggest that while chemotherapy can induce responses depending on regimen and patient characteristics, the presence of multiple metastases, especially including LM and PM, reduces long-term survival. New treatment regimens are being developed to address the challenges of advanced, initially unresectable metastatic CRC (mCRC), such as the combination of folinic acid, 5-FU, oxaliplatin, and irinotecan (FOLFOXIRI) with bevacizumab. For example, the TRIBE trial, a large Phase III clinical study involving 508 patients, compared this regimen to FOLFIRI + bevacizumab. The results showed a median PFS of 12.1 months for the FOLFOXIRI group compared to 9.7 months for the FOLFIRI group, while the OS was 31.0 months versus 25.8 months, respectively.[10] The inclusion criteria for this treatment require an Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or lower. Furthermore, the TRIBE trial included only patients aged 18–75 years, with those over 70 needing an ECOG score of 0. Meanwhile, an individual patient data meta-analysis conducted in 2020 with data from five eligible trials, including the TRIBE trial, concluded, with statistically significant results, that FOLFOXIRI + bevacizumab improves OS when compared with doublets + bevacizumab, with a median OS of 28.9 versus 24.5 months, longer PFS of 12.2 versus 9.9 months, and higher R0 resection rate, 16.4% versus 11.8%, respectively.[11] However, a higher burden of grade 3 and 4 adverse events was also observed, with some events being approximately double the rate compared to the control group.

According to the latest guidelines from the American Society of Clinical Oncology for patients with previously untreated, initially unresectable mCRC, doublet chemotherapy should be offered, while triplet therapy may be considered based on studies involving chemotherapy combined with anti-VEGF antibodies.[12] In the first-line setting, pembrolizumab is recommended for patients with mCRC that has microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) tumors. For microsatellite stable (MSS) or proficient mismatch repair (pMMR) left-sided *RAS* wild-type mCRC, chemotherapy combined with anti-EGFR therapy is recommended, whereas chemotherapy combined with anti-VEGF therapy is recommended for MSS or pMMR right-sided *RAS* wild-type mCRC. For patients with previously treated *BRAF* V600E-mutant mCRC that has progressed after at least one line of therapy, encorafenib + cetuximab is recommended. In cases of colorectal PM, cytoreductive surgery (CRS) + systemic chemotherapy may be recommended for selected patients, although the addition of oxaliplatin-based hyperthermic intraperitoneal chemotherapy (HIPEC) is not recommended. For patients with liver oligometastases who are not candidates for resection, stereotactic body radiation therapy may be recommended following systemic therapy, while selective internal radiation therapy is not routinely recommended for patients with unilobar or bilobar LM. In addition, perioperative chemotherapy or surgery alone should be offered to patients with mCRC who are candidates for potentially curative resection of LM. The guidelines emphasize the importance of a

multidisciplinary team management approach and shared decision-making to optimize patient care.

1.2. Combined treatment approaches and comparative outcomes

Given the limitations of chemotherapy alone, combined treatments have emerged as a more effective strategy. CRS aims to remove visible tumors, while HIPEC delivers heated chemotherapy directly into the abdominal cavity to target residual disease. For patients with both PM and LM studies showed significant survival benefits when CRS, HIPEC, and liver resection or ablation are used. For example, a systematic review and meta-analysis reported a median OS of 26.4 months with a 5-year OS rate of 25% for patients treated with CRS-HIPEC + local liver treatment, compared to 6–13 months with systemic chemotherapy alone, according to their metrics.[13] Another study, focusing on 565 patients, found that pre-operative systemic chemotherapy followed by combined surgery and HIPEC resulted in a 48-month median OS, highlighting the potential of multimodal strategies.[14] A total of 491 patients (91.9%) underwent pre-operative systemic chemotherapy before surgery to target peritoneal and/or LM before CRS with HIPEC and liver resection. The average interval between the diagnosis of LM or PM and the combined surgical intervention was 6 months, highlighting the urgent need for rapid adjunctive therapies to optimize the effectiveness of chemotherapy.

These outcomes contrast sharply with chemotherapy alone, suggesting that the efficiency of systemic chemotherapy is significantly enhanced when integrated into a multidisciplinary strategy. A clear example of this approach has been presented in a systematic review and pooled analysis of 11 studies regarding the potential of FOLFOXIRI + bevacizumab as a conversion therapy.[15] The eligibility for surgery after first-line chemotherapy in patients with unresectable mCRC was 39.1%, with 28.1% of patients achieving R0 resection.

1.3. Patient selection and challenges

Not all patients are candidates for combined treatments due to factors, such as disease extent, performance status, and comorbidities. The peritoneal cancer index (PCI) and completeness of cytoreduction score (CC score), as well as the number of liver lesions, are critical in determining eligibility. A higher PCI (>20), non-achievable complete cytoreduction (CC-0), or near-complete cytoreduction with residual tumor <2.5 mm (CC-1), as well as the presence of more than two or three liver lesions, often contraindicate CRS.[16] In addition, signet ring cell histopathology has also been considered a contraindication.[13] Systemic chemotherapy may serve as a bridge to surgery in some cases, converting unresectable disease into resectable, but its standalone efficiency remains limited for extensive multi-organ involvement. Given that approximately 25% of CRC cases are diagnosed at stage IV at first presentation,[17,18] there is an urgent need for adjunctive therapies that could increase the number of patients eligible for surgery.

1.4. Treatment-specific survival rates with peritoneal involvement

Survival rates vary significantly based on the treatment received, highlighting the importance of patient selection and multidisciplinary management. A 2021 review detailed outcomes of different treatment modalities for CRC patients with PM,[1] as summarized in Table 1. The review highlighted that CRS-HIPEC can significantly extend survival in selected patients, with median OS reaching up to 62.7 months in some case–control studies, compared to 12–24 months with systemic chemotherapy alone. However, the PRODIGE 7 trial in 2021 questioned the added value of oxaliplatin-based HIPEC over CRS alone, showing no significant difference in median OS (41.7 vs. 41.2 months), suggesting the need for a re-evaluation of the additional benefit of HIPEC within present practices and methodologies. In contrast, a Phase III randomized controlled trial (RCT) involving 245 ovarian cancer patients showed a HIPEC-benefited median OS (45.7 vs. 33.9 months); though differences in disease and protocol limit direct comparison.[19,20]

The design of the PRODIGE 7 trial has attracted criticism.[27,28] Methodological flaws included an overly optimistic sample size calculation that anticipated an 18-month survival benefit, a 12% cross-over rate from the CRS-only arm to HIPEC, and the potential for oxaliplatin resistance due to neoadjuvant FOLFOX treatment in 85% of patients. In addition, the post-chemotherapy assessment of the PCI may have misrepresented the initial disease burden. Pharmacologic issues were also noted, including an inadequate dose of 5-FU at only 400 mg/m^2, a limited HIPEC duration of just 30 min, which may have restricted oxaliplatin penetration and led to suboptimal bioavailability, and insufficient hyperthermia effect, even at 43°C.

To address these evidence gaps, the GECOP-MMC trial, a Phase IV, multicenter, randomized, open-label study, was initiated in March 2022.[29] This trial compares CRS-HIPEC with high-dose mitomycin-C (35 mg/m^2) to CRS alone in patients with limited PM from colon (not rectal) cancer, with a focus on preventing peritoneal recurrence. Study completion is estimated for 2029, representing a significant ongoing effort to clarify the efficacy of mitomycin-C HIPEC.

Table 1. Treatment-specific survival rates for peritoneal metastasis

Treatment	OS duration/rate	Study details/notes
Systemic chemotherapy alone	Median OS: 16.3 months	Limited effect in isolated PM, slightly improves OS[6]
Modern systemic chemotherapy	Optimal survival: 15.2 to 23.4 months	Reported for PM of colorectal origin with modern chemotherapy and supportive care[4,6]
CRS+mitomycin C-based HIPEC versus standard treatment	Median OS: 22.3 versus 12.6 months, p=0.032; 5-year OS: 54% for complete resection cases	RCT; 105 patients[21]
CRS+oxaliplatin-based HIPEC versus systemic therapy alone	Median OS: 62.7 versus 23.9 months, p<0.05; 5-year OS: 51% versus 13%	Case–control study post-FOLFOX introduction; 96 patients[22]
CRS+mitomycin C-based HIPEC versus systemic therapy alone	Median OS: 34.7 versus 16.8 months; p<0.001	Case–control study post-FOLFOX; 105 patients (38 in the control group)[23]
Surgery+EPIC (5-FU) versus FOLFOX alone	Median OS: 25 versus 18 months; p=0.04	Single-institution RCT; ended early with 48 eligible patients[24]
CRS+oxaliplatin-based HIPEC versus CRS alone	Median OS: 41.7 versus 41.2 months; p=0.99	PRODIGE 7 RCT; 265 patients; no significant difference[25]
Second-look surgery+prophylactic HIPEC versus standard follow-up	3-year OS: 79% versus 80%; 5-year OS: 68% versus 72%	PROPHYLOCHIP RCT; 150 patients; no significant difference[26]

Abbreviations: CRS: Cytoreductive surgery; EPIC: Early post-operative intraperitoneal chemotherapy; FOLFOX: Folinic acid, 5-fluorouracil, and oxaliplatin; HIPEC: Hyperthermic intraperitoneal chemotherapy; OS: Overall survival; PM: Peritoneal metastasis; RCT: Randomized controlled trial.

2. Anticancer mechanisms of selected medicinal mushrooms

Medicinal mushrooms have garnered attention for their potential anticancer properties, with *Coriolus versicolor* (now classified as *Trametes versicolor*), commonly known as turkey tail, standing out due to its extensive study and clinical use, particularly in Japan and China. Compounds, such as polysaccharide krestin (PSK) and polysaccharopeptide (PSP) from *T. versicolor* have been approved as adjuvant therapies in certain regions, suggesting their potential efficacy in enhancing treatment outcomes. This review explores the anticancer mechanisms of selected mushroom species—*T. versicolor* (turkey tail), *Ganoderma lucidum* (also known as *lingzhi* and *reishi*), *Grifola frondosa* (maitake), *Lentinula edodes* (shiitake), *Agaricus blazei* (sun mushroom), and *Inonotus obliquus* (chaga)—with an emphasis on evidence from *in vitro*, animal, and human studies. While *T. versicolor* is the focal point, findings from other species provide a broader context for mushroom-based therapies. A recent review, published in the *International Journal of Molecular Sciences* in 2022, highlights several anticancer mechanisms, targeting P-glycoprotein (P-gp)-mediated multidrug resistance, immune checkpoints (e.g., programmed cell death protein 1 [PD-1]/programmed death-ligand 1 [PD-L1] and cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]/cluster of differentiation 80 [CD80; also known as B7-1]), and signaling pathways (e.g., phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), Wnt/β-catenin, mitogen-activated protein kinase [MAPK], and nuclear factor kappa-light-chain-enhancer of activated B cells [NF-κB]), while bolstering immunomodulation.[30] To clarify their contributions, these mechanisms were organized into subsections that reflect either direct antitumor effects, targeting cancer cell survival and progression, or immune enhancement effects, boosting immune responses, with signaling pathways and immunomodulation integrated across both categories.

2.1. Suppression of multidrug resistance

P-glycoprotein, also known as multidrug resistance protein 1, encoded by the *ABCB1* gene, is an ATP-dependent efflux pump that contributes to multidrug resistance by expelling chemotherapeutic agents from cancer cells, reducing their intracellular concentration and efficacy. Multidrug resistance is a major challenge in cancer treatment, and inhibiting P-gp is a strategy to enhance drug effectiveness. Medicinal mushrooms, known for their bioactive compounds, have been studied for various therapeutic properties, including potential effects on cancer and drug resistance. Some medicinal mushrooms can inhibit P-gp to restore drug sensitivity. For example, extracts of several species of *Ganoderma* have been shown to induce apoptosis in both drug-sensitive (H69) and multidrug-resistant (VPA) human small-cell lung cancer cells, reducing the IC_{50} of etoposide and doxorubicin.[31] In addition, polysaccharides from *G. lucidum* (GL-PS) suppress the persistent activation of NF-κB, leading to a decrease in the expression of P-gp in cancer cells.[30,32] Similarly, two edible mushrooms, *Agaricus bisporus* and *Pleurotus ostreatus*, have been studied for their tryptophan

derivatives, specifically 5-hydroxy-*L*-tryptophan (5-HTP) and *L*-tryptophan (*L*-Trp). A 2021 study demonstrated that these compounds inhibit P-gp, with 5-HTP achieving 82% dye accumulation and *L*-Trp 47%.[33] Docking analysis revealed that serotonin, a 5-HTP metabolite, binds to P-gp with a minimum binding energy of −83.93 kcal/mol, while *L*-Trp shows a minimum binding energy of −64.38 kcal/mol. This suggests their potential as natural adjuvants to enhance chemotherapy by overcoming multidrug resistance. However, the study's *in vitro* focus and absence of *in vivo* or clinical cancer data limit immediate applicability, and bioavailability remains untested.

2.2. Blockade of immune checkpoints

Medicinal mushrooms can also enhance immune responses by inhibiting checkpoints that suppress T-cell activity, countering cancer immune evasion. β-glucan, a high-molecular-weight polysaccharide, serves as a significant pathogen-associated molecular pattern present in fungi, bacteria, and several other species,[34] enhancing both innate and adaptive immune responses. When used alongside PD-1/PD-L1 checkpoint-blocking antibodies, whole glucan particle β-glucan improves immune cell recruitment, balances T cell activation and tolerance, and delays tumor progression. This combination therapy has been shown to increase PFS in advanced cancer patients who previously discontinued anti-PD-1/PD-L1 therapy.[35] This finding is particularly relevant to MSI-H or dMMR tumors in CRC, where immunotherapy with agents, such as pembrolizumab—an anti-PD-1 antibody that inhibits the PD-1 receptor on T-cells, thereby enhancing the immune response against cancer cells—has demonstrated significant improvements in PFS compared to chemotherapy (median survival: 16.5 vs. 8.2 months; KEYNOTE-177 trial).[36] In addition, *G. lucidum* and its bioactive compounds can lower PD-1 protein levels in cultured human B-lymphocytes, indicating their potential for cancer prevention and treatment through immune modulation,[37] whereas PSP from *T. versicolor* inhibits CRC cell proliferation. Using human CRC cell lines (HCT116 and HT29), a 2022 study demonstrated that PSP significantly reduces cell growth by downregulating the expression of EGFR and PD-L1, along with key signaling molecules, such as signal transducer and activator of transcription 3 and c-Jun.[38] Moreover, PSP also enhanced T-cell-mediated killing of CRC cells by lowering PD-L1 levels, suggesting its potential as a prophylactic and therapeutic agent against CRC through immune modulation and EGFR pathway inhibition. Meanwhile, extracts from *I. obliquus* have also been shown to block the interactions between CTLA-4 and CD80, increasing T-cell activity to prevent immune evasion.[39]

2.3. Regulation of signaling pathways

Regulation of signaling pathways plays a dual role in cancer treatment. It contributes to direct antitumor effects by inducing apoptosis and inhibiting proliferation through pathways, such as PI3K/AKT, NF-κB, and Wnt/β-catenin. In addition, it enhances immune responses by modulating PD-L1 expression and immune cell infiltration through signaling, such as the MAPK pathway. Numerous signaling pathways are versatile, influencing both cancer and immune cells depending on the context.

Polysaccharides derived from edible and medicinal mushrooms enhance immune function and interact with various cell signaling pathways involved in cancer development, including NF-κB, MAPK, and PI3K/Akt. Given the limitations of present cancer treatments, these polysaccharides present a promising alternative due to their natural origin and low toxicity.[40] The PI3K/AKT pathway promotes cancer cell survival and resistance; hence, its inhibition induces apoptosis and sensitizes cells to therapy. It has been shown that ganoderic acid inactivates PI3K/AKT signaling, inducing apoptosis and autophagy in human glioblastoma cells.[41] Similarly, dysregulated Wnt/β-catenin signaling drives tumor growth and metastasis, and its inhibition reduces proliferation and enhances immune infiltration. Earlier studies demonstrated that *I. obliquus*-derived ergosterol peroxide reduces nuclear β-catenin in CRC cells, decreasing the expression of target genes, including *MYC*, *CCND1*, *CDK8*, and *VEGFA*.[42,43] The MAPK pathway contributes to oncogenesis and resistance to PD-1 blockade; therefore, its suppression enhances chemosensitivity and inhibits tumor growth. Previous research has found that the β-glucan lentinan, derived from *L. edodes*, suppresses cisplatin-induced PD-L1 expression through MAPK inhibition, restoring chemosensitivity in gastric cancer cells.[44] In addition, triterpene extracts from *G. lucidum* inhibit p38 MAPK phosphorylation, inducing autophagy in colon cancer cells.[45] Similarly, small molecules from *T. versicolor* inhibit the production of matrix metalloproteinase 3 (MMP-3), an enzyme that can facilitate tumor invasion and metastasis. This inhibition occurs indirectly by inactivating the p38 MAPK pathway.[46] It has also been reported that NF-κB promotes chemoresistance and tumor survival, and its inhibition induces apoptosis, thereby enhancing therapeutic efficacy. As mentioned earlier, GL-PS inhibits NF-κB, reducing P-gp expression.[32] Moreover, sulfated polysaccharides from *G. frondosa* induce apoptosis in HepG2 liver cancer cells by inhibiting Notch1 expression, preventing IκB-α degradation, blocking NF-κB/p65 translocation to the nucleus, and activating caspases 3 and 8, suggesting a Notch1/NF-κB/p65-mediated apoptotic pathway.[47]

2.4. Immunomodulation

Immunomodulation primarily enhances immune responses by stimulating immune cell activity and cytokine production through pathways, such as toll-like receptor (TLR) signaling and dectin-1. Some immunomodulatory compounds found in medicinal mushrooms directly inhibit cancer cell invasion, for example, by impairing the migratory capabilities of malignant cell lines. In relation to the immunomodulatory effects of medicinal mushrooms, *G. lucidum*-derived β-glucans and triterpenoids protect normal cells, reduce fatigue, and improve symptoms in patients with lung and breast cancer.[48,49] Similarly, extracts from *G. frondosa* have been shown to enhance the activity of natural killer (NK) cells and stimulate the production of various cytokines, including interferon (IFN)-γ and interleukin (IL)-12. A Phase I/II clinical trial involving breast cancer survivors demonstrated that oral administration of *G. frondosa* extract was well tolerated and resulted in significant immunological changes, with an optimal dose of 5–7 mg/kg/day associated with increased production of tumor necrosis factor (TNF)-α and both stimulatory (e.g., IL-2 and IFN-γ) and suppressive (e.g., IL-10) cytokines.[50] These findings suggest that *G. frondosa* extract may modulate immune responses, potentially benefiting cancer patients, although further research is needed to clarify its clinical significance and optimal dosing. Meanwhile, aqueous extracts of *L. edodes* can inhibit the proliferation of human breast cancer cells while enhancing the proliferation of rat thymocytes, indicating its potential as an immunostimulant.[51] Specifically, lentinan enhances cytotoxic T lymphocytes and macrophage proliferation.[52] Polysaccharides from *A. blazei* mitigate chemotherapy side effects in gynecological cancer patients receiving chemotherapy[53] and increase dendritic and T-regulatory cells in myeloma patients.[54]

Extracts from *T. versicolor*, especially PSP and PSK, exhibit significant immunomodulatory effects, enhancing antitumor immunity. PSP induces a pro-inflammatory cytokine profile, including TNF-α, IL-1β, IL-12, and IL-6, via pathways involving TLR2, Dectin-1, and TLR4, as evidenced by pre-clinical studies.[55] Notably, PSP increased TNF-α secretion >3.5-fold in human PBMCs *in vitro*,[56] demonstrating potent immunostimulation. Whereas under *in vivo* conditions, PSP-treated mice with H238-derived tumors showed elevated TNF-α expression in tumor specimens,[57] linking cytokine induction to tumor microenvironments and potential tumor necrosis effects. These findings underscore PSP's capacity to activate NK cells and overcome tolerogenic barriers, supporting its clinical use as an adjunct cancer therapy in Eastern cultures.

In contrast, small molecules derived from *T. versicolor* demonstrate significant immunomodulatory properties by inhibiting the production of TNF-α induced by lipopolysaccharide (LPS) while enhancing the levels of IFN-β induced by polyinosinic: polycytidylic acid in peripheral blood mononuclear adherent cells. These compounds exhibit both indirect anticancer effects by reducing MMP-3 production triggered by TNF-α in glioblastoma T98G cells and direct effects by impairing the invasive capabilities of various malignant cell lines, including T98G, A549, and MDA-MB-231.[46] The disruption of pro-inflammatory tumorigenic factors, as well as the anti-migratory effects of *T. versicolor* extract, have also been demonstrated in a wound healing assay, with a reduced production of IL-6, IL-8, and MMP-9 in cells stimulated by LPS, indicating the microenvironment-dependent modulatory capacity of these active compounds.[58] A small clinical trial involving 21 patients with advanced gastric cancer indicates that PSK can enhance OS, in part by inhibiting $CD57^+$ T cells, which are associated with a poor prognosis in this disease.[59] In another clinical trial, 30 rectal adenocarcinoma patients were randomly assigned to receive either standard chemoradiotherapy (CRT) or CRT with concurrent PSK administration. The findings indicated that the PSK group experienced a notable rise in NK cell counts in peripheral blood and cytotoxic T-cell counts in both peri-tumoral and normal mucosa, accompanied by a reduction in serum levels of immunosuppressive acidic protein. These findings suggest that combining PSK with pre-operative CRT may enhance immune function and reduce local recurrence, leading to improved survival outcomes.[60]

2.5. Induction of cell cycle arrest

Regarding cell cycle arrest, a critical mechanism for controlling cancer cell proliferation, research suggests that *T. versicolor* extracts can induce cell cycle arrest at various phases, impacting cancer cell division. PSP extracted from *T. versicolor* has been shown to induce S-phase cell arrest in HL-60 leukemia cells, enhancing the cell cycle-dependent activity and apoptotic cell death when combined with chemotherapy drugs, such as doxorubicin and etoposide.[61] The Memorial Sloan Kettering Cancer Center reported that *T. versicolor* blend inhibited cell proliferation and induced G2/M cell cycle arrest in an invasive breast cancer cell line, linked to suppression of urokinase plasminogen activator.[62,63] Another study noted that polysaccharides from *T. versicolor* induced cell cycle arrest at concentrations as low as 100 μg/mL *in vitro*, a significant effect given their large molecular weight.[64] These findings indicate that *T. versicolor* extracts potentially affect cell cycle regulatory genes, although the exact mechanisms may vary by cancer type and extract formulation.

Apoptosis induction, or programmed cell death, is a key strategy for eliminating cancer cells. Evidence leans toward *T. versicolor* extracts promoting apoptosis through multiple pathways. One example has already been highlighted in relation to cell cycle arrest, as these two mechanisms are interrelated.[61] Similarly, a commercially available PSP water extract has been shown to modulate apoptogenic and anti-apoptotic protein expression, inducing cell cycle changes and apoptosis in human leukemia HL-60 and U-937 cells.[65]

2.6. Anti-angiogenic properties

Anti-angiogenic effects involve inhibiting new blood vessel formation, which is essential for tumor growth and metastasis. *T. versicolor* extracts can impact angiogenesis by reducing pro-angiogenic factors. An *in vitro* study found that protein-bound polysaccharides from *T. versicolor* decreased the secretion of VEGF and monocyte chemoattractant protein-1 by breast cancer cells, thereby disrupting the tumor microenvironment and potentially inhibiting angiogenesis.[66] Similarly, an *in vivo* study directly showed that fungal PSP inhibited tumor angiogenesis in mice, reducing blood vessel growth and tumor expansion.[67]

2.7. Antimetastatic effects

Antimetastatic effects primarily target cancer cells directly by inhibiting invasion, migration, and angiogenesis, while also enhancing immune responses by activating immune cells. The protein-bound PSK derived from cultured mycelia of *T. versicolor* exhibits significant antimetastatic properties, as explored in a 1995 review by Kobayashi *et al*.[68] PSK, recognized for its significant role in inhibiting the early stages of cancer development, also demonstrates efficacy in impeding the dissemination of established tumors. Research indicates that PSK reduces metastasis in chemically induced sarcomas and the human prostate cancer cell line DU145M, as well as in lymphatic spread observed in murine leukemia P388, while concurrently enhancing survival rates in models of spontaneous metastasis. Furthermore, PSK has been shown to attenuate metastatic progression in controlled experimental settings involving rat hepatoma AH60C, mouse colon cancer (Colon 26), and mouse leukemia (RL male 1). The mechanisms underlying these effects are multifaceted—PSK inhibits tumor cell entry into the bloodstream by limiting invasion and adhesion, disrupts the binding of tumor cells to vascular endothelium by reducing platelet aggregation, restricts the motility of cancer cells post-circulation, and impedes growth at metastatic sites by obstructing angiogenesis, modulating immune signaling, and enhancing the activity of immune cells. In addition, PSK exhibits the capacity to neutralize deleterious superoxide radicals, thereby attenuating tumor aggressiveness in murine models. These properties highlight PSK's broad potential to combat metastasis, enhancing its value in cancer therapy. The review by Kobayashi *et al*.[68] also highlights the results from several murine models of LM, where suppression of liver lesions is observed after the administration of PSK. It also includes findings in mouse colon cancer (Colon 26), where a prolongation of the survival period is noted.[68-70]

Complementing this, a 2014 study examined the effects of aqueous extract from *T. versicolor* on metastatic breast cancer using the 4T1 mouse model.[71] Administering the extract at a daily dose of 1 g/kg for 4 weeks led to a 36% decrease in tumor weight and a 70.8% reduction in lung metastasis. In addition, the extract demonstrated protective effects on bone health, as indicated by increased bone volume observed in micro-computed tomography imaging. *In vitro* experiments revealed that the extract (at concentrations of 1–2 mg/mL) effectively inhibited the migration and invasion of 4T1 cells, significantly reducing MMP-9 activity. Furthermore, it enhanced immune function by increasing the production of cytokines, such as IL-2, IL-6, IL-12, TNF-α, and IFN-γ in spleen lymphocytes. Meanwhile, another study examined the direct effects of PSK on human colorectal adenocarcinoma cells (SW480 and HCT116) using protein microarrays.[72] After 96 h of PSK exposure, 14 proteins showed significant alterations, with ECA39 identified as a key candidate associated with PSK's antimetastatic effects. Together, these findings position *T. versicolor* as a promising agent against metastasis, targeting invasion, migration, angiogenesis, and immune modulation.

2.8. Clinical evidence and broader benefits

In the systematic review and meta-analysis by Zhong *et al*.,[73] the synergistic potential of *T. versicolor* and *G. lucidum* as adjunctive agents in cancer treatment was rigorously evaluated. This study, encompassing 23 RCTs and 4,246 cancer patients, demonstrated that natural products derived from these medicinal mushrooms significantly enhance therapeutic outcomes when combined with conventional cancer treatments. The meta-analysis revealed a reduced mortality risk (hazard ratio [HR]: 0.82; 95% CI: 0.72–0.94) and improved total efficacy (risk ratio [RR]: 1.30; 95% CI: 1.09–1.55) compared to control treatments, alongside immunomodulatory benefits, including elevated CD3 (mean difference [MD]: 9.03%; 95% CI: 2.10–16.50%) and CD4 (MD: 9.2%; 95% CI: 1.01–17.39%) levels. These effects are attributed to the synergistic action of bioactive compounds, such as polysaccharides (e.g., PSP and PSK) from *T. versicolor* and β-glucans and triterpenes from *G. lucidum*, all of which collectively enhance immune response and mitigate immunosuppression associated

with chemotherapy and radiotherapy (RT). In addition, this combination therapy improved patients' quality of life with minimal adverse effects, underscoring its safety profile and offering a compelling foundation for integrating medicinal mushrooms into comprehensive cancer care strategies. While these findings positioned *T. versicolor* and *G. lucidum* as promising adjunctive agents across various cancer stages and types, the present study highlights the need for further large-sample-size, high-quality RCTs across different continents.

3. Medicinal mushrooms as adjunctive agents in cancer treatment: a summary

Medicinal mushrooms have emerged as valuable adjunctive agents in cancer management, particularly due to their diverse bioactive compounds. Notably, *T. versicolor* and *G. lucidum* have been extensively studied for their potential to enhance conventional cancer treatments. These fungi exhibit various mechanisms that contribute to their anticancer effects as potent biological response modifiers, as summarized in Table 2.

Certain mushroom extracts have been shown to counteract multidrug resistance by inhibiting P-gp, thereby increasing the effectiveness of chemotherapeutic agents. This property is particularly beneficial in overcoming resistance in cancer cells. The effects are mediated through several mechanisms:

(i) Immune system activation: The ability of these mushrooms to modulate immune responses is significant. They can inhibit immune checkpoint proteins, allowing for a more robust T-cell activity against tumors. This immunological enhancement can complement existing immunotherapies, improving patient outcomes.

(ii) Influence on cellular signaling: Bioactive compounds from medicinal mushrooms interact with critical cellular signaling pathways that regulate cancer cell survival and proliferation. By modulating these pathways, they can induce cell death and enhance the sensitivity of cancer cells to treatment.

(iii) Support for immune function: Extracts from various mushroom species have demonstrated the capacity to boost immune function, leading to increased production of cytokines and improved overall health in cancer patients. This support can alleviate treatment-related side effects and enhance quality of life.

(iv) Cell cycle regulation and apoptosis: Research indicates that certain mushroom extracts can induce cell cycle arrest and promote programmed cell death in cancer cells, further contributing to their therapeutic potential.

(v) Angiogenesis inhibition: By reducing the secretion of pro-angiogenic factors, medicinal mushrooms can disrupt the formation of new blood vessels that tumors rely on for growth and metastasis.

(vi) Antimetastatic effects: The protein-bound polysaccharides PSP and PSK from *T. versicolor* exhibit significant antimetastatic properties, limiting tumor spread and enhancing survival in various cancer models. In addition, extracts from *T. versicolor* have shown potential in reducing tumor weight and metastasis while optimizing immunological health.

Clinical studies have highlighted the synergistic effects of combining these mushroom extracts with standard cancer therapies, leading to improved survival rates and better therapeutic responses. However, further research is essential to establish standardized treatment protocols and confirm the clinical benefits of these natural products. The integration of medicinal mushrooms into cancer treatment regimens offers a promising strategy for enhancing therapeutic efficacy and patient well-being.

4. Review of human studies on medicinal mushroom compounds in cancer patients

A thorough investigation of the available literature has been conducted to assess the safety and efficacy of medicinal mushroom compounds in cancer patients, specifically focusing on *T. versicolor* and CRC. This investigation utilized several regularly updated review summaries on the subject, including those from the Memorial Sloan Kettering Cancer Center,[62] the Whole Health Library website from the United States Department of Veterans Affairs,[74] and the Physician Data Query summary provided by the National Cancer Institute.[75] These review summaries are comprehensive, evidence-based resources that offer information on various aspects of cancer, including prevention, diagnosis, treatment, and supportive care.

4.1. *G. lucidum (reishi), G. frondosa* (maitake) D-fraction, *L. edodes* extract (lentinan), and active hexose correlated compound (AHCC) (derived from various Basidiomycetes)

The use of bioactive compounds derived from *G. lucidum* as adjuncts to chemotherapy has long been suggested.[76] Patients receiving *G. lucidum* extracts in combination with chemotherapy and/or RT were found to have 1.27 times higher likelihood of responding compared to those receiving conventional treatment alone, according to a systematic review by the Cochrane Library, last updated in 2016.[77,78] An improvement in quality of life and a stimulation of host immune-mediated responses were observed, with a marked increase in CD3, CD4, and CD8

Table 2. Medicinal mushrooms and their anticancer mechanisms

Mushroom species	Mechanism of action	Details
Trametes versicolor (turkey tail)	Suppression of multidrug resistance	Small molecules reduce MMP-3 production via p38 MAPK inactivation. Reducing tumor invasiveness may decrease the selective pressure for multidrug resistance development[46]
	Blockade of immune checkpoints	PSP downregulates PD-L1 and EGFR, enhancing T-cell-mediated killing of CRC cells[38]
	Regulation of signaling pathways	Small molecules inhibit p38 MAPK, reducing MMP-3 production[46]
	Immunomodulation	PSP and PSK enhance antitumor immunity, increasing TNF-α, IL-1β, IL-12, and IL-6 via TLR2, Dectin-1, and partially TLR4 pathways.[55-57] Small molecules inhibit LPS-induced TNF-α while enhancing IFN-β.[46] Disruption of pro-inflammatory tumorigenic factors with a reduced production of IL-6, IL-8, and MMP-9 in cells stimulated by LPS, indicating microenvironment-dependent modulatory capacity.[58] PSK enhances NK and cytotoxic T-cell counts, reducing immunosuppressive acidic protein in rectal cancer patients[60]
	Induction of cell cycle arrest	PSP induces S-phase arrest in HL-60 leukemia cells in combination with chemotherapy drugs.[61] Blend induces G2/M arrest in breast cancer cells, suppressing urokinase plasminogen activator.[62,63] Polysaccharides achieve arrest at 100 μg/mL[64]
	Apoptosis induction	PSP modulates apoptogenic/anti-apoptotic proteins, inducing apoptosis in human leukemia HL-60 and U-937 cells[65]
	Anti-angiogenic properties	Protein-bound polysaccharides reduce VEGF and MCP-1 secretion in breast cancer cells.[66] PSP inhibits tumor angiogenesis in mice[67]
	Antimetastatic effects	PSK reduces metastasis in sarcomas, prostate (DU145M), leukemia (P388, RL male 1), hepatoma (AH60C), and colon cancer (colon 26) by limiting invasion, adhesion, motility, and angiogenesis, enhancing immunity, and neutralizing superoxide.[68] Aqueous extract (1 g/kg, 4 weeks) reduces tumor weight by 36% and lung metastasis by 70.8%, while boosting cytokines (IL-2, IL-6, IL-12, TNF-α, and IFN-γ) in spleen lymphocytes; inhibits 4T1 cell migration/invasion (1–2 mg/mL) *in vitro*; and suppresses MMP-9.[71] PSK alters ECA39 in CRC cell lines (SW480, HCT116)[72]
Ganoderma lucidum (*reishi*)	Suppression of multidrug resistance	Induces apoptosis in drug-sensitive (H69) and multidrug-resistant (VPA) small-cell lung cancer cells.[31] Polysaccharide from *G. lucidum* inhibits NF-κB, reducing P-gp expression[32]
	Blockade of immune checkpoints	Lowers PD-1 protein levels in B-lymphocytes[37]
	Regulation of signaling pathways	Ganoderic acid inactivates PI3K/AKT, inducing apoptosis/autophagy in glioblastoma cells.[41] Triterpenes inhibit p38 MAPK phosphorylation, inducing autophagy in colon cancer cells.[45] Polysaccharides inhibit NF-κB, reducing P-gp[32]
	Immunomodulation	β-Glucans and triterpenoids protect normal cells, reduce fatigue, and improve symptoms in lung/breast cancer patients.[48,49]
Grifola frondosa (maitake)	Regulation of signaling pathways	Sulfated polysaccharides inhibit Notch1/NF-κB/p65 pathway, inducing apoptosis in HepG2 cells via caspase 3/8 activation[47]
	Immunomodulation	Enhances NK cell activity and cytokine production (e.g., IFN-γ and IL-12). Phase I/II trial shows increased TNF-α, IL-2, IFN-γ, and IL-10 at 5–7 mg/kg/day in breast cancer survivors[50]
Lentinula edodes (shiitake)	Regulation of signaling pathways	Lentinan suppresses MAPK, reducing cisplatin-induced PD-L1 expression and restoring chemosensitivity in gastric cancer cells[44]
	Immunomodulation	Aqueous extracts inhibit breast cancer cell proliferation and enhance thymocyte proliferation.[51] Lentinan boosts cytotoxic T lymphocytes and macrophage proliferation[52]
Agaricus blazei (sun mushroom)	Suppression of multidrug resistance	Tryptophan derivatives (5-HTP and *L*-tryptophan) inhibit P-gp, with 5-HTP achieving 82% dye accumulation[33]; results from related species *Agaricus bisporus*
	Immunomodulation	Polysaccharides mitigate chemotherapy side effects in gynecological cancers[53] and increase dendritic/T-regulatory cells in myeloma patients[54]
Inonotus obliquus (chaga)	Blockade of immune checkpoints	Blocks CTLA-4/CD80 interaction, increasing T-cell activity[39]
	Regulation of signaling pathways	Ergosterol peroxide reduces Wnt/β-catenin signaling, decreasing *MYC*, *CCND1*, *CDK8*, and *VEGFA* in CRC cells[42,43]

Abbreviations: 5-HTP: 5-Hydroxy-*L*-tryptophan; AKT: Protein kinase B; CD80: Cluster of differentiation 80; CRC: Colorectal cancer; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; EGFR: Epidermal growth factor receptor; IFN: Interferon; IL: Interleukin; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MCP-1: Monocyte chemoattractant protein 1; MMP-3: Matrix metalloproteinase 3; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NK cell: Natural killer cell; P-gp: P-glycoprotein; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; PI3K: Phosphoinositide 3-kinase; PSK: Polysaccharide krestin; PSP: Polysaccharopeptide; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

lymphocytes and a slight increase in NK cells. Notably, a few cases of minor adverse effects, including nausea and insomnia, were reported.

At present, there is an ongoing RCT sponsored by the Second Affiliated Hospital, Zhejiang University,[79] investigating the potential of *G. lucidum* spore powder as an adjunctive agent for osteosarcoma, a cancer with a low 5-year survival rate of <20%. This Phase II trial evaluates the efficacy of *G. lucidum* combined with doxorubicin and cisplatin chemotherapy in a multicenter, double-blind design. Previous research indicates that *G. lucidum* may inhibit tumor growth and metastasis without significant toxicity, supporting its use in combination therapy. For example, Japanese researchers investigated a water-soluble extract (MAK) from the mycelia of *G. lucidum* in patients with colorectal adenomas.[80] The study involved 123 patients receiving 1.5 g/day of MAK for 12 months, compared to 102 control patients who received no treatment. After 12 months, the treatment group showed a significant reduction in the number of adenomas (−0.42 ± 0.10) and a decrease in total adenoma size (−1.40 ± 0.64 mm). In contrast, the control group experienced an increase in both the number of adenomas (0.66 ± 0.10) and size (1.73 ± 0.28 mm) (p<0.01). These findings suggest that MAK may help suppress the development of premalignant colorectal adenomas. On the other hand, a *Ganoderma sinense* polysaccharide tablet, from a related species, has been approved in China since 2010 by the State Food and Drug Administration as an adjunctive therapy for addressing leukopenia and hematopoietic damage resulting from concurrent chemotherapy and radiation therapy.[81]

D-fraction from *G. frondosa*, a protein-bound polysaccharide containing β-glucans and protein, has demonstrated an improved response rate to chemotherapy, increasing from 12% to 28% in a Japanese non-randomized clinical study involving 165 patients suffering from various types of advanced cancers.[82,83] In a non-randomized case series, the effectiveness of a combination of *G. frondosa* D-fraction and whole *G. frondosa* powder was evaluated in cancer patients aged 22 to 57 with stages II–IV cancer.[84] The study found that 58.3% of liver cancer patients, 68.8% of breast cancer patients, and 62.5% of lung cancer patients experienced cancer regression or significant symptom improvement. In contrast, leukemia, stomach cancer, and brain cancer patients showed only a 10–20% improvement. In addition, the combination of *G. frondosa* extracts with chemotherapy enhanced immune-competent cell activities by 1.2–1.4 times compared to chemotherapy alone.

Lentinan, a polysaccharide extracted from the *L. edodes* mushroom, is recognized for its antitumor and immunostimulatory properties and is commonly used in cancer treatment. It has been approved as an adjuvant agent for cancer treatment in both China and Japan since the 1980s. An extensive review of over 9,474 patients from 135 independent studies in Chinese databases has summarized lentinan-associated cancer treatment cases over the past 12 years (2004–2016), utilizing data from China National Knowledge Infrastructure (CNKI), Chongqing VIP Chinese Scientific Journals Database (VIP), and Wanfang databases.[85] The results demonstrated clear improvements in quality of life and enhanced effects of chemotherapy and radiation therapy.

AHCC is a functional food supplement developed at the Faculty of Pharmaceutical Sciences, University of Tokyo, by Dr. Toshihiko Okamoto, in collaboration with researchers at Amino Up Chemical Co., Ltd. in Japan.[86] The primary component of AHCC is *L. edodes*, known for its long-standing reputation for healing properties. In addition, it contains various hybrids from the Basidiomycota phylum of fungi. A small randomized controlled study was conducted with 44 patients who had histologically confirmed advanced liver cancer, all receiving supportive care.[87] Among them, 34 patients received AHCC, while 10 received a placebo. The results indicated that patients treated with AHCC experienced significantly prolonged survival compared to the control group. In addition, quality of life improved in areas, such as mental stability and physical health after 3 months of treatment. Key clinical parameters, including albumin levels and lymphocyte percentages, also showed significant differences favoring the AHCC group. The study suggests that AHCC may enhance survival and prognosis in advanced liver cancer patients. Similarly, a pilot study examined the effects of AHCC in 11 patients with advanced cancer, including three each with prostate, breast, or ovarian cancer, and two with multiple myeloma.[88] All patients received conventional therapies alongside 3 g AHCC daily. After 2 weeks, 9 of the 11 patients exhibited a 2.5-fold increase in NK cell activity that sustained over time. In addition, the three prostate cancer patients showed a decline in prostate-specific antigen levels, indicating reduced malignancy. Normal levels were reached in 1–2 months post-treatment. Among the breast cancer patients, two experienced a significant decrease in CA-125 levels, with levels returning to normal 3–4 months after starting AHCC treatment. This study serves as a preliminary exploration of AHCC's potential effects in cancer therapy. Interestingly, encouraging results were obtained from a prospective cohort study of 269 hepatocellular carcinoma patients.[89] Of these, 113 received oral AHCC after curative surgery, showing a significantly prolonged recurrence-free period (HR = 0.639; 95% CI: 0.429–0.952; p=0.0277) and improved OS (HR = 0.421; 95% CI: 0.253–0.701; p=0.0009) compared to the control group.

4.2. *Coriolus/T. versicolor (turkey tail)*

With a long history in traditional Chinese medicine, *T. versicolor* is a medicinal fungus and is referred to as "*Yun Zhi*" in China. It is recognized for its immune-modulating properties, primarily through PSK and PSP, both of which are polysaccharides extracted from its mycelia. These extracts have been extensively researched for their potential as adjunctive agents in cancer therapies, enhancing the efficacy of conventional treatments, including chemotherapy and RT, while reducing adverse effects. In Asia, particularly Japan and China, these extracts have been integrated into clinical practices, with PSK approved for use in Japan since 1977 and PSP approved in China since 1987—when PSK's annual sales in Japan reached $357 million.[90,91] The PSP utilized in China is primarily derived from the COV-1 strain, while the PSK used in Japan comes from the CM-101 strain of *T. versicolor*. This review notes that PSK and PSP are used as adjuvant agents, with significant research from Asia focusing on colorectal, gastric, esophageal, lung, liver, and various advanced cancers.

A review of pre-clinical and clinical studies conducted in China, extracted from the Chinese VIP, CNKI, and Wanfang databases, concluded that PSP is an effective antitumor agent with minimal side effects, regardless of its administration method.[91] It helps alleviate adverse reactions from chemotherapy, such as bone marrow suppression, and significantly enhances patients' quality of life. A total of seven independent clinical studies were analyzed in this review, with one study from Japan, indicating that PSP has curative effects on various advanced cancers, including CRC, gastric, non-small cell lung, and primary liver cancers. It is commonly used in combination with chemotherapeutic drugs, such as XELOX (oxaliplatin + Xeloda) for advanced CRC, to reduce side effects, improve tolerance to treatment, and potentially prolong life.

Moreover, a systematic review and meta-analysis evaluated the efficacy of *T. versicolor* on the survival of cancer patients.[92] Analyzing data from 13 randomized, placebo-controlled trials, the study found that *T. versicolor* significantly improved survival rates compared to standard anticancer treatments alone, with a 9% absolute reduction in 5-year mortality. The effects on the overall 5-year survival rate were particularly notable in patients with breast cancer, gastric cancer, and CRC undergoing chemotherapy, while not so evident in esophageal and nasopharyngeal cancers. The analysis suggests that *T. versicolor* may enhance survival in certain cancer types, but further prospective studies are needed to optimize treatment protocols.

The 2015 systematic review by Fritz *et al.*[93] highlighted the promising potential of PSK, derived from the *T. versicolor* mushroom, as an adjuvant immunotherapy for lung cancer. Despite its established use in Japan, the review filled a significant gap by synthesizing English-language evidence on PSK's effects. The analysis included 31 reports from 28 studies, comprising six RCTs, five non-RCTs, and 17 pre-clinical studies. The majority of pre-clinical studies (15 out of 17) demonstrated PSK's anticancer effects through immunomodulation and direct tumor inhibition, while non-RCTs showed improvements in various survival metrics. Most RCTs reported beneficial impacts on immune parameters, tumor-related symptoms, and OS. Overall, the findings suggest that PSK may enhance immune function, reduce symptoms associated with tumors, and extend survival in lung cancer patients, underscoring the need for larger, more rigorous trials to further validate these encouraging results.

The need for further assessment and evaluation of relevant clinical data has also been highlighted in a 2022 systematic review published in the Cochrane Library, focusing on the use of PSK in CRC patients.[94] Although the report indicates that there is evidence suggesting a small effect on OS at 5 years, with an absolute risk reduction of 6% (95% CI: 1–11%), the impact of reduced adverse effects on this outcome could not be determined. Unfortunately, imprecision due to small sample sizes and indirectness related to the unknown relevance to present therapy standards downgraded the quality of evidence to "low-certainty," following the guidelines from the Grading of Recommendations Assessment, Development, and Evaluation system.[95]

Previous attempts to address the uncertainty in the field include a 2006 meta-analysis comprising 8,009 gastric cancer patients from eight RCTs, conducted by the Department of Epidemiological and Clinical Research Information Management, Kyoto University Graduate School of Medicine, to assess survival benefits after PSK adjunctive treatment.[96] The overall HR for eligible patients was 0.88 (95% CI: 0.79–0.98; p=0.018), with no significant heterogeneity observed (χ^2 [8] = 11.7; p=0.16). These findings indicate that adjuvant immunochemotherapy using PSK enhances the survival of patients following curative resection for gastric cancer. Another meta-analysis from the same year, with a focus on curatively resected CRC, reassessed the benefits of immunochemotherapy using the biological response modifier PSK.[97] This analysis included data from 1,094 patients enrolled in three clinical trials, all of whom were followed for at least 5 years post-surgery. The study compared outcomes of standard chemotherapy with chemotherapy + PSK, evaluating OS and disease-free survival through intent-to-treat analysis. The results indicated an OS RR of 0.71 (95% CI: 0.55–0.90; p=0.006) and a disease-free survival RR of 0.72 (95% CI:

0.58–0.90; p=0.003). These findings suggest that adjuvant immunochemotherapy with PSK can significantly improve both overall and disease-free survival in patients with curatively resected CRC.

In relation to CRC patients, two observational studies have reported significant results. In the retrospective analysis of 63 patients, 39 received post-operative uracil/tegafur (UFT) alone (control group) and 24 were treated with UFT + PSK (PSK group).[98] The results showed a significant improvement in the 3-year relapse-free survival rate, with 76.2% in the PSK group compared to 47.8% in the control group (p=0.041). In addition, the OS rate was notably higher in the PSK group at 80.8%, versus 52.8% in the control group (p=0.0498). Subset analyses indicated particularly favorable outcomes for patients with colon tumors and those with lower pre-operative lymphocyte percentages. Adverse drug reactions were minimal, with all reported reactions being grade 2 or lower. The second study by Sakai *et al.*[99] evaluated the long-term survival benefits of combining PSK with oral fluoropyrimidines in patients with curatively resected CRC. In this retrospective analysis, the 10-year OS rate for the PSK group was significantly higher at 81.9%, compared to 50.6% in the control group receiving only fluoropyrimidines. Notably, in Dukes' C cases, the PSK group also demonstrated superior survival rates. For patients with lymphatic invasion graded ly2–ly3 or venous invasion graded v2–v3, the PSK group achieved a 10-year survival rate of 80.6%, markedly better than the 25.9% in the control group. Cox's proportional hazard model analysis further confirmed a significant difference in prognosis between the two groups. The findings suggest that post-operative adjuvant immunochemotherapy with PSK significantly enhances long-term survival, particularly for patients with advanced disease features, and the authors recommend its use in these cases.

4.3. Efficacy of adjuvant immunochemotherapy with PSP/PSK: a meta-analysis of selected RCTs

This section highlights the key findings from the meta-analysis conducted in the present study that evaluates the efficacy of PSP and PSK as adjuncts to standard chemotherapy or RT in the post-operative treatment of patients with esophageal, gastric, and colon cancer, focusing on 5-year OS (detailed methodologies and data provided in the Supplementary File Section S1). The analysis initially considered 13 RCTs[59,100-111]; however, only 11 were finally included for evaluation after excluding two studies (Tsang *et al.*,[100] and Miyake *et al.*,[101]) that did not meet the inclusion criteria, as detailed in the "Relevant studies not evaluated in the meta-analysis" section of the supplementary documentation. The evaluated studies, listed in Table 3, span gastric, colorectal, and esophageal cancers, with seven RCTs selected for the final meta-analysis based on their reporting of 5-year OS.

In the meta-analysis, to estimate the pooled relative RR of survival, the following steps were followed[112]:

(i) The RR for each study, defined as the ratio of event probabilities (p_1/p_2), was log-transformed:

$$\ln\left(RR\right)=\ln\left(\frac{p_1}{p_2}\right) \quad \text{(I)}$$

(ii) The standard error (SE) of ln(RR) was derived from the 95% CI bounds:

$$\text{SE}=\frac{\ln\left(\text{Upper CI}\right)-\ln\left(\text{Lower CI}\right)}{2\times 1.96} \quad \text{(II)}$$

(iii) The variance was calculated as the square of the SE:

$$\text{Var}((\ln(RR)) = \text{SE}^2 \quad \text{(III)}$$

(iv) Each study's weight (w_i) was computed as the inverse of the variance:

$$\text{w}_\text{i} = \frac{1}{\text{Var}\left(\ln\left(RR\right)\right)}=\frac{1}{\text{SE}^2} \quad \text{(IV)}$$

(v) The pooled estimate was obtained by a weighted average of ln(RR_i) across studies:

$$\ln\left(RR_{\text{pooled}}\right)=\frac{\sum w_i \ln\left(RR_i\right)}{\sum w_i} \quad \text{(V)}$$

(vi) Heterogeneity was assessed using:

$$Q=\sum w_i\left(\ln\left(RR_i\right)-\ln\left(RR_{\text{pooled}}\right)\right)^2 \quad \text{(VI)}$$

(vii) The between-study variance was estimated, where k is the number of studies:

$$\tau^2=\frac{Q-\left(k-1\right)}{\sum w_i-\frac{\sum w_i^2}{\sum w_i}} \quad \text{(VII)}$$

τ^2 is set to 0 if $Q < k - 1$, indicating no additional variance beyond sampling error, with I^2 statistic as:

$$I^2=\frac{Q-\left(k-1\right)}{Q}\times 100\% \quad \text{(VIII)}$$

(viii) The SE of the pooled estimate was calculated as:

Table 3. Meta-analysis of selected randomized controlled trials

Study ID	Cancer type (study design)	Endpoint	Number of patients	Risk ratio (95% CI)	Normalized weight (%)
Akagi and Baba[59]	Gastric (CT+PSK vs. CT)	3-year OS	21	4.98 (0.97–25.56)	-
Niimoto *et al.*[102]	Gastric (CT+PSK vs. CT)	5-year OS	579	The risk ratio could not be obtained due to a lack of public access[b].	-
Torisu *et al.*[103a]	Colorectal (PSK vs. PSK-P [placebo])	5-year OS	111	1.47 (0.94–2.31)	1.28
Mitomi *et al.*[104a]	Colorectal (CT+PSK vs. CT)	5-year OS	448	1.12 (1.01–1.26)	21.17
Nakazato *et al.*[105a]	Gastric (CT+PSK vs. CT)	5-year OS	262	1.22 (1.02–1.45)	8.37
Ogoshi *et al.*[106a]	Esophageal (RT+PSK vs. RT)	5-year OS	87	1.06 (0.64–1.75)	1.02
Ogoshi *et al.*[106a]	Esophageal (RT+CT+PSK vs. RT+CT)	5-year OS	87	1.28 (0.70–2.34)	0.71
Toge and Yamaguchi[107a]	Gastric (non-stratified)	5-year OS	751	1.10 (0.99–1.22)	23.73
Toge and Yamaguchi[107a]	Gastric (stratified: granulocyte/ lymphocyte≥2.0)	5-year OS	364	1.24 (1.05–1.46)	-
Ito *et al.*[108a]	Colorectal (CT+PSK vs. CT)	5-year OS	446	1.03 (0.95–1.13)	34.40
Ohwada *et al.*[109a]	Colorectal CT+PSK vs. CT)	5-year OS	205	1.13 (0.96–1.34)	9.31
Shichinohe *et al.*[110]	Colorectal (CT+PSK vs. CT)	3-year OS	256	0.98 (0.91–1.06)	-
Ogawa *et al.*[111]	Colorectal (CT+PSK vs. CT)	3-year DFS	186	1.13 (0.91–1.42)	-
Subtotal (pooled)[a]	-	5-year OS	2,397	1.10 (1.04–1.15)	100

Notes: [a]Studies that were included in the meta-analysis. [b]Survival rate significantly higher in the PSK group ($p<0.01$), with 5-year survival significantly increased ($p<0.05$).[102] The hazard ratio for overall survival is reported in the study by Zhong *et al.*[73]
Abbreviations: CT: Chemotherapy; DFS: Disease-free survival; OS: Overall survival; PSK: Polysaccharide krestin; RT: Radiotherapy.

$$SE_{\ln(RR_{pooled})} = \sqrt{\frac{1}{\sum w_i}} \quad \text{(IX)}$$

(ix) The 95% CI for ln (RR_{pooled}) was computed as:

$$\ln(RR_{pooled}) \pm 1.96 \times SE_{\ln(RRpooled)} \quad \text{(IX)}$$

These bounds were then exponentiated to yield the pooled RR and its 95% CI on the original scale.

By applying this methodology, the meta-analysis yielded a pooled RR of 1.10 (95% CI: 1.04–1.15), indicating a survival benefit for patients receiving PSP/PSK in combination with standard treatment compared to those receiving standard treatment alone. This result is visualized in a forest plot in Figure 1. The weights in the forest plot are allocated based on the inverse of the variance of the log-transformed RR ($1/SE^2$), reflecting each study's precision. Studies with smaller standard errors, such as that by Ito *et al.*[108] with a normalized weight of 34.40%, contribute more significantly to the pooled estimate due to their higher reliability, while those with larger standard errors, such as that by Ogoshi *et al.*[106] with normalized weights of 1.02% and 0.71% for the RT and RT + chemotherapy (CT) arms, respectively, contribute less.

Heterogeneity was assessed using Cochran's Q statistic, which yielded a value of 5.59 with seven degrees of freedom (8 comparisons minus 1), indicating no significant heterogeneity ($p=0.59$). The Q statistic heterogeneity test evaluates the variation in study results that exceeds what would be expected by chance. A non-significant p-value (>0.05) suggests that there is no evidence of heterogeneity, indicating that the effect sizes are consistent across studies. The inclusion of two independent comparisons from Ogoshi *et al.*[106]—RT + PSK versus RT and RT + CT + PSK versus RT + CT—increases the total number of comparisons to eight, justifying the adjustment in degrees of freedom. In addition, the I^2 statistic, at 0%, further reinforces the absence of heterogeneity, as it quantifies the proportion of variation due to between-study differences. A value of 0% implies that all variability is attributable to sampling error. This low heterogeneity enhances the reliability of the pooled RR and supports its generalizability across the included post-operative cancer cohorts. However, the consistency does not preclude potential subgroup-specific effects that could be explored in future analyses. These findings underscore the potential of PSP and PSK as adjuvant agents.

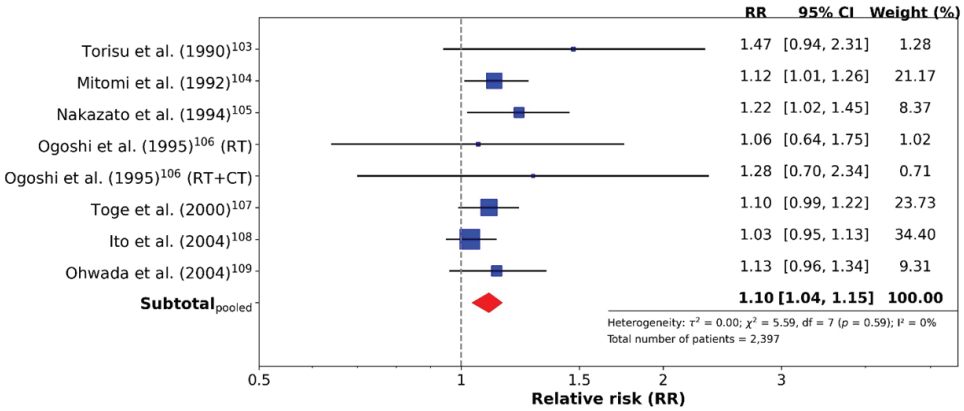

Figure 1. Forest plot of survival relative risk (RR) associated with polysaccharide krestin (PSK)/polysaccharopeptide (PSP). It summarizes data across seven studies, encompassing a total of 2,397 *patient*s. Each blue square represents the RR from an individual study, with the size of the square reflecting the study's weight. Each blue square represents the RR for an individual comparison between interventions in the meta-analysis. The horizontal lines indicate the 95% confidence intervals (CIs). The red diamond represents the pooled RR, which is 1.10 (95% CI: 1.04–1.15). The vertical dashed line at RR = 1 signifies no effect, while RR >1 favors the treatment group (standard treatment + PSP/PSK). Heterogeneity statistics displayed in the plot reveal no significant variability among the studies (p=0.59, I^2 = 0%), indicating a consistent effect of PSP/PSK on survival. Therefore, a random-effects model is unnecessary, as a fixed-effect model adequately represents the data.
Abbreviations: CT: Chemotherapy; RT: Radiotherapy.

5. Extraction methods and composition of medicinal mushroom-derived polysaccharides and other bioactive compounds

Medicinal mushrooms are known to contain a diverse array of bioactive compounds that contribute to their therapeutic potential. Among these, polysaccharides are particularly significant, including α-glucans and β-glucans, specifically β-1,3- and β-1,6-*D*-glucans. Other important polysaccharides include heteroglycans, peptidoglycans (e.g., ganoderan B and C), and polysaccharides (e.g., PSP and PSK). In addition to polysaccharides, terpenes and triterpenoids also play a crucial role in the bioactivity of medicinal mushrooms. Notable examples include ganoderic acids, ganodermic acids, ganodermic alcohols, lucidones, and lucinedic acids in the genus *Ganoderma*. Proteins and peptides are also essential components, with fungal immunomodulatory proteins recognized for their cytotoxic and immunomodulatory effects, and lectins, which specifically bind to mono- and oligosaccharides, being another important class. In addition, peptides, such as *G. lucidum* peptide, along with glycoproteins, such as *G. frondosa* glycoprotein-3a from *G. frondosa*, contribute to the bioactive profile of these mushrooms. Furthermore, phenolic compounds, known for their antioxidant properties, are present in various species of medicinal mushrooms. Fatty acids, including conjugated linoleic acid, further enhance the chemical diversity of these fungi. Steroids and their derivatives, including ergosterol and antcin A, are also noteworthy, while ergothioneine, an amino acid derivative, contributes separately to their bioactive spectrum. Diterpenoids, particularly erinacines A–I, are recognized for their bioactive properties, as are benzyl alcohol derivatives, such as hericenones C–H. Finally, other significant compounds include laccases, which are copper-containing oxidases, as well as nucleosides and nucleotides that can influence platelet aggregation. Flavonoids and the α-glucosidase inhibitor SKG-3 from *G. lucidum* further exemplify the wide range of bioactive compounds found in medicinal mushrooms, highlighting their diverse pharmacological potential.[113,114] The following section explores the main bioactive compounds used in clinical research and evaluates their pharmacological equivalence based on extraction methodologies and standardization techniques, with a primary focus on *T. versicolor*.

5.1. Comprehensive analysis of medicinal compounds in selected mushrooms

This section, based on the most frequently utilized medicinal mushroom species or extracts in human clinical studies, provides a detailed examination of the medicinal compounds found in *G. lucidum*, *G. frondosa* D-fraction, lentinan (*L. edodes* extract), AHCC, and *T. versicolor*. The analysis aims to elucidate the chemical nature of these compounds, offering a thorough resource for understanding their pharmacological relevance.

5.1.1. G. lucidum (reishi)

G. lucidum, a widely studied medicinal mushroom, is known for its diverse bioactive compounds, particularly

triterpenoids and polysaccharides. Triterpenoids, such as ganoderic acids (including A, C, F, H, and T), lucidenic acids, and ganodermanondiol, are derivatives of lanosterol, a tetracyclic triterpene, and are noted for their antitumor properties. GL-PS, including β-glucans (specifically β-1,3- and β-1,6-*D*-glucans) and α-1,3-*D*-glucans, as well as peptidoglycans ganoderan B and C, are sugar polymers with glucose as a major component, exhibiting strong immune-strengthening effects. GL-PS can be extracted from *G. lucidum* spores, fruiting bodies, mycelium, and fermentation broth using various methods, including hot water extraction, which is commonly employed to obtain the soluble polysaccharides.[115-118]

5.1.2. G. frondosa (maitake) D-fraction

The protein-bound D-fraction from *G. frondosa* is characterized as an acid-insoluble, alkali-soluble, and hot water-extractable fraction. It contains both a β-1,6-*D*-glucan with β-1,3 branches and a β-1,3 main chain having β-1,6 branches, forming a heavy proteoglycan with a molecular weight of approximately 1,000 kDa. This compound is a β-glucan conjugated with protein, known for its immune-activating properties, particularly in cancer therapy. It is standardized for oral administration and has been studied for its antitumor effects. This compound's safety and efficacy are supported by various studies, emphasizing its role as a biological response modifier.[82,83,119,120]

5.1.3. Lentinan

Lentinan, extracted from *L. edodes*, is a β-1,3-*D*-glucan with two β-1,6-glucopyranoside branches for every five β-1,3-glucopyranoside linkages. It is a polysaccharide with a molecular weight of approximately 500 kDa. It is known for its immunomodulatory and antitumor effects, often used as an adjuvant in cancer therapy, particularly in Japan and China. Its chemical structure is detailed in previous studies,[121,122] which discussed its host-mediated antineoplastic activity through immune stimulation. This compound's ease of availability and low toxicity make it a significant focus in medicinal mushroom research.[85]

5.1.4. AHCC

As mentioned earlier, AHCC is a functional food supplement synthesized at the University of Tokyo by Dr. Toshihiko Okamoto and researchers from Amino Up Chemical Co., Ltd. Its main ingredient is *L. edodes*, recognized for its healing properties, along with various hybrids from the Basidiomycota fungal phylum. AHCC has a composition rich in α-1,4-glucans (20–30%) with partially acylated sugar moieties. The product also includes various polysaccharides, such as β-1,3-glucan, along with trace amounts of protein, amino acids, lipids, and minerals, with a mean molecular weight of approximately 5 kDa. These components work synergistically to enhance immune responses, particularly the activity of NK cells and T cells. Its production involves a patented culturing process.[86,123,124]

5.1.5. T. versicolor (turkey tail)

Turkey tail, scientifically known as *T. versicolor*, previously classified as *C. versicolor*, is rich in polysaccharides, with PSP and PSK being the most commonly known. Both are protein-bound polysaccharides with a molecular weight of approximately 100 kDa. Some reports indicate that PSP has a polysaccharide-to-peptide ratio of 90:10, comprising mannose, xylose, galactose, and fructose, while PSK has a 60:40 ratio, comprising mannose, xylose, galactose, arabinose, and rhamnose; however, these ratios, as well as their monosaccharide compositions, might vary considerably across studies due to differences in extraction methods, fungal strains (e.g., COV-1 for PSP and CM-101 for PSK), and analytical techniques.

These structurally diverse β-glucan-based compounds, with a main primary partial structure as illustrated in Figure 2, are well-known for their immunomodulatory and anticancer properties.[55,64,90,113,125,126] Generally, in a broader polysaccharide fraction beyond PSP/PSK, *C. versicolor* polysaccharides (CVP) have a molecular weight of 500–1000 kDa, with a β-1,4/1,3-*D*-glucan main chain and β-1,6 branches. The monosaccharide composition of CVP predominantly contains glucose in the form of β-*D*-glucopyranose, along with small amounts of mannose, fructose, rhamnose, and fucose. In addition, the strain, processing technology, and extraction method of CVP influence its monosaccharide types, molecular weight, and chemical composition.[127] Further research is needed to investigate the pharmacological activities of small molecular weight compounds.[46,64]

Polysaccharides from *T. versicolor* are commercially extracted from mushrooms or mycelia grown on solid substrates, as well as from mycelial biomass produced through submerged fermentation. The primary clinically approved polysaccharide preparations, PSK and PSP, are derived from mycelial biomass cultivated under submerged conditions, processed into dried powder after hot water extraction.[127,128] The extraction and purification of CVP involve several techniques, each employing distinct chemical and physical principles to isolate these bioactive compounds from raw materials, such as fruiting bodies and mycelium. According to the study by Jing *et al.*,[127] the most commonly used extraction method for CVP is hot water extraction. The optimal extraction conditions are a solid-

to-liquid ratio of 1:30 and an extraction temperature of 90°C for 120 min (twice), followed by ethanol precipitation, resulting in a yield of 5.38%. This yield can be increased to up to 16.7% with mechanical crushing and liquid nitrogen for particles under 200 mesh in size. Other methods include enzyme extraction, which is operationally more complex and can achieve yields of 9.58% at 52°C with an enzyme concentration of 2.50%, a pH of 5.5, and an enzymolysis time of 37 min, generally using cellulase, proteases, and pectinase. In addition, ultrasonic extraction can be optimized to achieve extraction rates up to 13.6% at a 1:45 solid-to-liquid ratio, 30 min of extraction time, and an ultrasonic power of 450 W through the response surface method, although high-intensity shock waves may have structural impacts. The purification process for crude CVP includes the removal of proteins and pigments through methods, such as ethanol fractionation, column chromatography, and ion exchange chromatography, resulting in a refined product suitable for further pharmacological use. These processes are commercially utilized in the production of high-quality, standardized extracts. It is important to note that although some of these polymers have distinct structures, they cannot be differentiated based on their physiological activity.[128]

5.2. Reproducibility and consistency of medicinal mushroom extracts for clinical applications

The therapeutic potential of medicinal mushrooms, including *T. versicolor*, *G. lucidum*, *G. frondosa*, and *L. edodes*, depends on the consistent delivery of bioactive compounds, such as polysaccharides and triterpenoids, for clinical applications.[113] Variability in fungal raw materials and extraction processes poses significant challenges to achieving reproducible and reliable extracts. This section examines these challenges and proposes strategies to ensure consistency, balancing scientific rigor with economic feasibility for clinical adoption.

Variability in fungal source material is a primary obstacle to reproducibility. Differences in strains, growth substrates, and environmental conditions, such as temperature or cultivation methods, can significantly alter bioactive compound profiles. Extraction methods, including hot water, ultrasonic, or enzyme-assisted techniques, further contribute to variability by affecting yield and molecular integrity. In addition, inconsistent analytical methods for characterizing compounds can also lead to discrepancies in reported compositions, hindering standardization.[114,117,127,128]

Standardized cultivation practices are critical for addressing these issues. Controlling substrate composition and environmental parameters minimizes variability in chemical profiles. Among the cultivation practices, two common techniques for mycelium cultivation are solid-state fermentation and submerged fermentation. Solid-state fermentation often utilizes substrates, such as sawdust combined with rice bran or wheat bran. On the other hand, submerged fermentation tends to be more efficient, resulting in greater mycelial productivity within a shorter duration, requiring less physical space for production, and enabling improved quality control.[119]

Optimized, uniform extraction protocols ensure consistent yields and bioactivity. Analytical tools, such as high-performance liquid chromatography and mass spectrometry, enhance quality control by enabling precise characterization of bioactive fractions.[127] In applications requiring high consistency, purified compounds offer reliable solutions; however, the associated purification costs may pose challenges to scalability. Economically, while crude extracts are less expensive, they tend to exhibit greater variability and are not suitable for clinical use. Purification costs for these extracts can be substantial, covering raw materials, solvents, energy for extraction, and specialized equipment costs. Furthermore, labor

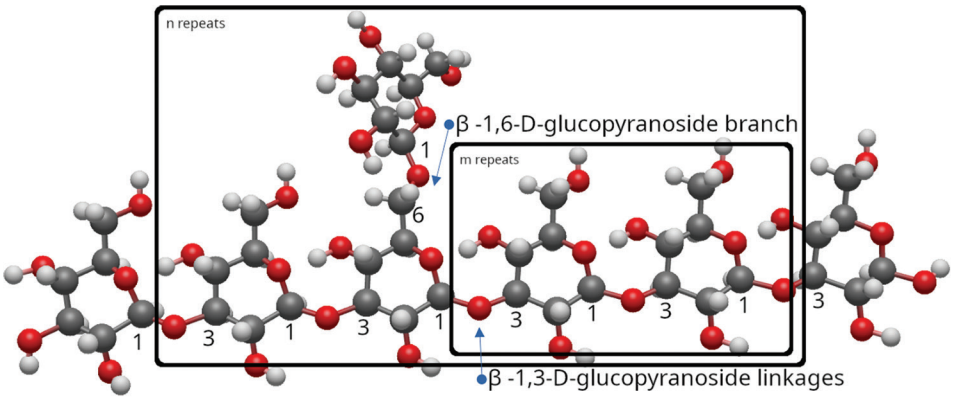

Figure 2. Repeat unit of the partial structure of polysaccharide krestin/polysaccharopeptide, featuring m and n repeats.[55] The β-*D*-glucan molecular structure was generated using the Avogadro software. Carbon atoms in black, oxygen in red, and hydrogen in white.

for production and testing, as well as laboratory analysis for purity and potency, adds to the expense.[129] Costs can be further increased due to adherence to certifications for organic or good manufacturing practices (GMP) standards. While exact figures are difficult to assess due to their proprietary nature and vary by scale and species, these costs are often reflected in the higher price of premium mushroom supplements. Recent biotechnological advances, such as precision fermentation and omics-based strategies,[130] may provide more affordable solutions in the near future, enhancing scalability.

In clinical settings, where predictable therapeutic outcomes are essential, reproducibility is paramount. Standardized extracts, such as *T. versicolor*-derived PSP and PSK, exhibit significant immunomodulatory and anticancer potential. Their integration into medical practices requires robust standardization. Future research should prioritize cost-effective standardization and synthetic analogs to facilitate routine clinical use, unlocking the full therapeutic benefits of medicinal mushrooms. Rigorous quality control, including batch testing for bioactivity and adherence to GMP, is critical for clinical-grade reliability.

6. Surgical eligibility enhancement: a proposal for the use of medicinal mushroom extracts as an adjunctive therapy to optimize pre-operative systemic chemotherapy outcomes

6.1. Proposal

The author posits that medicinal mushroom extracts, which encompass a variety of bioactive compounds, particularly PSP and PSK derived from *T. versicolor*, as well as polysaccharides, alongside small molecules, from species, such as *G. lucidum*, *G. frondosa*, *L. edodes*, *A. blazei*, and *I. obliquus*, may serve as adjunctive agents to enhance pre-operative systemic chemotherapy in patients with initially unresectable mCRC involving LM and PM. This approach aims to increase tumor response rates, improve surgical eligibility (e.g., achieving R0 resection via CRS and liver resection/ablation), and mitigate treatment-related toxicities, ultimately extending OS beyond present benchmarks. The hypothesis leverages the mushroom extracts' multifaceted anticancer properties to optimize conversion therapy outcomes in this challenging dual-metastasis subgroup.

6.2. Clinical context, rationale, and limitations of present therapies

CRC with LM (15–25% at diagnosis) and PM (8–15% during initial treatment) is associated with poor prognosis, with a median OS of 6–9 months untreated and 12–24 months with modern systemic chemotherapy alone (e.g., FOLFOX and FOLFIRI) or targeted therapies. Although aggressive regimens, such as FOLFOXIRI + bevacizumab, improve outcomes, with an OS of 31.0 months and a PFS of 12.1 months as demonstrated in the TRIBE trial involving 508 patients, it is important to note that these results are not limited to patients with LM and PM and only apply to patients who are fit to receive this treatment. Nevertheless, conversion to resectable disease within this cohort occurs in only 39.1% of cases, with an R0 resection rate of 28.1%, underscoring the importance of patient selection in optimizing therapeutic strategies. Combined approaches, such as CRS-HIPEC with liver resection, extend OS to 26–48 months in selected patients, but eligibility is restricted by high PCI (>20), multiple liver lesions (>2–3), and incomplete cytoreduction (CC >1). Therefore, enhancing pre-operative tumor shrinkage is critical to broaden surgical candidacy (refer to Section 1 for citations).

6.3. Medicinal mushroom extracts: mechanisms and evidence

Medicinal mushroom compounds, such as PSP/PSK and small molecules from *T. versicolor*, GL-PS and triterpenoids from *G. lucidum*, β-glucans from *G. frondosa*, lentinan from *L. edodes*, polysaccharides from *A. blazei*, and ergosterol peroxide from *I. obliquus* collectively exhibit potent anticancer effects, as detailed in pre-clinical and clinical studies. Their activity as biological response modifiers can suppress multidrug resistance (e.g., P-gp inhibition), block immune checkpoints (e.g., PD-L1 and CTLA-4), regulate oncogenic pathways (e.g., PI3K/AKT, Wnt/β-catenin, MAPK, and NF-κB), and enhance immunomodulation (e.g., NK cell activation and Th1 cytokine production). Furthermore, they can induce apoptosis, inhibit angiogenesis, and reduce metastasis. Clinical trials and meta-analyses of over 8,000 patients (e.g., Oba *et al.*,[96] 8,009 gastric cancer patients; Zhong *et al.*,[73] 4,246 patients across cancers; and Sakamoto *et al.*,[97] 1,094 CRC patients), have reported improved OS (e.g., the meta-analysis in the present review indicates a 4–15% relative increase in the probability of survival at 5 years) and tolerability when these compounds were used in combination with chemotherapy, primarily in post-operative CRC and other cancers.

6.4. Potential in conversion therapy

While most clinical data on mushroom extracts pertain to post-operative settings, their ability to enhance chemotherapy efficacy suggests a pre-operative role. For example, the immune-enhancing effects and

chemosensitizing properties of PSP/PSK could amplify tumor responses to FOLFOXIRI + bevacizumab, potentially increasing the 39.1% surgical eligibility rate observed in systematic reviews. In patients with LM and PM, these extracts might counteract liver immunosuppression (e.g., regulatory T-cells) and peritoneal tumor burden, reducing PCI and lesion counts to surgically manageable levels. Synergy with PD-1 inhibitors further suggests compatibility with modern therapies, such as pembrolizumab in MSI-H mCRC, though this requires further validation.

6.4.1. Safety and practical integration

Mushroom extracts, such as PSP/PSK (1–3 g/day), lentinan, and AHCC, are well-tolerated, with only mild side effects (e.g., nausea) and extremely rare severe events, as evidenced by extensive use in Japan and China. Their compatibility with chemotherapy supports their integration into pre-operative treatment regimens; however, standardization is crucial to ensure consistency. This includes standardizing extraction methods—such as hot water extraction—and the source materials used, including fruiting bodies, mycelium, and submerged fermentation broths, with the latter typically preferred in industrial settings to maximize yield and production. Combining these extracts with present regimens may mitigate toxicities (e.g., grade 3–4 events doubled with FOLFOXIRI), thereby improving patient tolerance, treatment response, and treatment duration.

6.4.2. Implementation and testing

The author argues that a Phase II trial in patients with unresectable mCRC with PM/LM would be highly valuable. Participants should be randomized to receive standard chemotherapy (e.g., FOLFOXIRI + bevacizumab) with or without a mushroom extract cocktail (e.g., PSP/PSK 2–3 g/day or GL-PS 1–2 g/day) for a duration that allows for adequate assessment of treatment response, potentially ranging from 24 to 48 weeks. This proposed Phase II trial aims for a 10–15% increase in the conversion rate to resectable disease (e.g., from 39% to 49–54%), with 80% power and a two-sided alpha of 0.05.

The primary endpoint, conversion rate, is defined by a PCI <20, 2–3 liver lesions, and a CC of 0/1. The baseline conversion rate in the control arm for patients who are fit to undergo a FOLFOXIRI + bevacizumab regimen was 39%, as highlighted in the systematic review and pooled analysis of prior studies, including the TRIBE trial.[15] It is important to consider varying conversion rates for different patient cohorts.

A two-sample proportion test (e.g., Chi-square) will be used to compare conversion rates across arms. The sample size is calculated using the following formula[131]:

$$n = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \cdot \left(p_1\left(1-p_1\right) + p_2\left(1-p_2\right)\right)}{\left(p_1 - p_2\right)^2} \quad \text{(XI)}$$

Where n is the sample size per arm, $Z_{1-\alpha/2}$ is 1.96 for a two-sided α of 0.05, $Z_{1-\beta}$ is 0.84 for 80% power, p_2 represents the control arm conversion rate set at 0.39, and p_1 represents the experimental arm set at 0.49 or 0.54 for a 10% or 15% increase, respectively. For a 10% increase (p_1=0.49), the required sample size is approximately 383 per arm, while it is approximately 170 per arm for a 15% increase (p_1=0.54). To account for a 10% dropout rate, the sample size is inflated by dividing by 0.9. Thus, ultimately, for a 10% increase, the adjusted sample size is 426, while it is 189 for a 15% increase.

A sample size of 200–250 patients per arm is proposed, prioritizing a 15% increase for feasibility in a Phase II setting, while acknowledging that detecting a 10% increase requires a larger sample. The range reflects practical constraints and variability in effect size assumptions. The sample size is powered for the primary endpoint of conversion rate to resectable disease, with overall response rate and PFS as exploratory endpoints. Secondary endpoints include OS, toxicity (e.g., a comparative analysis of grade 3–4 adverse reactions per Common Terminology Criteria for Adverse Events v5.0), and quality of life. If several endpoints are analyzed, adjustments for multiple comparisons (e.g., Bonferroni correction) may be applied to control the overall alpha level.

6.4.3. Predicted outcomes and implications

If effective, this adjunctive therapy could elevate R0 resection rates beyond the 28.1% observed with FOLFOXIRI + bevacizumab, potentially aligning OS with CRS-HIPEC outcomes (26–48 months) or even surpassing them in a broader population. By enhancing tumor shrinkage and reducing immunosuppression, mushroom extracts could redefine conversion therapy, offering a low-toxicity, biologically plausible adjunct to multidisciplinary care.

6.4.4. Limitations and future directions

The absence of direct pre-operative trials for mCRC limits present evidence, requiring extrapolation from post-operative data. Variability in extract composition and the unique challenges of dual metastases (e.g., liver vascularity and peritoneal spread) necessitate tailored studies. Future research should assess the potential of mushroom extracts in combination with targeted therapies (e.g., anti-VEGF, anti-EGFR), immunotherapies (e.g., pembrolizumab in MSI-H cases), or other bioactive compounds (Supplementary File Section S2) to enhance efficacy across mCRC subtypes.

7. Conclusion

This review examines the challenges of managing mCRC with LM and PM, where systemic chemotherapy alone yields a median OS of 12–24 months, and conversion to resectable disease remains limited (e.g., 39.1% in patients eligible for FOLFOXIRI + bevacizumab, not limited to those with LM and PM). Combined approaches, such as CRS-HIPEC extend OS to 26–48 months in selected cases, but eligibility is often constrained by disease extent. The author proposes that medicinal mushroom extracts (e.g., PSP/PSK from *T. versicolor* and polysaccharides from *G. lucidum*) may enhance pre-operative chemotherapy outcomes by increasing tumor response, surgical eligibility, and patient tolerability. This approach is supported by pre-clinical data on multidrug resistance suppression, immune modulation, and pathway regulation, among others, as well as clinical evidence from meta-analyses (>8,000 patients) showing survival benefits. The meta-analysis in this study, presented in Supplementary File Section S1 and involving 2,397 patients, indicates a 10% relative increase in 5-year survival with the application of PSP/PSK compared to standard treatment (RR = 1.10; 95% CI: 1.04–1.15), based on post-operative data. The review proposes a Phase II trial to evaluate this approach. While promising, the lack of direct pre-operative data and variability in metastatic site responses highlight the need for further research to validate and optimize this strategy in mCRC management.

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Conflict of interest

The author declares no conflict of interest.

Author contributions

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Consent for publication

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Availability of data

All data supporting the findings of this study's meta-analysis are provided in the Supplementary File.

Further disclosure

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