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How far have we explored fungi to fight cancer?

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ABSTRACT

The use of fungal cultures have been well documented in human history. Although its used in healthcare, like penicillin and statins, have saved countless of lives, but there is still no fungal products that are specifically indicated for cancers. Research into fungal-derived materials to curb cancers in the recent decades have made a considerable progress in terms of drug delivery vehicles, anticancer active ingredients and cancer immunotherapy. Various parts of the organisms have successfully been exploited to achieve specific tasks. Apart from the identification of novel anticancer compound from fungi, its native capsular structure can also be used as drug cargo to achieve higher oral bioavailability. This review summarises the anticancer potential of fungal-derived materials, highlighting the role of capsular polysaccharides, proteins, and other structures in variety of innovative utilities to fit the current pharmaceutical technology. Many bioactive compounds isolated from fungi have also been formulated into nanoparticles to achieve greater anticancer activity. The progress of fungal compounds and their analogues in clinical trials is also highlighted. In addition, the potential of various fungal species to be developed for anticancer immunotherapy are also discussed.

1. Introduction

Anticancer drug discovery from natural products has been ongoing for decades [1–4]. Many important anticancer drugs have been successfully isolated from plant sources such as vincristine and vinblastine which are isolated from the Madagascar periwinkle, *Catharanthus roseus*. With the advancement of knowledge in the field of chemistry, anticancer drug discovery is now not limited to isolation and direct application of bioactive compounds, but also extends to structural modification of these compounds for improved pharmacokinetic and pharmacodynamics profile. Total synthesis of structural mimics is thoroughly investigated with the aim to increase the production of those bioactive compounds for clinical application [5,6]. Computational methods also allow rapid screening of a large compound library and visualise the binding of bioactive compounds through modelling/simulation [7].

Despite the successful stories of drug discovery from plant sources, there are issues associated with the use of plant-derived natural products. For instance, bioactive compounds are often produced in very low quantities resulting a serious supply issue. Metabolite composition of these active compounds are also impacted by environment, making the reisolation of a desired compound problematic. Indeed, total synthesis of bioactive compounds are tedious, time-consuming and cost-ineffective. To resolve the abovementioned problems, researchers have investigated microorganisms such as fungi as a source to fight cancer. There are several practical advantages to employ fungi to fight cancer. Fungi typically respond well to routine culture techniques, and hence productivity amplification is relatively easy. Fungi can be grown large amount in a bioreactor and stored indefinitely, ensuring long-term

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availability of the source organism and supply of desired bioactive compounds [8,9]. In this review, we aim at providing an update how different parts of fungi were employed to combat cancer. The discussion mainly focuses on the use of fungi materials as construction of delivery system for anticancer drugs and stimulate anticancer immune responses. Since many other research groups have extensively reviewed anticancer properties of secondary metabolites of fungi, we would also like to provide the update on how nanotechnology could improve the selectivity of these compounds as cancer targeted therapy. The latest development of fungal metabolites and their analogues in cancer clinical trials is also highlighted in this review.

2. The relevance of fungal-derived components to oncological perspective

The use of fungi to treat human diseases have been dated back since ancient time. Fungi provide a generous source of bioactive compounds that are currently being studied and clinically used. Numerous fungal-derived compounds such as mycotoxins, antifungal agents have been reported in the literature in the past few decades. In the field of cancer, perhaps the most notable one is paclitaxel, though it was originally isolated from the bark of *Taxus brevifolia* in 1965. Although the anti-tumoural activity of paclitaxel was confirmed in 1977 in mouse melanoma B16 model, Food and Drug Administration (FDA) only approved its usage as an oncology drug 3 decades later. In a clinical trial of 15 advanced breast cancer anthracyclines resistant patients, a paclitaxel treatment with a 5 cycles median has induced 3 complete responses and 4 partial responses. These values account for an overall response rate of 47 %. Although the drug is not without noxious effects, but the results of the clinical trial is already sufficient to merit significance in oncological field considering the lack of effective treatment at that time. Apart from its use against advance breast cancer, other neoplasms like refractory ovarian cancer and AIDS-related Kaposi's sarcoma were also indicated. Novel compounds revealing remarkable activities are still being discovered and have been reviewed in multiple occasions [10].

Cancer chemotherapeutic drugs have often been lamented as highly cytotoxic to rapidly dividing cells. Neoadjuvant treatment for cancer consists largely of drug combinations, each of which has distinct mode of action [11]. While these drugs in combination may be appealing for their synergistic effects against the cancerous cells, their cytotoxic effects are also extended to harm healthy dividing cells in the patients as well. As a result, patients may suffer a range of adverse drug effect that include anaemia, emesis, fatigue, compromised immune system and alopecia. Due to its inability to distinguish between normal cells and cancerous cells, chemotherapeutic drugs crippled all rapidly dividing cells in the body. One of the purposes of the advancement in pharmaceutical technology has to do with reducing off-target accumulation of anticancer compounds to minimise its side-effect [12]. In the following sections, we discuss different types of fungal components that are used directly or indirectly to achieve effective anticancer drug delivery from the perspective of effectiveness, toxicity and immunological activity.

3. Fungal polysaccharides

Polysaccharides are the macromolecules that involved in variety cellular functions. Its presence as structural support component is evident in all 3 domains of life. Being a source of energy in both the eukaryotes and prokaryotes, polysaccharides are major component of cell wall. In microorganisms, polysaccharides are the major constituents of cell envelope. The distribution of polysaccharides around the surface of the microorganisms is shown to influence host-pathogen interactions. The classification of polysaccharides ranged from linear to branched, and from homopolymers to heteropolymers. A simple polysaccharide composed of at least 10 monosaccharides that are linked together by glycosidic bonds.

In fungal cells, the structure of polysaccharides exhibits high degree of complexity due to various conjugation on the polymers. The association between conjugated polysaccharides to the microbial virulence has been consolidated and were among the earliest determinant of virulence in microorganisms in the early 90s'. However, various beneficial usage of the fungal polysaccharide has been exploited especially in the context of human healthcare. In this section, the relevance of fungal components, including the polysaccharides, proteins and organelles for anticancer purposes is reviewed (Fig. 1) and summarised in Table 1.

3.1. Matrix ingredients for drug delivery systems

In the past few decades, pharmaceutical technology has been focusing on using nanosized materials to entrap anticancer drugs. Compared to fungal capsule, which are measured in micron, nanosizing of materials can substantially enhance the surface area of the matrix for housing the anticancer drugs. Other advantages of nanoparticles such as injectable-friendly does offer greater advantage in both efficacy and safety over conventional pharmaceutical agents. Recently, Huang and colleagues claimed to have formulated fungal beta-glucan molecules into nanoparticles [13]. Although the preparation process was merely mixing and incubation, but the electron microscopy and dynamic light scattering have verified the morphology and the size reduction of the preparation. Apart from activating macrophages via increased expression of IL-1β, TNF-α, IFN-γ, the nanoparticles also demonstrated increased uptake into MCF-7 cell lines. Notwithstanding, the team showed that chirality has different immune-potentiating ability. Sathiyaseelan et al. (2020) has utilised the chitosan oligosaccharides extracted from fungus *Cunninghamella elegans* to fabricate silver nanoparticles. The reaction of amino group of chitosan with silver nanoparticles enhanced the *in vitro* anti-cancer activity against lung A549 cancer cells (IC_{50} value of 71.2 μg/mL) [14].

3.2. Conjugated polysaccharide for cancer-targeting drug delivery system

The tumour microenvironment is complex. Due to the rapid rate of cellular growth and angiogenesis at the vicinity of a tumour, the cells are often arranged in a disorganised pattern that reveals uneven size of cell fenestrations. The large fenestration between cells could result in the accumulation of submicron materials, to which nanoparticles may be useful. Nanoparticles exploit the disorganised cellular arrangement at the tumour site and accumulate at the target tissue. To further reduce the off-target distribution, researchers performed modifications to the nanoparticles surfaces via various techniques, typically based on the overexpressed receptors on tumour cell. In 2017, the polysaccharide portion of *Auricularia auricular* was extracted and linked to histidine (His) molecules to produce pH-sensitive drug delivery systems [15]. The His molecule is regarded as a pH-sensitive molecule due to the presence of an imidazole group. Having an electron lone pair on the unsaturated nitrogen of the imidazole ring, His molecule is amphoteric by protonation-deprotonation. With a size of about 160 nm and 80 % of paclitaxel entrapment efficiency, the drug entrapped within the core is released up to 88 % at pH 5.0. Although leakage of drug was detected even at pH7.4, but the *in vivo* test using tumour-bearing mice showed that the histidine-polysaccharide micelles significantly inhibited tumour growth compared to paclitaxel alone [15].

In a separate study conducted by Qiu et al. (2018), the extracted polysaccharides were conjugated with folic acid (FA) [16]. The use of FA as a homing-ligand for cancers was based on the high expression of folate receptors in epithelial, ovarian, cervical, breast, lung, kidney, colorectal and brain tumours. FA is a small (441 Da) molecule but quite stable over a broad range of temperatures and pH values. Apart from cost-effective and non-immunogenic, it retains the ability to bind to the folate receptor after conjugation with drugs or diagnostic markers [17]. FA conjugated-*Auricularia auricular* polysaccharide formulated with diaminedichloroplatinum (CDDP) was able to enhance the antitumor potency of CDDP with reduced side effects when tested on cervical

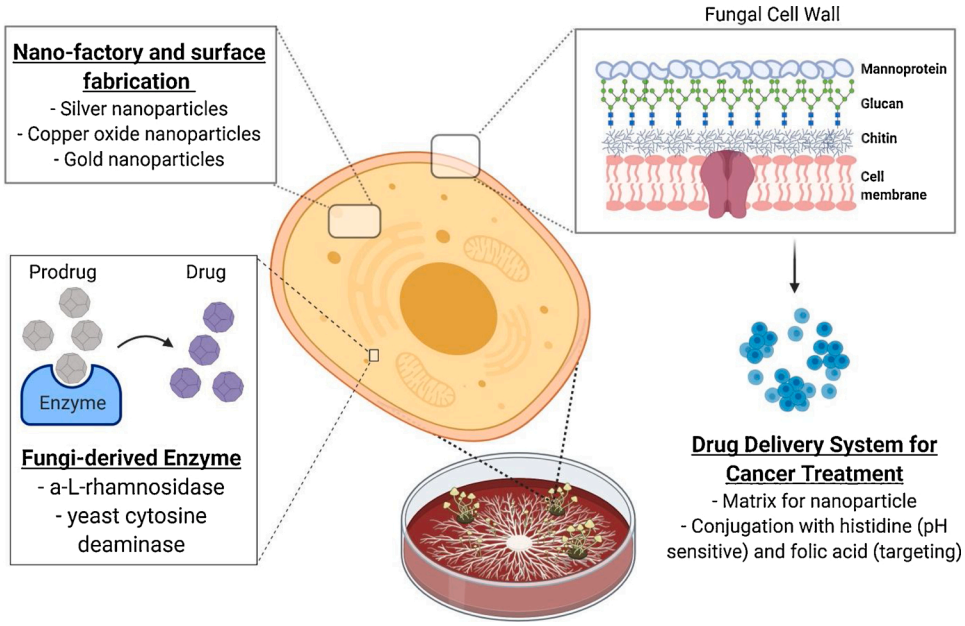

Fig. 1. The roles of different fungal components in cancer treatment. The cell wall comprises of polysaccharides acts as the matrix for nanoparticle for pharmaceutical agents with some conjugations on the nanoparticles surface; the fungal proteins act as reducing and capping agents for metal nanoparticles; and fungal enzymes act as the prodrug activator (Created with BioRender.com).

carcinoma in mice. The kidneys of treated group had higher antioxidant activities. Moreover, the complex induced higher expression of interleukin-2, interleukin-4 and interferon-gamma in mice. An increased expression of Bax and caspase-3 protein, while decreased expression of Bcl-2 protein were demonstrated in the tumour cells in tested nude mice. Indeed, the FA-AAP-CDDP complex has a higher intra-tumoral accumulation. The composition of FA-AAP-CDDP was shown to retain their intrinsic function even after chemical conjugation may reflect a new direction for folate receptor targeted polymers to improve drug efficacy and simultaneously reduce its adverse effects.

4. Fungal proteins

In nature, the fungi secrete enzymes to digest the complicated macromolecules and absorb them as source of nutrition and energy. As more and more of the novel fungal proteins are being added into the repository, researchers have expressed their interest in investigating the use of these proteins in drug development for cancer treatment [18,19].

4.1. Fungal proteins mixture for the biosynthesis of nanoparticles

Recently, researchers have been promoting "green technologies" that involved biological approach in the production of metal nanoparticles for cancer treatment. The fungi contain enzymes or proteins that could act as reducing agents both on the fungal surfaces and inside the cell. These reducing agents are utilised to synthesise metal nanoparticles from metal salts [18,20]. As compared to other microorganisms such as algae and bacteria, fungi are considered as an attractive nano-factory since they are more stable, cost-effective and could withstand bioreactor's conditions during scale-up production [20].

Several studies have been carried out to biosynthesise metal nanoparticles using fungal *Trichoderma* species which is commonly present in soil as biofertiliser. Study by Adebayo-Tayo et al. (2019) has successfully formulated silver nanoparticles with a diameter of 10 nm in spherical shape using *Trichoderma viride*. The silver nanoparticles were cytotoxic towards human cervix carcinoma Hep-2C cells with an IC_{50} value of 54 μg/mL [21]. Similarly, another type of metal nanoparticles, copper oxide nanoparticle were produced in the same species of fungus but with a larger in diameter (up to 130 nm) [19]. Under the exposure of near-infrared laser, the copper oxide nanoparticles exhibited photothermal effect by inducing apoptosis with increased levels of reactive oxygen species (ROS), caspase-3 and Bcl-2 proteins.

Saravanakumar and the research team utilised *Trichoderma harzianum* as a nano-factory to synthesise zinc oxide nanoparticles (ZnO) [22] and chitosan nanoparticles [23]. Interestingly, the study showed that the ZnO nanoparticles, with average diameter of 30 nm, was effective against human lung carcinoma A549 with IC_{50} of 56 μg/mL, meanwhile did not kill the normal NIH3T3 cell line [22]. Both ZnO and chitosan nanoparticles also exhibited anti-bacterial activity against *Staphylococcus aureus* and *Salmonella enterica* suggesting that these nanoparticles could penetrate the bacterial cell wall and cause cell death [22,23]. The other *Trichoderma* species, *T. atroviride* was used to produce silver nanoparticles which showed *in vitro* efficacy against breast cancer [24].

Apart from *Trichoderma* species, more fungi have been explored to greenly synthesise nanoparticles. Hu et al. (2020) has successfully synthesised silver nanoparticles with diameter less than 50 nm using endophytic fungus *Talaromyces purpureogenus*. The silver nanoparticles were proven to exhibit cytotoxicity against human lung A549 cancer cells and cell wound healing activity in Swis albino mouse embryo tissue NIH3T3 cells [25].

Gold nanoparticles have been widely used in biomedical applications such as drug delivery, imaging and diagnosis due to the properties of oxidation resistance, biocompatible and good stability in various environmental conditions [26]. Likewise, the gold nanoparticles can be produced by various species of fungi. The production of gold nanoparticle from gold ions via phytochelation is possible under the presence of glutathione [27]. Subsequently, by conjugation with antibody, the gold nanoparticles can target and bind to the surface markers of liver cancer cells, suggesting that the nanoparticles could be a potential imaging agent for cancer detection

Apart from the potential use in cancer diagnosis, fungal protein-derived gold nanoparticles exhibited great potential in cancer treatment as well. The gold nanoparticles synthesised from endophytic fungi *Fusarium solani* has shown cytotoxic effect against human breast cancer MCF-7 cells and cervical cancer HeLa cells with IC_{50} values of 0.8 and 1.3 μg/mL, respectively. The study suggested that the gold nanoparticle induced apoptosis and G_1/S phase cell cycle arrest against MCF-7 cells. Further, it was non-toxic to normal human embryonic kidney (HEK)

Table 1
The use of different fungal components for cancer treatment.

Fungus species	Components	Purpose/Mechanism	Type of cancer cell	Outcome	Reference
Fungal Polysaccharides					
Saccharomyces cerevisiae	beta-glucan	Synthesis of doxorubicin-loaded nanoparticles	MCF-7	• Activate macrophages via upregulation of IL-1β, TNF-α, IFN-γ expression • Increase cellular uptake	[13]
Cunninghamella elegans	chitosan oligosaccharides	Fabrication of silver nanoparticles	A549	• IC_{50} value of 71.2 μg/mL at 24 h	[14]
Auricularia auricular	water-soluble polysaccharides	Synthesis of doxorubicin-loaded nanoparticles	MCF-7	• IC_{50} value of 0.005 μg/mL at 72 h	[15]
		Synthesis of folic acid-loaded nanoparticles	HeLa	• Inhibit tumour growth in BALB/c nude mice	[16]
Fungal Protein					
Trichoderma viride	Aldehydes, amino acids, ethers, esters, carboxylic acids, hydroxyl groups, phenol	Synthesis of silver nanoparticles	Hep-2C	• IC_{50} value of 54 μg/mL at 72 h	[21]
Trichoderma asperellum	NM	Synthesis of copper oxide nanoparticles	A549	• Induce apoptosis via upregulation of ROS, caspase-3 and Bcl-2 protein expressions	[19]
Trichoderma harzianum	NM	Synthesis of zinc oxide nanoparticles	A549	• IC_{50} of 56 μg/mL at 6 h	[22]
Talaromyces purpureogenus	NM	Synthesis of silver nanoparticles	A549	• IC_{50} of 376.24 μg/mL at 24 h	[25]
Fusarium solani	NM	Synthesis of gold nanoparticles	MCF-7	• IC_{50} of 0.8 μg/mL at 48 h • induce apoptosis and G_1/S cell cycle arrest	[28]
			HeLa	• IC_{50} of 1.3 μg/mL	
Pleurotus tuber-regium	polysaccharides–protein complexes	Surface capping for selenium nanoparticles	MCF-7	• IC_{50} value of 3.7 μM at 72 h • Induce apoptosis and PARP cleavage	[34]
	hyperbranched polysaccharide β-D-glucan	Surface capping for selenium nanoparticles	NM	• Improve colloidal stability	[32]
Polyporus rhinoceros	polysaccharides-protein complexes (PSP)	Surface capping for selenium nanoparticles	A549	• IC_{50} value of 4.1 μM at 72 h • Induce G_2/M cell cycle arrest and apoptosis via upregulated caspase-3 and -8	[36]
Amanita muscaria	ibotenic acid, muscimol, hydroxypyrollidone derivatives, and polysaccharide peptides	Synthesis of silver and ultrasmall iron oxide nanoparticles	HeLa cells	• 50 % and 40 % cell viability at 48 and 72 h, respectively	[37]
Acremonium species	α-L-rhamnosidase (RhaL1)	Production of prodrugs for FUDR, Ara C and Hydrea	MDA-MB-231 cells	• Reduce to 60 %, 70 % and 35 % viability at 100 μM FUDR, Ara-C and Hydrea at 30 h, respectively	[73]
NM	yeast cytosine deaminase (yCD)	Conversion of 5- FC into 5-FU	SUM149 and MCF10A	• Reduce to 45 and 47 % viability in SUM149 and MCF10A, respectively	[42]
			HT29	• Complete tumour regression in 60 % of the tumour in nude female mice with 500 mg/kg of 5-FC	[43]
Fungal Organelle/Metabolites					
Saccharomyces cerevisiae	Yeast Cell Wall	Synthesis of cabazitaxel-loaded yeast cell wall microparticles	NM	• Promote controlled drug release and high stability in simulated gastrointestinal environment	[47]
		Synthesis of cisplatin-loaded yeast capsule	A549	• Reduce tumour weight wihout toxicity at 6 mg/kg in BALB/c mice	[48]
		Synthesis of MYD88 and TNF mRNA loaded carrier	primary human macrophage	• Induce pro-inflammatory cytokine expression in M2 macrophages	[51]
Monascus pilosus	Monascin	Bioactive component	Skin papilloma	• Inhibit mouse skin carcinogenesis by UVB and TPA	[59]
	Ankaflavin	Bioactive component	HepG2	• IC_{50} of 15 μg/mL at 48 h and induce apoptosis	[60]
			MIA-Pa-Ca-2	• IC_{50} of 19 μM at 48 h, induce apoptosis	[63]
Xylaria psidii	5-methylmellein	Bioactive component	PC3, HCT-116, MCF-7	• IC_{50} of 5.7, 2 and 2.9 μM in PC-3, HCT-116 and MCF-7 cells, respectively at 72 h, induce apoptosis and ROS	[64]
Flammulina velutipes	Sterols (ergosterol and 2223-dihydroergosterol)	Bioactive component	HepG2 and A549	• IC_{50} of 9.3 and 20.4 μg/mL in HEPG2 and A549 cells, respectively	[65]
Xyralia primorskensis	Xylaranic acid	Bioactive component	A549	• IC_{50} of 39.4 μg/mL at 24 h • Induced apoptosis via upregulation of p53 and caspase-3 and downregulation of BCL-2 genes	[66]
Inonotus obliquus	Chaga extract	Bioactive component and coating of palladium nanoparticle	HeLa	• Reduce cell viability to 40 %	[68]
Aspergillus molds	gliotoxin	Bioactive component	PC3, DU-145	• Inhibit angiogenesis and suppress tumour growth	[69]
Trichoderma atroviride	TM2	Bioactive component	PC3	• Reduce cell viability to 40.5 % • Induce apoptosis via activation of caspase-3 and inhibition of BCL-2	[72]

Abbreviation: ROS, reactive oxygen species; NM, not mentioned; FUDR, 2′-deoxy-5-fluorouridine; Ara C, cytosine arabinoside; Hydrea, hydroxyurea; UVB, Ultraviolet B; TPA, 12-O-tetradecanoylphorbol-13-acetate.

cells with cell viability more than 60 % even at high dose of 4 μg/mL [28].

Since nanoparticle biosynthesis provides vast advantages such as minimised toxicity, biocompatibility, cost-effective and environmental-friendly, it has been regarded as the alternative to the traditional way of nanoparticle synthesis [29,30]. In spite of the advantages mentioned, the parameters required to optimise its production for monodispersed nanoparticles is no less onerous than the conventional preparation method. Issues related to total yield, choice of biological source, incubation time, pH and temperature can directly influence the nanoparticles' characteristic and morphology [29].

4.2. Nanoparticle surface capping with protein

Macromolecules originated from fungi serve as a good surface decoration for inorganic nanoparticles such as gold, selenium and iron oxides nanoparticles in the process of crystal nucleation and biosynthesis of these nanoparticles [31]. The macromolecules particularly polysaccharides and proteins offer vast advantages as capping agent for size controlling, protection of nanoparticle against plasma protein adsorption and clearance, stability improvement, prolongation in systemic circulation and enhancement in cellular uptake. On top of that, the capping agent originated from fungi are less likely to stimulate immune response as well.

Wu and the research team has utilised the polysaccharides-protein complexes (PSP) which was extracted from sclerotia of mushroom *Pleurotus tuber-regium* (PTR) to decorate the surface of selenium nanoparticles. The PSP was proven to have high number of terminal hydroxyl groups and imino groups which enabled it to bind to the nanomaterials or cell membrane, hence preventing the precipitation of the nanoparticles and enhancing the cellular uptake [32,33]. The selenium nanoparticles capped with PSP showed average diameter of <50 nm and exhibited specific cytotoxicity against MCF-7 cells with IC_{50} value of 3.7 μM while non-toxic towards normal cell Hs68 human fibroblast with IC_{50} value of more than 100 μM [34]. Similar study was conducted by Zhang et al. (2010) in which the selenium nanoparticles were capped with hyperbranched polysaccharide β-D-glucan extracted from sclerotia of PTR. The hyperbranched polysaccharide nanoparticles exhibited mean particle diameter of 24 nm with improved colloidal stability [32]. However, no further efficacy study was reported since then.

Interestingly, similar study investigated the use of water soluble PSP extracted from another type of edible mushroom sclerotia, *Polyporus rhinoceros* as the capping agent for selenium nanoparticles [35,36]. The study proved that the capping enhanced the cytotoxicity of selenium nanoparticles against human lung adenocarcinoma A549 cells with IC_{50} value of 4.1 μM. The study also suggested that the PSP could be used as the targeting moiety in drug delivery as well as intrinsic anti-cancer agent [36].

Apart from edible mushroom, the extract from toxic mushroom was also explored as the capping agent for nanoparticles. Ivashchenko and co-workers used the extract of *Amanita muscaria* for the synthesis of silver and ultrasmall iron oxide nanoparticles. The addition of glucans and muscimol from *A. muscaria* to the surface of nanoparticles showed enhanced cytotoxic activity in spheroid HeLa cells with 50 % and 40 % cell viability after treated with 7.5 mg/ml of the nanoformulation for 48 and 72 h, respectively [37].

4.3. Fungal enzyme as prodrug activator

One of the approaches to alter a bioactive compound's solubility, pharmacokinetic properties and toxicity is via prodrug design. Enzymatic approach such as glycosylation and rhamnosylation are some of the feasible strategies to design prodrug [38]. In comparison to glycosylation, rhamnosylation is less commonly explored as the process requires nucleotide donor UDP-Rha or dTDP-Rha which could not be achieved through chemical synthesis [39]. Li and colleagues explored the use of fungi-derived enzyme, α-L-rhamnosidase (RhaL1) in production of prodrugs for anti-cancer agents including 2′-deoxy-5-fluorouridine (FUDR), cytosine arabinoside (Ara C) and hydroxyurea (Hydrea) via one-step rhamnosylation process [40]. Interestingly, these prodrugs only exhibited cytotoxic effect against breast cancer MDA-MB-231 cells after exposure to α-L-rhamnosidase, suggesting a targeted drug delivery at tumour sites.

Another example of enzyme-activated prodrug therapy is the yeast cytosine deaminase (yCD). It is a prodrug converting enzyme that catalyse the deamination of cytosine to uracil. Researchers have explored the use of this enzyme in converting the non-toxic prodrug 5-fluorocytosine (5-FC) to cytotoxic 5-fluorouracil (5-FU) [41]. Lieser and the team has found that the cytotoxic effect of the yCD and 5-FC was almost the same as treatment with 5-FU alone [42]. As 5-FU has always been associated with long-term side effects such as gastrointestinal and cardiovascular toxicities, the treatment of yCD/5-FC may offer a better option with minimised toxicity [42]. Even though cytosine deaminase can be found in both bacteria and yeast, yCD has better therapeutic effect than bCD as yCD is more efficient in converting the 5-FC to 5-FU and more thermostable than bCD [43]. Another novel approach is to manipulate human mesenchymal stem cells (MSC) exosome as a cargo to deliver yCD. Upon internalisation by tumour cells, yCD-UPRT converts nontoxic 5-FC to 5-FU at the site of the tumour to kill cancer cells but did not inhibit the growth of noncancerous human skin fibroblast and dental pulp MSCs [44].

5. Fungal organelles

5.1. Yeast cell wall capsular microparticles

With the aim to enhance the selectivity of drug toward tumour, many chemotherapeutic drugs have been packaged into drug cargo for *in vivo* test. The strategy seemed to achieve considerable success since the targeting capability is not solely relying on the selectivity of drug alone. Perhaps the first drug capable of with targeting was doxorubicin. Marketed in 1995, the liposomal formulation of doxorubicin can be concentrated in tumour lesion 10 times more than free drug [45]. Apart from liposomes, many other nature-like carriers have been investigated. This includes lipids, proteins, virus, lipoproteins and even whole cells, primarily for cell-tracking purposes. Yeast cell wall particles (YPs) are a new class of natural carriers proposed. Yeasts are cells whose membrane consists of b-1,3-D-glucan polymers associated with mannose-containing proteins and chitin. Like other biomolecules, YPs are well tolerated by living systems and can be processed into small fragments in macrophages. Moreover, b-1,3-D-glucan is an excellent targeting vector towards the dectin-1 receptor, which is exposed on the membrane of several phenotypes of antigen presenting cells [46].

Saccharomyces cerevisiae is a species of fungus and has been instrumental in winemaking, baking, and brewing since ancient times. Due to its capsules that comprised primarily of b-1,3-D-glucan, it is one of the most studied yeasts for YPs constructions. Being a single cell microorganism, its capsule was deemed promising to be processed into a carrier system. In a study conducted by Ren et al. (2018), the yeast cells were processed into porous and hollow yeast cell wall microparticles, which was used to harbour nanoparticles [47]. Within the nanoparticles contained cabazitaxel, a cytotoxic chemotherapy used for prostate cancer. The strategy was to exploit the polysaccharides that can be recognised by the dectin-1 (apical membrane receptor) which is highly expressed on macrophages and intestinal M cells. Thus, achieving macrophage-targeted oral delivery of cabazitaxel (CTX). The association of the nanoparticles with YPs is via electrostatic self-deposition and was verified using electron microscopes. The YPs was able to achieve longer sustained release of cabazitaxel in comparison to its nanoparticulate form at intestinal pH. Although the oral bioavailability CTX-YPs formulation was 32.1 %, but it was 5.7 times higher than that of CTX alone. Hence, this nano-in-micro carrier system is believed to become a

hopeful alternative strategy for increasing the oral absorption of small molecule drugs. Cisplatin, a platinum-based chemotherapeutic agent, has also showed a similar results [48]. Zhou and colleague demonstrated that although the formulation was administered orally, YCs could accumulate in A549 human lung carcinoma xenografts in mice, achieving by monocyte/macrophage-mediated translocation via the lymphatic system. This result was comparable with IV cisplatin administration. This biomimetic approach serves as an effective strategy for targeted oral chemotherapies. Compare to the use of bacteria as carriers for active pharmaceutical ingredients, yeast offers more advantages such as smaller size, easy cultivation, shorter growth rate and most important less toxic as they do not contain toxic cellular component such as lipopolysaccharides and super antigens that are commonly present in gram-negative and gram-positive bacteria, respectively [49].

5.1.1. Compound solubility and compatibility to capsular microparticles

The compatibility of drug carriers with a drug model is determined by the general properties of drug molecules. The hydrophilic nature of the polysaccharidic membrane typically does not allow the incorporation of amphiphilic chemicals as typically done for lipid-based particles e.g., liposomes. Even for lipid-based particles, attempts to stably include hydrophilic molecules in the particle core was unsuccessful due to the long alkyl chain in lipids and high porosity of the wall. Nevertheless, the peculiar chemical stability of yeast walls can undertake a new loading procedure in which the inner cavity of the particle may act as a microreactor, thus allowing the formation of large size self-assembling systems (e.g., emulsions) that, once formed, remain entrapped in the particle. These properties allow the fungi capsule to deliver both amphipathic and lipophilic molecules. A study conducted in 2011 successfully incorporated and verified the imaging capability of lipophilic rhodamine-DPPE and water insoluble paramagnetic complex Gd-DOTAMA(C18)$_2$ [50]. These dyes are used for laser-scanning confocal microscopy and magnetic resonance imaging (MRI), respectively.

In addition to delivering small molecules, YPs are also compatible with macromolecules such as nucleic acid. The baker's yeast *Saccharomyces cerevisiae* has been developed as a live functional nucleic acid vaccine vehicle for oral and subcutaneous delivery [51]. The yeast vehicle derived from *S. cerevisiae* was loaded with *MYD88* and *TNF* mRNA for targeting the primary human macrophage that plays crucial roles in tumour progression. The study proved that internalisation of these mRNA has yielded a two-fold increase in the secretion of pro-inflammatory TNF-α protein in the M2-macrophage, suggesting that baker's yeast could be developed as an effective vaccine delivery system for cancer immunotherapy [51].

5.1.2. Route of administration

In clinical practices, chemotherapeutic drugs are often parenterally administered. However, capsular microparticles are larger and hence intravenous administration may increase the risk of embolism. Although the administration of capsular microparticles is only restricted to oral administration, considerable bioavailability could still be achieved [47]. This is attributed to a specialised cell type present in the Peyer's patches along the lining of the intestinal lumen, the M cells. As one of the lymphoid structures, antigen uptake by M cells contributes to not only mucosal but also systemic immune responses. The uptake of particulate materials (i.e., microorganisms) by M cells was suggested to be one of the mechanisms involved in explaining the increase bioavailability. This route bypasses the liver first-pass metabolism as it travels through the lacteal after taken up by the macrophages, instead of traveling in blood stream. Although there is evident implying that the uptake of microorganisms by M cells in PPs may play a role in the immune-induced targeting, but the extend of orally-dose particles that gets into the systemic circulation is still unclear [52].

5.2. Secreted extracellular vesicles

Nanoparticles are often prepared from a defined composition of chemicals in controlled conditions. However, nanoparticulate structure resemble extracellular vesicles secreted by fungi was recently discovered [53,54]. The extracellular vesicles secreted by *Arthrobotrys oligospora* were composed of glycoprotein mixture, with about 3% glycoaminoglycan and 55 % protein. Wang et al. (2014) had investigated the vesicles' immunostimulatory activities, cytotoxic mechanisms and *in vitro* immunochemotherapeutic effects. The nanoparticles of different charges revealed an enhanced secretion of proinflammatory cytokines and chemokines from RAW264.7 macrophages and splenocytes, including TNF-α. Nevertheless, FNP2 has stronger cytotoxicity and induced apoptosis and cell cycle arrest. The study incorporated doxorubicin into the FNP2. Albeit the mechanism of incorporation is simple via electrostatic interactions, but such method may not be most preferred in *in vivo* settings. Normal cells could still be exposed to the doxorubicin since the anticancer drug is adsorbed on the surface of the carriers rather than shield within the core. Furthermore, plasma protein interaction with the nanoparticles is a dynamic process, causing displacement of the attached drug resulting in burst release.

6. Enhanced anticancer activities of fungal secondary metabolites with nanotechnology

There has been a significant increase in the number of anticancer agents isolated from endophytic fungi following the first report of the production of paclitaxel by a fungus [55]. The classification and efficacy of anticancer agents isolated from fungus have been discussed by a great number of authors in literature [10,56–58]. Despite the promising preclinical data, the pharmacological use of these anticancer agents in clinical setting is hindered by their low water solubility, poor bioavailability and toxicity. Therefore, a suitable carrier system is desired for its delivery towards cancer cells. In recent years, nanocarriers based delivery systems have gained considerable interest for delivery of chemotherapeutic agents due to their size, shape and selectively accumulation in tumours by enhanced permeation and retention effect which leads to enhanced anticancer efficacy with reduced side effects (Fig. 2 **and** Table 1). In this section, the use of nanotechnology in improving the abovementioned limitations is discussed.

Monascus yellow pigments (MYPs) are hydrophobic secondary metabolites isolated from *Monascus* species. Monascin (MNS) and ankaflavin (ANK), the two major bioactive components of MYPs isolated from red-mold rice have been reported to inhibit peroxynitrite- and UVB-induced mouse skin carcinogenesis [59] as well as the growth of HepG2 liver cancer and A549 lung cancer cells [60]. Due to the hydrophobicity, a multicompartmental nanovehicle comprised of MNS/ANK-loaded casein micelles (CAS MCs) enveloped within phytosomal resveratrol (RSV) bilayer was developed [PC-CAS MCs(F_2)] [61]. Casein has an amphiphilic nature being composed of hydrophobic and hydrophilic amino acids readily self-assembles in aqueous solution into nanosized micelles [62]. Besides acting as an RSV carrier enhancing its release, the outer phytosomal bilayer enveloping CAS MCs also acts as an additional barrier toward MYPs release resulting in reduced initial burst of MYPs from interior core compared with single-compartment MCs. For comparison, both compounds (RSV and MYPs) were simultaneously incorporated into the hydrophobic core of CASMCs without bilayer envelope [CAS MCs (F_1)]. Results showed that CAS MCs (F_1) (IC_{50} = 23.2 μg/mL) and PC-CAS MCs (F_2) (IC_{50} = 20.5 μg/mL) were more potent in inhibiting the growth of MCF-7 breast cancer cells in comparison to free RSV/MYPs combination (IC_{50} = 35.0 μg/mL). Interestingly, PC-CAS MCs (F_2) was the most effective formulation to suppress breast tumour growth in ehrlich ascites tumour -inoculated mice (60 mm^3) as compared to CAS MCs (F_1) (402 mm^3), free RSV (883 mm^3), free MYPs (1025 mm^3) and free RSV/MYPs combination (762 mm^3) [61]. The astounding efficacy of PC-CAS MCs (F_2) was attributed

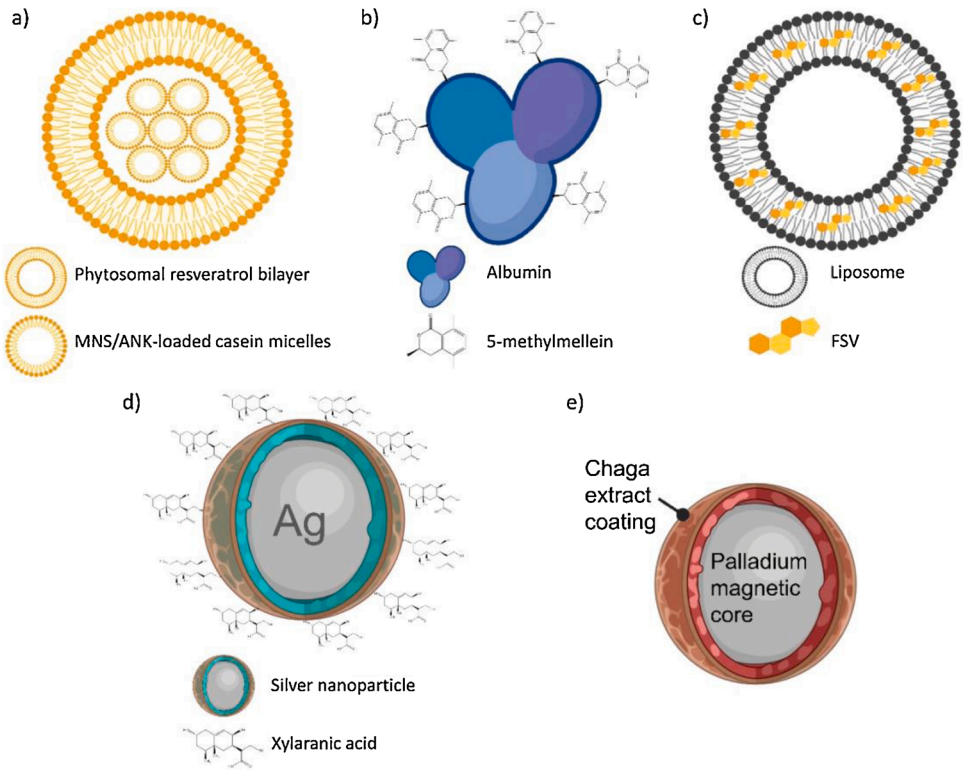

Fig. 2. Nanoformulation of fungal secondary metabolites for enhanced pharmacokinetic profiles and targeted therapy. (a) A multi-compartmental nanovehicle comprised of MNS/ANK-loaded casein micelles enveloped within phytosomal resveratrol bilayer was developed. The outer phytosomal bilayer enveloping casein micelles acts as an additional barrier toward MNS/ANK release resulting in reduced initial burst of MNS/ANK from interior core compared with single-compartment, thus leading to higher efficacy, reduced toxicity and may overcome the multidrug resistance. (b) 5-methylmellein was formulated with albumin-based nanoparticles. The enhancement in cytotoxicity was due to increased endocytosis of 5-methylmellein albumin-based nanoparticles in cancer cells. (c) FVS-loaded liposomes was found to have increased bioavailability and widely distributed to liver and spleen. (d) Xylaranic acid was formulated into silver nanoparticle to improve its low water solubility and poor bioavailability. (e) Aqueous extract palladium nanoparticles were synthesised to kill tumour by dual actions: anticancer effects from chaga mushroom and hyperthemic effect by manipulating magnetic properties of palladium. Abbreviation: MNS : Monascin; ANK : Ankaflavin; FSV: *Flammulina velutipes* (Created with BioRender.com).

to the biphasic mode of release with a higher initial burst release of RSV followed by about a slow released after 4 h, MYPs were slowly released from the core which exert a more prolonged tumour growth suppressive effect and enhanced uptake of PC-CAS MCs (F2) into cancer cells. The level of tumour growth biomarker aromatase, VEGF, NF-κB and CD1 were the lowest in PC-CAS MCs (F_2) group. In addition, PC-CAS MCs (F_2) group also showed the highest level of caspase-3 [61]. Taken all these together, coadministration of MYPs with RSV can enhance the anticancer effects of both drugs via modulating different signalling pathways, thus leading to higher efficacy, reduced toxicity and may overcome the multidrug resistance.

5-methylmellein isolated from *Xylaria psidii*, an endophytic fungus of the medicinal plant *Aegle marmelos* demonstrated anticancer activity but it is sparingly soluble in water [63]. FDA-approved formulation albumin-based nanoparticles (BSANPs) were then used to formulate this compound [64]. The advantages of BSANPs are nontoxic, biodegradable, biocompatible, hydrophilicity and can specifically bind to a cell surface glycoprotein receptor (E.g.: albondin, gp60) which is often highly expressed on cancer cells' plasma membrane. In this study, BSANPs alone was not toxic towards cancer cells up to 10 mg/mL. Nevertheless, the incorporation of 5-methylmellein into BSANPs resulting in a higher percentage of cell inhibition towards PC-3 (1.3 μg/mL), HCT-116 (< 0.5 μg/mL) and MCF-7 (< 0.5 μg/mL) cancer cell lines in comparison to 5-methylmellein alone with increased rate of apoptosis, mitochondrial membrane potential loss and generation of high reactive oxygen species. The enhancement in cytotoxicity of 5-methylmellein BSANPs may be due to increased endocytosis of 5-methylmellein BSANPs in these cancer cells which is further supported by other similar studies [64]. These findings collectively indicated that BSA nanoparticles may serve as promising drug delivery system for improving the efficacy of 5-methylmellein.

Flammulina velutipes (Curt. ex Fr.) Sing. has been extensively studied for its nutritional components and biological activities for several decades. Nevertheless, the potential anticancer effects of sterols extracted from this species (FVS) were not well documented. In 2013, Yi et al. (2013) determined the anticancer effect of FVS *in vitro* and evaluated the pharmacokinetics profile of FVS-loaded liposomes *in vivo* [65]. FVS which is mainly composed of ergosterol and 2223-dihydroergosterol, inhibited the growth of HepG2 liver and A549 lung cancer cells with IC_{50} values of 9.3 and 20.4 μg/mL, respectively. To improve its poor solubility, FVS was encapsulated in liposomes (FVSL). After oral administration in Kunming mice, FVSL was mainly distributed in liver and spleen. In comparison to its two free sterol, the relative bioavailability of ergosterol and 2223-dihydroergosterol in FVSL was significantly increased to 163 and 244 %, respectively [65]. These findings support the fact that FVS, a potential nutraceutical and an effective drug for the treatment of liver cancer, could be encapsulated in liposomes for improved solubility and bioavailability.

Xyralia primorskensis is a fungus of genus Xylaria, belongs to the family Xylariaceae. Xylaranic acid, a major terpenoid compound isolated from *Xyralia primorskensis* was reported to possess antimicrobial, antioxidant and anticancer activities. Nevertheless, its terpenoid nature limits its pharmacological use due to its low water solubility and poor bioavailability. As such, effort has been performed to improve these limitations by encapsulating xylaranic acid into silver nanoparticle (XA-AgNPs). In anticancer study, XA-AgNPs was more potent towards A549 lung cancer cells terms of IC_{50} value as compared to xylaranic acid alone (25 μg/mL vs 39 μg/mL). The study also suggested that XA-AgNPs induced apoptosis in A549 lung cancer cells via upregulation of tumour suppressor p53 and caspase-3 genes and downregulation of BCL-2 gene in a time- and dose-dependent manner. Despite the promising data, the safety profile of XA-AgNPs was not evaluated in the study [66].

Inonotus obliquus, also known as chaga mushroom, is a parasitic mushroom in birch trees. Chaga mushroom has wide biological activities including anticancer [67]. To enhance the delivery efficiency and maximize the characteristics of chaga mushroom, its aqueous extract palladium nanoparticles (chaga-PdNPs) were synthesised [68]. The purpose of this formulation was to kill tumour by dual actions: anticancer effects from chaga mushroom and hyperthemic effect by manipulating magnetic properties of palladium. In this study, hyperthermic cancer cell death was achieved only in the designated region by laser irradiation. Thus, it was verified that chaga-PdNPs could cause

selective cancer cell death via photothermal conversion in addition to the anticancer effect of surface-coated chaga extract. Similar work was also performed on gliotoxin, a secondary metabolite produced by *Aspergillus* molds with the purpose to reduce the toxicity of this compound. Gliotoxin has been shown to inhibit angiogenesis and suppress tumour growth in mice with prostate cancer xenografts [69]. In this study, gliotoxin bound to magnetic nanoparticles retained a high anti-tumour activity and selectivity towards cancer cells [70,71].

Trichorderma atroviride, the filamentous cosmopolitan fungus, produced the metabolite TM2 which showed anti-prostate cancer efficacy. Saravanakumar et al. (2021) improved the therapeutic effect of TM2 by encapsulating the fungal metabolite in chitosan nanoparticles. The drug delivery system with diameter of around 188 nm, exhibited better cell death in PC3 cells via stimulation of ROS, DNA damage and caspase 3 [72].

7. Promising fungal metabolites in Cancer Clinical trials

A number of fungal metabolites and/or their analogues such as anguidine, aphidicolin, fumagillin, illudin S, irofulven, rhizoxin, wortmannin, plinabulin (NPI-2358, a semisynthetic analogue of Phenylahistin) and sonolisib (PX-866, a synthetic analogue of wortmannin) have progressed to various stages of cancer clinical trials. Nevertheless, only plinabulin and sonolisib could be translated into clinically used drugs due to their low toxicities and high efficacies. The remaining of the compounds failed in clinical trials due to serious toxicities and/or poor water solubility. In the meantime, apicidin, chaetocin, cotylenin A, destruxin, gliocladicillins, myriocin and palmarumycin have otained promising results in the preclinical testing. Based on the search in https://clinicaltrials.gov, only FTY-720 (Fingolimod/Gilenya, a synthetic analogue of myriocin) has progressed into clinical trials so far. To know more about the history of the abovementioned compounds, it is recommended to read the review papers written by Banerjee and Paruthy (2017) [74] and Kornienko et al. (2015) [75]. In this section, we intend to provide the latest update of plinabulin, sonolisib and fingolimod in clinical trials.

Phenylahistin consists of phenylalanine and isoprenylated dehydrohystidin, which was isolated from Aspergillus ustus as racemic mixture [76]. This compound exerts anti-tumour activity by disrupting tumour vascular endothelial cell architecture resulting in selective collapse of established tumour vasculature [77]. In 2006, plinabulin was evaluated in Phase I clinical trial by examining the safety, pharmacokinetics and pharmacodynamics of escalating doses of plinabulin in patients with refractory solid tumours or lymphoma [NCT00322608 at https://clinicaltrials.gov] [78]. At the recommended phase II dose of 30 mg/m^2 which was escalated from 2 mg/m^2, plinabulin significantly decreased tumour blood flow, tumour pain and showed a favorable safety profile [79]. Following the promising data in this trial, plinabulin has been actively evaluated for lung cancer in combination with other anticancer agents. A Phase I study of plinabulin + docetaxel

Table 2
Cancer clinical trials of plinabulin, sonolisib and fingolimod.

Compound Name	Trial Design	Study Population	Outcome	References
Plinabulin (NPI-2358)	Phase I study to assess the recommended Phase 2 dose of plinabulin combined with docetaxel	13 Patients including 8 with NSCLC	- Among patients with NSCLC, 2 achieved a partial response and 4 demonstrated lesser decreases in tumour measurements	[77]
	Phase I study to evaluate the safety, pharmacokinetics and pharmacodynamics of escalating doses of plinabulin	38 patients with various types of solid tumours or lymphoma	- Decreased tumour blood flow, tumour pain and showed a favorable safety profile	[79]
	Phase I/II study to evaluate the efficacy of plinabulin + docetaxel in patients with advanced NSCLC	172 patients	- Study is completed, but no results posted	[80]
	Randomized blinded phase III assessment of second or third-line chemotherapy with docetaxel + plinabulin compared to docetaxel + placebo in patients with advanced NSCLC	559 patients	- Study is ongoing	[81]
	Phase I study of nivolumab in combination with escalating doses of plinabulin in patients with metastatic NSCLC	38 patients	- Study is ongoing	[82]
	Phase I/II study of nivolumab, ipilimumab and plinabulin in patients with recurrent SCLC	55 patients	- Study is ongoing	[83]
Sonolisib (PX-866)	Phase II study in recurrent glioblastoma	33 patients	- Response rate was low - 21 % of patients obtained durable stable disease	[85]
	Phase II study in patients with recurrent or metastatic castration-resistant prostate cancer	68 patients	- Sonolisib had modest single agent activity - Adding abiraterone acetate to sonolisib did not reverse the cancer resistance - Well tolerated	[86]
	Phase I study to evaluate for maximum-tolerated dose, safety, pharmacodynamics, pharmacokinetics and antitumor activity	84 patients with various types of tumours	- Associated with prolonged stable disease, particularly when using a continuous dosing schedule	[87]
	Randomized, Phase II trial of cetuximab with or without sonolisib in patients with metastatic colorectal carcinoma	85 patients	- No improvement in terms of progression-free survival, objective response rate, or overall survival. - The combination had greater toxicity	[88]
	Randomized phase II study evaluated sonolisib combined with cetuximab in patients with advanced, refractory head and neck squamous cell cancer	83 patients	- No improvement in terms of progression-free survival, objective response rate, or overall survival.	[89]
	Phase I/II combination therapies (sonolisib + vemurafenib) in advanced BRAF-mutant melanoma	24 patients	- Study is completed, but no results posted	[90]
	Phase I/II combination therapies (sonolisib + docetaxel) in NSCLC and squamous cell carcinoma of the head and neck	223 patients	- Study is completed, but no results posted	[91]
Fingolimod (FTY-720)	Early Phase I to evaluate the safety of fingolimod with radiation and temozolomide in newly diagnosed high grade glioma	5 patients	- Study is completed, but no results posted	[94]
	Phase I to study how well fingolimod in preventing chemotherapy-induced nerve pain (neuropathy) in patients with breast cancer who are taking paclitaxel	20 patients	- Study is ongoing	[95]

Abbreviation: NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

demonstrated that this combination is tolerable. Of the 8 patients with non-small cell lung cancer (NSCLC), 2 achieved a partial response and 4 demonstrated lesser decreases in tumour measurements [77]. Clinical trial [NCT00630110] on patients with advanced NSCLC with primary outcome endpoint comparing overall survival of patients treated with docetaxel to patients treated with docetaxel + plinabulin has been completed, but results are not posted yet [80]. Randomized blinded phase III assessment of second or third-line chemotherapy with docetaxel + plinabulin compared to docetaxel + placebo in patients with advanced NSCLC [NCT02504489] is ongoing but not recruiting [81]. It is hoping that a positive outcome could be achieved in this phase III study. Currently patient recruitment is ongoing for Phase I study of nivolumab in combination with escalating doses of plinabulin in patients with metastatic NSCLC [NCT02812667] [82] and Phase I/II study of nivolumab, ipilimumab and plinabulin in patients with recurrent SCLC [NCT03575793] [83] (Table 2).

Sonolisib is an analogue of Wortmannin, which inhibits phosphatidylinositol 3-kinases and phosphatidylinositol 3-kinase-related kinases [84]. Sonolisib has been used in trials studying the treatment of glioblastoma [85], prostate cancer [86], advanced solid tumours [87], metastatic colorectal carcinoma [88], relapsed or metastatic head and neck squamous cell cancer [89], advanced BRAF-mutant melanoma [NCT01616199] [90] and NSCLC [NCT01204099] [91]. In a first-in-human Phase I study to evaluate for maximum-tolerated dose, safety, pharmacodynamics, pharmacokinetics and antitumor activity, sonolisib was well tolerated and was associated with prolonged stable disease, particularly when using a continuous dosing schedule [87]. Nevertheless, subsequent Phase II clinical trials are somehow disappointing. Sonolisib did not meet the predefined efficacy endpoints for glioblastoma study except for 21 % of the participants obtained prolonged stable disease [85]. Indeed, the addition of sonolisib to cetuximab did not improve progression-free survival, objective response rate and overall survival in metastatic colorectal carcinoma [88] as well as relapsed or metastatic head and neck squamous cell cancer [89]. A study also evaluated the combination of abiraterone acetate + sonolisib in patients with rising prostate-specific antigen. Although sonolisib had modest single agent activity, nevertheless, adding abiraterone acetate to sonolisib did not reverse the cancer resistance [86]. Combination therapies in advanced melanoma (sonolisib + vemurafenib, NCT01616199] [90] and NSCLC (sonolisib + docetaxel, NCT01204099] [91] have been completed, but no results of these studies have been posted yet.

Fingolimod is a potent immunosuppressant which was approved as a first-line therapy for relapsing forms of multiple sclerosis in 2010 [92]. Evidences in preclinical studies demonstrated that fingolimod is more than just an immunosuppressant in which it could be used to treat cancer [93]. A safety study of fingolimod with radiation and temozolomide in newly diagnosed high grade glioma [NCT02490930] has been completed in 2017, but reports are not published [94]. Currently patient recruitment is ongoing to evaluate how well fingolimod works in preventing chemotherapy-induced neuropathy in patients with breast cancer who are taking paclitaxel [NCT03941743] [95]. It is hoping that a positive outcome could be achieved in these two studies.

8. Fungus for development of cancer vaccine in immunotherapy

Cancer treatment vaccines are therapeutic vaccines designed to activate the patient's own immune system to mount an attack against established malignant or virally (human papillomavirus, hepatitis B and C virus, etc.) infected cells in the body, offering the potential of cancer eradication and prevention of cancer recurrence. These cancer treatment vaccines are different from the vaccines that are used to fight against infectious diseases, instead of the utilisation of killed or attenuated organisms as source of immunogens, cancer vaccines are made up of whole or parts of cancer cells or pure antigens (certain proteins on the cancer cells). The fundamental purpose of cancer vaccine is to trigger immune response, stimulate tumour- or viral-specific cytotoxic T lymphocyte ($CD4^+$ and $CD8^+$) responses, reducing the disease burden, thus achieving the ultimate goal of therapeutic vaccine. The advancement in molecular biology and protein chemistry have enabled the expression of heterologous proteins in cells of prokaryotes and eukaryotes without compromising the ability of the expressed protein to induce immune response. In particular, yeast or unicellular fungus have emerged as ideal model for expression of heterologous proteins in vaccine development due to their highly efficient heterologous gene expression system, availability of genome sequence, responsiveness to genetic manipulation and cost-effectiveness in large-scale manufacturing. Yeast is highly suitable to be used in the development of cancer vaccine, as it can induce immunologic responses through expression of tumour-specific or tumour-associated antigens. The work done based on different mechanisms are discussed in this section.

8.1. Yeast cell wall component induced immunologic response

Although yeast is non-pathogenic in nature, it has been shown to induce immunologic responses in mammals. It is deduced that the polysaccharides β-1, 3-D-glucan (BGs) and mannan on the yeast cell wall possessed strong adjuvant properties, allowing detection by pattern recognition receptor and phagocytosis by antigen-presenting cells (APCs). Studies have demonstrated that yeasts are avidly taken up by APCs and macrophages, such as dendritic cells (DCs) for presentation to major histocompatibility complex (MHC) class I and class II pathways [96]. The MHC presentation could be recognised by cytotoxic T-cells, resulting in subsequent proliferation, maturation and activation of $CD4^+$ and $CD8^+$ T cells. In the presence of yeast, the interaction signals due to engagement of T-cell receptor and peptide-MHC complex together with interaction between DC stimulatory molecules and T-cell ligands could be enhanced, leading to increased expression of costimulatory molecules on DCs [96]. In addition, the activated $CD4^+$ helper T cells release immunostimulatory Th-1 type inflammatory cytokines, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α), further inducing activation of $CD8^+$ killer T cells for the elimination of abnormal cells [62]. The therapeutic performance of $CD8^+$ T cells is achieved via release of cytotoxins perforin and granzymes for cell death, and interaction with surface protein Fas ligands on abnormal cells for cell apoptosis. Therefore, the activation of $CD4^+$ and $CD8^+$ T cells by yeast cell wall components has demonstrated the suitability and potential of yeast-based therapeutic vaccine for combating cancer.

8.2. Engineered yeast for expression of heterologous protein

Yeasts are widely used in the production of recombinant proteins for biomedical applications as they combine the advantages of unicellular organisms (easy to grow and manipulate genetically into expressing viral or tumour antigens) with the ability to perform eukaryotic post-translational modifications. *Saccharomyces cerevisiae* is the first and well-established model yeast expression system developed in the 1980s and has been engineered to express a wide variety of recombinant proteins. For instance, *S. cerevisiae* is popular in expressing cancer-related antigens in the form of virus-like particle, purified protein and also the use of whole recombinant yeast for vaccine development as shown in Table 3. Human papillomavirus (HPV) has been known to cause cancer (cervical, vulvar, vaginal, penile, anal and oropharyngeal) while yeast-based vaccine is useful in cancer prevention with the first yeast-based vaccine against HPV formulated with HPV16 L1 virus-like particles approved by the US Food and Drug Administration (FDA) in 2006 [97]. *S. cerevisiae* expressing recombinant HPV L1 protein could be applied as prophylactic HPV vaccine as L1 is the major capsid protein with the ability to spontaneously self-assemble into virus-like particle. A great deal of research was performed focusing on the engineering of *S. cerevisiae* in expressing HPV types 16, 18 and 58 antigens for vaccine development [98–100]. In addition, *S. cerevisiae* was also engineered to

Table 3
Expression of cancer-related antigens by different yeast species for vaccine development.

Yeast species	Antigen/Immunogen		Cancer Type	Strategy	Reference
Saccharomyces cerevisiae	Human Papillomavirus (HPV)	HPV16 L1	Cervical, vulvar, vaginal, penile, anal, and oropharyngeal	Virus like particle	[98,100,113,114]
				Purified protein	[115]
		HPV18 L1		Virus like particle	[116]
		HPV58 L1			[99]
	Hepatitis B virus	Hepatitis B surface antigen (HBsAg)	Liver	Virus like particle	[102,117,118,119]
				Purified protein	[101]
		Hepatitis B core antigen (HBcAg)		Purified protein	[120]
		Hepatitis B X, surface (S) and core antigen (X-S-Core)		Whole recombinant yeast	[103]
	Hepatitis C virus (HCV)	HCV NS3-Core fusion protein	Liver, head and neck	Whole recombinant yeast	[121]
Pichia pastoris	Human Papillomavirus (HPV)	HPV16 L1	Cervical, vulvar, vaginal, penile, anal and oropharyngeal	Virus-like particle	[122,123,124]
				Purified protein	[125]
		HPV16 L1-L2		Purified protein	[126]
		HPV18 L1		Virus-like particle	[122,123]
		HPV58		Virus-like particle	[127]
	Hepatitis B virus	Hepatitis B surface antigen (HBsAg)	Liver	Virus-like particle	[128,129,130]
				Purified protein	[131,132,133,134,135]
		Hepatitis B core protein		Virus-like particle	[136,137]
	Hepatitis C virus (HCV)	HCV core protein (HCcAg)	Liver, head and neck	Virus-like particle	[138]
				Purified protein	[139]
		HCV CoreE1E2		Purified protein	[140]
Hansenula polymorpha	Human Papillomavirus (HPV)	HPV16 L1	Cervical, vulvar, vaginal, penile, anal, and oropharyngeal	Virus-like particle	[141]
				Purified protein	[142]
		HPV16 L1-L2		Purified protein	[126]
		HPV52 L1		Virus-like particle	[143]
		HPV6/11		Virus-like particle	[38]
	Hepatitis B virus	Hepatitis B surface antigen (HBsAg)	Liver	Purified protein	[144]
				Whole recombinant yeast	[145]
Saccharomyces cerevisiae	Brachyury (GI-6301)		Chordoma, Epithelial-mesenchymal transition	Whole recombinant yeast	[146,147]
	Human MART-1		Melanoma	Whole recombinant yeast	[107,148]
	Carcinoembryonic antigen (CEA)		Colon, prostate, ovarian, lung, thyroid, liver	Whole recombinant yeast	[109,149,150]
	KRAS		Lung	Whole recombinant yeast	[111]
	BCR-ABL(T315I)		Leukaemia	Whole recombinant yeast	[110]

express Hepatitis B virus-related antigen for use as prophylactic vaccines [101,102]. It is noteworthy that the whole recombinant yeast is a suitable vector in overcoming the storage, transportation and shelf-life issues of peptide-based vaccines as the cellular environment could provide long-term stability and maintenance of protein structure. With this intention, King and colleagues (2014) developed yeast-based vaccine expressing hepatitis B virus X, surface and core antigens (X-S-Core) that could elicit functional adaptive immune responses in patients with chronic hepatitis B infection [103]. The whole recombinant yeast-based immunotherapy was also employed for the expression of Hepatitis C virus NS3-core fusion protein to induce potent antigen-specific proliferative and cytotoxic T cell responses in mice [104].

Apart from the expression of viral antigens, *S. cerevisiae* was also engineered to express cancer-associated proteins that can augment the number of cytotoxic T-cells crucial for recognition and elimination of tumour cells. In preclinical study, *S. cerevisiae* expressing brachyury (embryonic transcription factor) using whole recombinant yeast approach was applied to activate human T cells *in vitro*, resulting in stabilisation of disease, decreased tumour density and reduced serum carcinoembryonic antigen (CEA) in colorectal carcinoma patient [105]. Furthermore, the recombinant brachyury-yeast vaccine also elicits brachyury-specific $CD4^+$ and $CD8^+$ T-cell responses, contributing positively to preventing cancer progression by halting the epithelial-mesenchymal transition [106]. Prophylactic melanoma vaccine was generated using whole recombinant *S. cerevisiae* expressing MART-1, stimulating both $CD4^+$ and $CD8^+$ T-cells simultaneously but no reduction of regulatory T cells was observed, suggesting that the yeast vaccine cannot prevent immunotolerance in tumour microenvironment [107]. Injection of heat-killed *S. cerevisiae* expressing the tumour-associated antigen CEA has been shown to induce CEA-specific immune responses, reducing tumour burden, and extending overall survival rate [108,109]. Studies were also performed on expressing the lung cancer- and leukaemia-associated proteins (KRAS and BCR-ABL) in whole recombinant yeast for vaccine development [110,111]. Although *S. cerevisiae* has been applied in the development of various kinds of vaccine, issues related to plasmid instability, low protein yields and hyperglycosylation of proteins have led to the development of alternative expression systems, including two methylotrophic yeasts, *Pichia pastoris* and *Hansenula polymorpha* [112]. Similarly, *P. pastoris* and *H. polymorpha* were also engineered to express HPV-, Hepatitis B and C virus-related proteins as illustrated in Table 3. All these studies have demonstrated that the expression of tumour-specific or tumour-associated antigens in yeast and the application of whole recombinant yeast could elicit specific immune response, reduce tumour burden and extend survival rate. Hence, yeast-based vaccine holds promising potential in cancer immunotherapy.

9. Challenges and future perspective

Even with the claims of economic and environmental friendly, the clinical translation of fungi metabolites and bioengineered nanoparticles still remains challenging. For bioengineered metal

nanoparticles, one of the main obstacles is the unclear mechanism underlying the synthesis in cellular components, which indirectly hamper the standardisation of the experimental parameters during the synthesis process such as temperature, concentration of precursor metal ions and pH [18]. These parameters directly influence the physicochemical properties of nanoparticles, including the size, polydispersity, morphology and stability [151]. Despite efficacious against a wide range of cancers, more studies are needed to standardise synthesis protocol that is applicable for large-scale production. It is notable that a significant number of fungi metabolites have advanced to clinical trials. However, similar to other potential compounds, researchers still face difficulties to translate promising preclinical results into expected human responses due to poor pharmacokinetic properties and solubility. Researchers have attempted to formulate fungal metabolites and modifying their structures to solve the abovementioned issues. Nevertheless, this has to be evaluated in animal study and subsequently in clinical trials to prove their efficacy. Hopefully pharmaceutical companies would take the lead to bring all these promising analogues/formulations to benefit cancer patients.

Many yeast-based vaccines are currently in different phases of clinical trials with a few being commercialised. For instance, the quadrivalent HPV (HPV4) vaccine (Gardasil™) produced using *S. cerevisiae* expressing HPV L1 gene (type 6, 11, 16 and 18) has been licensed by the U.S. Food and Drug Administration on June 8, 2006 [152]. On the other hand, GS-4774 and GI-5005 are recombinant yeast-based vaccine producing HBsAg and HCV NS3-Core fusion protein, respectively. Both vaccines have undergone Phase II clinical trial [153,154]. Apart from virus-related antigen, recombinant yeast-brachyury (GI-6301) and yeast-KRAS (GI-4000) have completed Phase II trials [155,156]. Yeast-CEA (GI-6207) displayed well tolerance and increased T lymphocyte response at Phase I trial [157]. Although yeast-based vaccines have undergone different phases of clinical trials, some vaccine development did not progress further. GS-4774 did not show any clinical benefit at the Phase II trial, GI-5005 has been combined with standard of care (SOC) as a triple therapy to treat prior non-responders while GI-6301 demonstrated low conditional power for statistical difference at the planned end of accrual. For GS-4774, Phase II study was carried out with chronic Hepatitis B patients who were virally suppressed with approved HBV oral antiviral (OAV) drugs for more than 1 year and remained under the OAV treatment throughout the trial. This could prevent the detection of the effect of vaccine-induced immune response on HBsAg productions [158]. Therefore, it is crucial to take careful consideration in designing appropriate immunization protocols focusing on primary efficacy endpoints and correct selection of recruited populations. In addition, rational treatment combinations of vaccine with drug should also be explored to elucidate the potential therapeutic effect.

10. Conclusion

In the pursuit of finding new ways to treat cancers, method based on small molecule chemotherapeutics is not the only strategy that gained significant importance. The current chemotherapeutics regimen may be appealing for their synergistic effects against the cancerous cells, but their cytotoxic effects are also extended to harm healthy cells in the patients due to the lack of target specificity. While researcher may explore new drug candidate for better cancer targeting, other researcher incorporate technology into the existing drugs to improve their targeting. In this regard, all parts of fungal structures have been exploited to serve as component for constructing drug carriers that target cancers, directly or indirectly. In terms of the compound preference, both hydrophilic and hydrophobic compounds are compatible to the fungal capsule and can achieve higher oral bioavailability than ingesting the compounds alone. When the polysaccharides of fungi are extracted and incorporated in nanoparticulate structure, its surfaces can be functionalised with targeting ligands to further increase cancer specificity. Apart from fungal capsule and polysaccharides, the enzymes in fungi lysate are also used to synthesis inorganic nanoparticles through green synthesis. It should be noted that green synthesis is an emerging field of bionanotechnology that is touted to be more economic and environmental-friendly than chemical and physical methods. yCD, another class of enzyme, is a popular choice for prodrugs activator in fungal anticancer research.

Cancer immunotherapeutic treatment is a competing field to small molecule treatment. With the recent advance and success in clinical trials, the potential of immunotherapy is gradually revealed and draw the attention of pharmaceutical company. Unicellular fungus is an alternative model for the expression of heterologous proteins in vaccine development. This strategy can be achieved because fungi can be engineered to induces immunologic responses by the tumour-specific or tumour-associated antigens expressed on their surfaces. In summary, although currently there is no chemotherapeutics agents based on fungal metabolites, it appears to only be a matter of time before the rest of the fungal component will be added to the anti-cancer pharmaceutical armamentarium.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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References

[1] N.S. Yarla, A. Bishayee, G. Sethi, P. Reddanna, A.M. Kalle, B.L. Dhananjaya, K.S. V.G.K. Dowluru, R. Chintala, G.R. Duddukuri, Targeting arachidonic acid pathway by natural products for cancer prevention and therapy, Semin. Cancer Biol. 40-41 (2016) 48–81.
[2] K. Andrijauskaite, M.J. Wargovich, Role of natural products in breast cancer related symptomology: targeting chronic inflammation, Semin. Cancer Biol. (2020).
[3] D. Tewari, P. Patni, A. Bishayee, A.N. Sah, A. Bishayee, Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: a novel therapeutic strategy, Semin. Cancer Biol. (2019).
[4] D. Kashyap, H.S. Tuli, M.B. Yerer, A. Sharma, K. Sak, S. Srivastava, A. Pandey, V. K. Garg, G. Sethi, A. Bishayee, Natural product-based nanoformulations for cancer therapy: opportunities and challenges, Semin. Cancer Biol. 69 (2021) 5–23.
[5] M.M. Pompeo, J.H. Cheah, M. Movassaghi, Total synthesis and anti-cancer activity of all known communesin alkaloids and related derivatives, J. Am. Chem. Soc. 141 (36) (2019) 14411–14420.
[6] S. Scheeff, D. Menche, Total syntheses of the archazolids: an emerging class of novel anticancer drugs, Beilstein J. Org. Chem. 13 (2017) 1085–1098.
[7] W. Cui, A. Aouidate, S. Wang, Q. Yu, Y. Li, S. Yuan, Discovering anti-cancer drugs via computational methods, Front. Pharmacol. 11 (2020), 733.
[8] M. Berovic, B.B. Podgornik, Chapter 9 - cultivation of medicinal fungi in bioreactors, in: M. Petre (Ed.), Mushroom Biotechnology, Academic Press, San Diego, 2016, pp. 155–171.
[9] P. Vrabl, C.W. Schinagl, D.J. Artmann, B. Heiss, W. Burgstaller, Fungal growth in batch culture – what we could benefit if we start looking closer, Front. Microbiol. 10 (2391) (2019).
[10] S.K. Deshmukh, V. Prakash, N. Ranjan, Marine fungi: a source of potential anticancer compounds, Front. Microbiol. 8 (2018) 2536.
[11] M. Bahrami, T. Karimi, G. Yadegarfar, A. Norouzi, Assessing the quality of existing clinical practice guidelines for chemotherapy drug extravasation by appraisal of guidelines for research and evaluation II, Iran. J. Nurs. Midwifery Res. 24 (6) (2019) 410.
[12] C.-Y. Zhao, R. Cheng, Z. Yang, Z.-M. Tian, Nanotechnology for cancer therapy based on chemotherapy, Molecules 23 (4) (2018) 826.
[13] J. Huang, C. Wu, S. Tang, P. Zhou, J. Deng, Z. Zhang, Y. Wang, Z. Wang, Chiral active β-Glucan nanoparticles for synergistic delivery of doxorubicin and immune potentiation, Int. J. Nanomed. 15 (2020) 5083.
[14] A. Sathiyaseelan, K. Saravanakumar, A.V.A. Mariadoss, M.H. Wang, Biocompatible fungal chitosan encapsulated phytogenic silver nanoparticles

enhanced antidiabetic, antioxidant and antibacterial activity, Int. J. Biol. Macromol. 153 (2020) 63–71.
[15] Y. Wang, P. Li, F. Chen, L. Jia, Q. Xu, X. Gai, Y. Yu, Y. Di, Z. Zhu, Y. Liang, A novel pH-sensitive carrier for the delivery of antitumor drugs: histidine-modified auricularia auricular polysaccharide nano-micelles, Sci. Rep. 7 (1) (2017) 1–10.
[16] J. Qiu, H. Zhang, Z. Wang, D. Liu, S. Liu, W. Han, J.M. Regenstein, L. Geng, The antitumor effect of folic acid conjugated-Auricularia auricular polysaccharide-cisplatin complex on cervical carcinoma cells in nude mice, Int. J. Biol. Macromol. 107 (2018) 2180–2189.
[17] A.A. John, S.K. Jaganathan, M. Ayyar, N.P. Krishnasamy, R. Rajasekar, E. Supriyanto, Folic acid decorated chitosan nanoparticles and its derivatives for the delivery of drugs and genes to cancer cells, Curr. Sci. (2017) 1530–1542.
[18] P. Khandel, S.K. Shahi, Mycogenic nanoparticles and their bio-prospective applications: current status and future challenges, J. Nanostructure Chem. 8 (4) (2018) 369–391.
[19] K. Saravanakumar, S. Shanmugam, N.B. Varukattu, D. MubarakAli, K. Kathiresan, M.-H. Wang, Biosynthesis and characterization of copper oxide nanoparticles from indigenous fungi and its effect of photothermolysis on human lung carcinoma, J. Photochem. Photobiol. B, Biol. 190 (2019) 103–109.
[20] K.S. Siddiqi, A. Husen, Fabrication of metal nanoparticles from Fungi and metal salts: scope and application, Nanoscale Res. Lett. 11 (1) (2016) 98.
[21] B.C. Adebayo-Tayo, G.E. Ogunleye, O. Ogbole, Biomedical application of greenly synthesized silver nanoparticles using the filtrate of *Trichoderma viride*: anticancer and immunomodulatory potentials, Polim. Med. 49 (2) (2019) 57–62.
[22] K. Saravanakumar, E. Jeevithan, X. Hu, R. Chelliah, D.-H. Oh, M.-H. Wang, Enhanced anti-lung carcinoma and anti-biofilm activity of fungal molecules mediated biogenic zinc oxide nanoparticles conjugated with β-D-glucan from barley, J. Photochem. Photobiol. B, Biol. 203 (2020) 111728.
[23] K. Saravanakumar, R. Chelliah, D. MubarakAli, E. Jeevithan, D.-H. Oh, K. Kathiresan, M.-H. Wang, Fungal enzyme-mediated synthesis of chitosan nanoparticles and its biocompatibility, antioxidant and bactericidal properties, Int. J. Biol. Macromol. 118 (2018) 1542–1549.
[24] K. Saravanakumar, M.-H. Wang, Trichoderma based synthesis of anti-pathogenic silver nanoparticles and their characterization, antioxidant and cytotoxicity properties, Microb. Pathog. 114 (2018) 269–273.
[25] X. Hu, K. Saravanakumar, T. Jin, M.H. Wang, Mycosynthesis, characterization, anticancer and antibacterial activity of silver nanoparticles from endophytic fungus *Talaromyces purpureogenus*, Int. J. Nanomedicine 14 (2019) 3427–3438.
[26] M. Kitching, M. Ramani, E. Marsili, Fungal biosynthesis of gold nanoparticles: mechanism and scale up, Microb. Biotechnol. 8 (6) (2015) 904–917.
[27] N.A. Byzova, I.V. Safenkova, E.S. Slutskaya, A.V. Zherdev, B.B. Dzantiev, Less is more: a comparison of antibody–gold nanoparticle conjugates of different ratios, Bioconjug. Chem. 28 (11) (2017) 2737–2746.
[28] P. Clarance, B. Luvankar, J. Sales, A. Khusro, P. Agastian, J.C. Tack, M.M. Al Khulaifi, H.A. Al-Shwaiman, A.M. Elgorban, A. Syed, H.J. Kim, Green synthesis and characterization of gold nanoparticles using endophytic fungi *Fusarium solani* and its *in-vitro* anticancer and biomedical applications, Saudi J. Biol. Sci. 27 (2) (2020) 706–712.
[29] P. Singh, Y.-J. Kim, D. Zhang, D.-C. Yang, Biological synthesis of nanoparticles from plants and microorganisms, Trends Biotechnol. 34 (7) (2016) 588–599.
[30] G. Gahlawat, A.R. Choudhury, A review on the biosynthesis of metal and metal salt nanoparticles by microbes, RSC Adv. 9 (23) (2019) 12944–12967.
[31] H. Zhang, H. Zhou, J. Bai, Y. Li, J. Yang, Q. Ma, Y. Qu, Biosynthesis of selenium nanoparticles mediated by fungus *Mariannaea* sp. HJ and their characterization, Colloids Surf. A Physicochem. Eng. Asp. 571 (2019) 9–16.
[32] Y. Zhang, J. Wang, L. Zhang, Creation of Highly Stable Selenium Nanoparticles Capped with Hyperbranched Polysaccharide in Water, Langmuir 26 (22) (2010) 17617–17623.
[33] W. Zhang, Z. Chen, H. Liu, L. Zhang, P. Gao, D. Li, Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*, Colloids Surf. B Biointerfaces 88 (1) (2011) 196–201.
[34] H. Wu, X. Li, W. Liu, T. Chen, Y. Li, W. Zheng, C.W.-Y. Man, M.-K. Wong, K.-H. Wong, Surface decoration of selenium nanoparticles by mushroom polysaccharides–protein complexes to achieve enhanced cellular uptake and antiproliferative activity, J. Mater. Chem. 22 (19) (2012) 9602–9610.
[35] D. Zeng, J. Zhao, K.-H. Luk, S.-T. Cheung, K.-H. Wong, T. Chen, Potentiation of *in vivo* anticancer efficacy of selenium nanoparticles by mushroom polysaccharides surface decoration, J. Agric. Food Chem. 67 (10) (2019) 2865–2876.
[36] H. Wu, H. Zhu, X. Li, Z. Liu, W. Zheng, T. Chen, B. Yu, K.-H. Wong, Induction of apoptosis and cell cycle arrest in A549 human lung adenocarcinoma cells by surface-capping selenium nanoparticles: an effect enhanced by polysaccharide–protein complexes from *Polyporus rhinocerus*, J. Agric. Food Chem. 61 (41) (2013) 9859–9866.
[37] O. Ivashchenko, Ł. Przysiecka, B. Peplińska, M. Jarek, E. Coy, S. Jurga, Gel with silver and ultrasmall iron oxide nanoparticles produced with *Amanita muscaria* extract: physicochemical characterization, microstructure analysis and anticancer properties, Sci. Rep. 8 (1) (2018) 13260.
[38] Y. Mao, Y. Zhang, Z. Luo, R. Zhan, H. Xu, W. Chen, H. Huang, Synthesis, Biological Evaluation and Low-Toxic Formulation Development of Glycosylated Paclitaxel Prodrugs, Molecules 23 (12) (2018) 3211.
[39] S. Li, H. Wang, J. Ma, G. Gu, Z. Chen, Z. Guo, One-pot four-enzyme synthesis of thymidinediphosphate-l-rhamnose, Chem. Commun. 52 (97) (2016) 13995–13998.
[40] L. Xu, X. Liu, Y. Li, Z. Yin, L. Jin, L. Lu, J. Qu, M. Xiao, Enzymatic rhamnosylation of anticancer drugs by an α-L-rhamnosidase from *Alternaria* sp. L1 for cancer-targeting and enzyme-activated prodrug therapy, Appl. Microbiol. Biotechnol. 103 (19) (2019) 7997–8008.
[41] T.S. Stolworthy, A.M. Korkegian, C.L. Willmon, A. Ardiani, J. Cundiff, B. L. Stoddard, M.E. Black, Yeast cytosine deaminase mutants with increased thermostability impart sensitivity to 5-fluorocytosine, J. Mol. Biol. 377 (3) (2008) 854–869.
[42] R.M. Lieser, W. Chen, M.O. Sullivan, Controlled epidermal growth factor receptor ligand display on Cancer suicide enzymes via unnatural amino acid engineering for enhanced intracellular delivery in breast Cancer cells, Bioconjug. Chem. 30 (2) (2019) 432–442.
[43] E. Kievit, E. Bershad, E. Ng, P. Sethna, I. Dev, T.S. Lawrence, A. Rehemtulla, Superiority of yeast over bacterial cytosine deaminase for Enzyme/Prodrug gene therapy in Colon Cancer xenografts, Cancer Res. 59 (7) (1999) 1417–1421.
[44] U. Altanerova, J. Jakubechova, K. Benejova, P. Priscakova, V. Repiska, A. Babelova, B. Smolkova, C. Altaner, Intracellular prodrug gene therapy for cancer mediated by tumor cell suicide gene exosomes, Int. J. Cancer 148 (1) (2021) 128–139.
[45] A. Gabizon, H. Shmeeda, Y. Barenholz, Pharmacokinetics of pegylated liposomal doxorubicin, Clin. Pharmacokinet. 42 (5) (2003) 419–436.
[46] M.K. Mansour, J.M. Tam, N.S. Khan, M. Seward, P.J. Davids, S. Puranam, A. Sokolovska, D.B. Sykes, Z. Dagher, C. Becker, A. Tanne, J.L. Reedy, L.M. Stuart, J.M. Vyas, Dectin-1 activation controls maturation of β-1,3-glucan-containing phagosomes, J. Biol. Chem. 288 (22) (2013) 16043–16054.
[47] T. Ren, J. Gou, W. Sun, X. Tao, X. Tan, P. Wang, Y. Zhang, H. He, T. Yin, X. Tang, Entrapping of nanoparticles in yeast cell wall microparticles for macrophage-targeted oral delivery of cabazitaxel, Mol. Pharm. 15 (7) (2018) 2870–2882.
[48] X. Zhou, K. Ling, M. Liu, X. Zhang, J. Ding, Y. Dong, Z. Liang, J. Li, J. Zhang, Targeted delivery of cisplatin-derived nanoprecursors via a biomimetic yeast microcapsule for tumor therapy by the oral route, Theranostics 9 (22) (2019) 6568–6586.
[49] M.N. Moghadam, B. Khameneh, B.S.F. Bazzaz, *Saccharomyces cervisiae* as an efficient carrier for delivery of bioactives: a review, Food Biophys. 14 (3) (2019) 346–353.
[50] S. Figueiredo, J.N. Moreira, C.F. Geraldes, S. Rizzitelli, S. Aime, E. Terreno, Yeast cell wall particles: a promising class of nature-inspired microcarriers for multimodal imaging, Chem. Commun. 47 (38) (2011) 10635–10637.
[51] M. Seif, J. Hoppstädter, F. Breinig, A.K. Kiemer, Yeast-mediated mRNA delivery polarizes immuno-suppressive macrophages towards an immuno-stimulatory phenotype, Europe J. Pharm. Biopharm. 117 (2017) 1–13.
[52] E.E. Kenngott, R. Kiefer, N. Schneider-Daum, A. Hamann, M. Schneider, M. J. Schmitt, F. Breinig, Surface-modified yeast cells: a novel eukaryotic carrier for oral application, J. Control. Release 224 (2016) 1–7.
[53] Y. Wang, S. Yi, L. Sun, Y. Huang, M. Zhang, Charge-selective fractions of naturally occurring nanoparticles as bioactive nanocarriers for cancer therapy, Acta Biomater. 10 (10) (2014) 4269–4284.
[54] K. Zhao, M. Bleackley, D. Chisanga, L. Gangoda, P. Fonseka, M. Liem, H. Kalra, H. Al Saffar, S. Keerthikumar, C.-S. Ang, Extracellular vesicles secreted by *Saccharomyces cerevisiae* are involved in cell wall remodelling, Commun. Biol. 2 (1) (2019) 1–13.
[55] A. Stierle, G. Strobel, D. Stierle, Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew, Science 260 (5105) (1993) 214–216.
[56] L. Chen, Q.-Y. Zhang, M. Jia, Q.-L. Ming, W. Yue, K. Rahman, L.-P. Qin, T. Han, Endophytic fungi with antitumor activities: their occurrence and anticancer compounds, Crit. Rev. Microbiol. 42 (3) (2016) 454–473.
[57] C. Florean, M. Dicato, M. Diederich, Immune-modulating and anti-inflammatory marine compounds against cancer, Semin. Cancer Biol. (2020).
[58] F. Uzma, C.D. Mohan, A. Hashem, N.M. Konappa, S. Rangappa, P.V. Kamath, B. P. Singh, V. Mudili, V.K. Gupta, C.N. Siddaiah, S. Chowdappa, A.A. Alqarawi, E. F. Abd_Allah, Endophytic Fungi—alternative sources of cytotoxic compounds: a review, Front. Pharmacol. 9 (2018).
[59] T. Akihisa, H. Tokuda, M. Ukiya, A. Kiyota, K. Yasukawa, N. Sakamoto, Y. Kimura, T. Suzuki, J. Takayasu, H. Nishino, Anti-tumor-Initiating effects of monascin, an azaphilonoid pigment from the extract of *Monascus pilosus* fermented rice (Red-Mold rice), Chem. Biodivers. 2 (10) (2005) 1305–1309.
[60] N.-W. Su, Y.-L. Lin, M.-H. Lee, C.-Y. Ho, Ankaflavin from monascus-fermented red rice exhibits selective cytotoxic effect and induces cell death on hep G2 cells, J. Agric. Food Chem. 53 (6) (2005) 1949–1954.
[61] S.W. El-Far, M.W. Helmy, S.N. Khattab, A.A. Bekhit, A.A. Hussein, A.O. Elzoghby, Phytosomal bilayer-enveloped casein micelles for codelivery of monascus yellow pigments and resveratrol to breast cancer, Nanomedicine 13 (5) (2018) 481–499.
[62] M. Sahlan, M. Tejamaya, R. Arbianti, T. Baruji, R. Adawiyah, H. Hermansyah, The effects of nano-casein encapsulation and productions of a controlled-release on eugenol containing bio-pesticide toxicity, key engineering materials, Trans Tech Publ (2021) 115–127.
[63] D. Arora, N. Sharma, V. Singamaneni, V. Sharma, M. Kushwaha, V. Abrol, S. Guru, S. Sharma, A.P. Gupta, S. Bhushan, S. Jaglan, P. Gupta, Isolation and characterization of bioactive metabolites from *Xylaria psidii*, an endophytic fungus of the medicinal plant Aegle marmelos and their role in mitochondrial dependent apoptosis against pancreatic cancer cells, Phytomedicine 23 (12) (2016) 1312–1320.
[64] D. Arora, A. Kumar, P. Gupta, G. Chashoo, S. Jaglan, Preparation, characterization and cytotoxic evaluation of bovine serum albumin nanoparticles encapsulating 5-methylmellein: a secondary metabolite isolated from *Xylaria psidii*, Bioorg. Med. Chem. Lett. 27 (23) (2017) 5126–5130.

[65] C. Yi, M. Fu, X. Cao, S. Tong, Q. Zheng, C.K. Firempong, X. Jiang, X. Xu, J. Yu, Enhanced oral bioavailability and tissue distribution of a new potential anticancer agent, *Flammulina velutipes* sterols, through liposomal encapsulation, J. Agric. Food Chem. 61 (25) (2013) 5961–5971.
[66] M. Adnan, M. Patel, M.N. Reddy, E. Alshammari, Formulation, evaluation and bioactive potential of *Xylaria primorskensis* terpenoid nanoparticles from its major compound xylaranic acid, Sci. Rep. 8 (1) (2018) 1740.
[67] S. Arata, J. Watanabe, M. Maeda, M. Yamamoto, H. Matsuhashi, M. Mochizuki, N. Kagami, K. Honda, M. Inagaki, Continuous intake of the Chaga mushroom (*Inonotus obliquus*) aqueous extract suppresses cancer progression and maintains body temperature in mice, Heliyon 2 (5) (2016) e00111.
[68] Y.-G. Gil, S. Kang, A. Chae, Y.-K. Kim, D.-H. Min, H. Jang, Synthesis of porous Pd nanoparticles by therapeutic chaga extract for highly efficient tri-modal cancer treatment, Nanoscale 10 (42) (2018) 19810–19817.
[69] K.M. Reece, E.D. Richardson, K.M. Cook, T.J. Campbell, S.T. Pisle, A.J. Holly, D. J. Venzon, D.J. Liewehr, C.H. Chau, D.K. Price, W.D. Figg, Epidithiodiketopiperazines (ETPs) exhibit *in vitro* antiangiogenic and *in vivo* antitumor activity by disrupting the HIF-1α/p300 complex in a preclinical model of prostate cancer, Mol. Cancer 13 (1) (2014) 91.
[70] L. Comas, E. Polo, M.P. Domingo, Y. Hernández, M. Arias, P. Esteban, L. Martínez-Lostao, J. Pardo, J. Martínez de la Fuente, E.M. Gálvez, Intracellular delivery of biologically-active fungal metabolite gliotoxin using magnetic nanoparticles, Materials Basel (Basel) 12 (7) (2019).
[71] Z. Ma, X. Xiang, S. Li, P. Xie, Q. Gong, B.-C. Goh, L. Wang, Targeting hypoxia-inducible factor-1, for cancer treatment: recent advances in developing small-molecule inhibitors from natural compounds, Semin. Cancer Biol. (2020).
[72] K. Saravanakumar, A.V.A. Mariadoss, A. Sathiyaseelan, K. Venkatachalam, X. Hu, M.-H. Wang, pH-sensitive release of fungal metabolites from chitosan nanoparticles for effective cytotoxicity in prostate cancer (PC3) cells, Process. Biochem. 102 (2021) 165–172.
[73] L. Xu, X. Liu, Y. Li, Z. Yin, L. Jin, L. Lu, J. Qu, M. Xiao, Enzymatic rhamnosylation of anticancer drugs by an α-L-rhamnosidase from *Alternaria* sp. L1 for cancer-targeting and enzyme-activated prodrug therapy, Appl. Microbiol. Biotechnol. 103 (19) (2019) 7997–8008.
[74] S. Banerjee, S.B. Paruthy, in: J.-M. Mérillon, K.G. Ramawat (Eds.), Preclinical and Clinical Perspective on Fungal Metabolites and Their Analogs as Anticancer Agents – From Bench to Bedside, Fungal Metabolites, Springer International Publishing, Cham, 2017, pp. 669–700.
[75] A. Kornienko, A. Evidente, M. Vurro, V. Mathieu, A. Cimmino, M. Evidente, W.A. L. van Otterlo, R. Dasari, F. Lefranc, R. Kiss, Towards a Cancer drug of fungal origin, Med. Res. Rev. 35 (5) (2015) 937–967.
[76] K. Kanoh, S. Kohno, T. Asari, T. Harada, J. Katada, M. Muramatsu, H. Kawashima, H. Sekiya, I. Uno, (−)-Phenylahistin: a new mammalian cell cycle inhibitor produced by *aspergillus ustus*, Bioorg. Med. Chem. Lett. 7 (22) (1997) 2847–2852.
[77] M. Millward, P. Mainwaring, A. Mita, K. Federico, G.K. Lloyd, N. Reddinger, S. Nawrocki, M. Mita, M.A. Spear, Phase 1 study of the novel vascular disrupting agent plinabulin (NPI-2358) and docetaxel, Invest. New Drugs 30 (3) (2012) 1065–1073.
[78] I. Nereus Pharmaceuticals, Phase I Study of the Vascular Disrupting Agent NPI-2358 Administered Via Intravenous Infusion in Patients With Advanced Solid Tumor Malignancies or Lymphoma, clinicaltrials.gov, 2011.
[79] M.M. Mita, M.A. Spear, L.K. Yee, A.C. Mita, E.I. Heath, K.P. Papadopoulos, K. C. Federico, S.D. Reich, O. Romero, L. Malburg, M. Pilat, G.K. Lloyd, S.T. C. Neuteboom, G. Cropp, E. Ashton, P.M. LoRusso, Phase 1 first-in-human trial of the vascular disrupting agent plinabulin(NPI-2358) in patients with solid tumors or lymphomas, Clin. Cancer Res. 16 (23) (2010) 5892–5899.
[80] I. Nereus Pharmaceuticals, Study of the Vascular Disrupting Agent NPI-2358 in Combination with Docetaxel in Patients With Advanced Non-Small Cell Lung Cancer, clinicaltrials.gov, 2011.
[81] I. BeyondSpring Pharmaceuticals, Randomized Blinded Phase III Assessment of Second or Third-Line Chemotherapy with Docetaxel + Plinabulin Compared to Docetaxel + Placebo in Patients With Advanced Non-Small Cell Lung Cancer and With at Least One Measurable Lung Lesion, clinicaltrials.gov, 2021.
[82] L. Bazhenova, A Phase I Study of Nivolumab in Combination with Escalating Doses of Plinabulin in Patients With Metastatic Non-Small Cell Lung Cancer (NSCLC), clinicaltrials.gov, 2019.
[83] J. Malhotra, A Phase I/II Study of Nivolumab, Ipilimumab and Plinabulin in Patients with Recurrent Small Cell Lung Cancer: Big Ten Cancer Research Consortium. BTCRC-LUN17-127, clinicaltrials.gov, 2020.
[84] P. Workman, P.A. Clarke, F.I. Raynaud, R.L.M. van Montfort, Drugging the PI3 kinome: from chemical tools to drugs in the clinic, Cancer Res. 70 (6) (2010) 2146–2157.
[85] M.W. Pitz, E.A. Eisenhauer, M.V. MacNeil, B. Thiessen, J.C. Easaw, D. R. Macdonald, D.D. Eisenstat, A.S. Kakumanu, M. Salim, H. Chalchal, J. Squire, M.S. Tsao, S. Kamel-Reid, S. Banerji, D. Tu, J. Powers, D.F. Hausman, W. P. Mason, Phase II study of PX-866 in recurrent glioblastoma, NeuroOncology 17 (9) (2015) 1270–1274.
[86] S.J. Hotte, K.N. Chi, A.M. Joshua, D. Tu, R.J. Macfarlane, R.W. Gregg, J. D. Ruether, N.S. Basappa, D. Finch, M. Salim, E.W. Winquist, V. Torri, S. North, C. Kollmannsberger, S.L. Ellard, B.J. Eigl, A. Tinker, A.L. Allan, K. Beja, M. Annala, J. Powers, A.W. Wyatt, L. Seymour, A phase II study of PX-866 in patients with recurrent or metastatic castration-resistant prostate Cancer: canadian Cancer trials group study IND205, Clin. Genitourin. Cancer 17 (3) (2019) 201–208.
[87] D.S. Hong, D.W. Bowles, G.S. Falchook, W.A. Messersmith, G.C. George, C. L. O'Bryant, A.C.H. Vo, K. Klucher, R.S. Herbst, S.G. Eckhardt, S. Peterson, D. F. Hausman, R. Kurzrock, A. Jimeno, A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-Kinase inhibitor, in patients with advanced solid tumors, Clin. Cancer Res. 18 (15) (2012) 4173–4182.
[88] D.W. Bowles, M. Kochenderfer, A. Cohn, L. Sideris, N. Nguyen, V. Cline-Burkhardt, I. Schnadig, M. Choi, L. Nabell, A. Chaudhry, R. Ruxer, A. Ucar, D. Hausman, L. Walker, A. Spira, A. Jimeno, A. Randomized, Phase II trial of cetuximab with or without PX-866, an irreversible oral phosphatidylinositol 3-Kinase inhibitor, in patients with metastatic colorectal carcinoma, Clin. Colorectal Cancer 15 (4) (2016) 337–344, e2.
[89] A. Jimeno, K. Shirai, M. Choi, J. Laskin, M. Kochenderfer, A. Spira, V. Cline-Burkhardt, E. Winquist, D. Hausman, L. Walker, R.B. Cohen, A randomized, phase II trial of cetuximab with or without PX-866, an irreversible oral phosphatidylinositol 3-kinase inhibitor, in patients with relapsed or metastatic head and neck squamous cell cancer, Ann. Oncol. 26 (3) (2015) 556–561.
[90] I. Cascadian Therapeutics, Phase 1/2 Study of PI-3K Inhibition with PX-866 Combined with Vemurafenib (BRAF Inhibitor) in Patients With BRAF-mutant Cancer Including Advanced Melanoma, clinicaltrials.gov, 2018.
[91] I. Cascadian Therapeutics, Phase 1/2 Study of PX-866 and Docetaxel in Patients with Solid Tumors, clinicaltrials.gov, 2018.
[92] C.R. Strader, C.J. Pearce, N.H. Oberlies, Fingolimod (FTY720): a recently approved multiple sclerosis drug based on a fungal secondary metabolite, J. Nat. Prod. 74 (4) (2011) 900–907.
[93] T. Takasaki, K. Hagihara, R. Satoh, R. Sugiura, More than just an immunosuppressant: the emerging role of FTY720 as a novel inducer of ROS and apoptosis, Oxid. Med. Cell. Longev. 2018 (2018). Article-ID 4397159.
[94] H. Sidney Kimmel, Comprehensive Cancer Center at Johns, A Safety Study of Fingolimod With Radiation and Temozolomide in Newly Diagnosed High Grade Glioma, clinicaltrials.gov, 2017.
[95] C. Mayo, Prevention of Paclitaxel-Associated Neuropathy With Fingolimod: a Pilot Trial, clinicaltrials.gov, 2020.
[96] E.A. Speakman, I.M. Dambuza, F. Salazar, G.D. Brown, T cell antifungal immunity and the role of C-type lectin receptors, Trends Immunol. 41 (1) (2020) 61–76.
[97] R. Roden, T.C. Wu, How will HPV vaccines affect cervical cancer? Nat. Rev. Cancer 6 (10) (2006) 753–763.
[98] H.J. Kim, S.Y. Kim, S.J. Lim, J.Y. Kim, S.J. Lee, H.-J. Kim, One-step chromatographic purification of human papillomavirus type 16 L1 protein from *Saccharomyces cerevisiae*, Protein Expr. Purif. 70 (1) (2010) 68–74.
[99] H.-L. Kwag, H.J. Kim, D.Y. Chang, H.-J. Kim, The production and immunogenicity of human papillomavirus type 58 virus-like particles produced in *Saccharomyces cerevisiae*, J. Microbiol. 50 (5) (2012) 813–820.
[100] M.C. Patel, K.K. Patkar, A. Basu, K.M. Mohandas, R. Mukhopadhyaya, Production of immunogenic human papillomavirus-16 major capsid protein derived virus like particles, Indian J. Med. Res. 130 (3) (2009) 213–218.
[101] J. Jung, M. Iijima, N. Yoshimoto, M. Sasaki, T. Niimi, K. Tatematsu, S.Y. Jeong, E. K. Choi, K. Tanizawa, S.I. Kuroda, Efficient and rapid purification of drug- and gene-carrying bio-nanocapsules, hepatitis B virus surface antigen L particles, from *Saccharomyces cerevisiae*, Protein Expr. Purif. 78 (2) (2011) 149–155.
[102] N. Hadiji-Abbes, M. Martin, W. Benzina, H. Karray-Hakim, C. Gergely, A. Gargouri, R. Mokdad-Gargouri, Extraction and purification of hepatitis B virus-like M particles from a recombinant *Saccharomyces cerevisiae* strain using alumina powder, J. Virol. Methods 187 (1) (2013) 132–137.
[103] T.H. King, C.B. Kemmler, Z. Guo, D. Mann, Y. Lu, C. Coeshott, A.J. Gehring, A. Bertoletti, Z.Z. Ho, W. Delaney, A. Gaggar, G.M. Subramanian, J. G. McHutchison, S. Shrivastava, Y.-J.L. Lee, S. Kottilil, D. Bellgrau, T. Rodell, D. Apelian, A whole recombinant yeast-based therapeutic vaccine elicits HBV X, s and core specific t cells in mice and activates human t cells recognizing epitopes linked to viral clearance, PLoS One 9 (7) (2014) e101904-e101904.
[104] X. Guo, J.-Y. Zhong, J.-W. Li, Hepatitis C virus infection and vaccine development, J. Clin. Exp. Hepatol. 8 (2) (2018) 195–204.
[105] J. Jezkova, The Role of Brachyury in Colorectal Cancer Cells, Prifysgol Bangor University, 2015.
[106] D.H. Hamilton, J.M. David, C. Dominguez, C. Palena, Development of cancer vaccines targeting brachyury, a transcription factor associated with tumor epithelial-mesenchymal transition, Cells Tissues Organs (Print) 203 (2) (2017) 128–138.
[107] A. Tanaka, J.D. Jensen, R. Prado, H. Riemann, Y.G. Shellman, D.A. Norris, L. Chin, C. Yee, M. Fujita, Whole recombinant yeast vaccine induces antitumor immunity and improves survival in a genetically engineered mouse model of melanoma, Gene Ther. 18 (8) (2011) 827–834.
[108] R. Lázaro-Gorines, J. Ruiz-de-la-Herrán, R. Navarro, L. Sanz, L. Álvarez-Vallina, A. Martínez-Del-Pozo, J.G. Gavilanes, J. Lacadena, A novel carcinoembryonic antigen (CEA)-targeted trimeric immunotoxin shows significantly enhanced antitumor activity in human colorectal cancer xenografts, Sci. Rep. 9 (1) (2019) 1–13.
[109] C. Remondo, V. Cereda, S. Mostböck, H. Sabzevari, A. Franzusoff, J. Schlom, K. Y. Tsang, Human dendritic cell maturation and activation by a heat-killed recombinant yeast (*Saccharomyces cerevisiae*) vector encoding carcinoembryonic antigen, Vaccine 27 (7) (2009) 987–994.
[110] M.R. Bui, V. Hodson, T. King, D. Leopold, S. Dai, V. Fiolkoski, S. Oakes, R. Duke, D. Apelian, A. Franzusoff, J. DeGregori, Mutation-specific control of BCR-ABL T315I positive leukemia with a recombinant yeast-based therapeutic vaccine in a murine model, Vaccine 28 (37) (2010) 6028–6035.
[111] J.E. Chaft, A. Litvak, M.E. Arcila, P. Patel, S.P. D'Angelo, L.M. Krug, V. Rusch, A. Mattson, C. Coeshott, B. Park, D.M. Apelian, M.G. Kris, C.G. Azzoli, Phase II study of the GI-4000 KRAS vaccine after curative therapy in patients with stage I-

III lung adenocarcinoma harboring a KRAS G12C, G12D, or G12V mutation, Clin. Lung Cancer 15 (6) (2014) 405–410.
[112] E. Çelik, P. Çalik, Production of recombinant proteins by yeast cells, Biotechnol. Adv. 30 (5) (2012) 1108–1118.
[113] S.N. Kim, H.S. Jeong, S.N. Park, H.-J. Kim, Purification and immunogenicity study of human papillomavirus type 16 L1 protein in *Saccharomyces cerevisiae*, J. Virol. Methods 139 (1) (2007) 24–30.
[114] M.-K. Woo, S.-J. Hur, S. Park, H.-J. Kim, Study of cell-mediated response in mice by HPV16 L1 virus-like particles expressed in Saccharomyces cerevisiae, J. Microbiol. Biotechnol. 17 (10) (2007) 1738–1741.
[115] M.-A. Park, H.J. Kim, H.-J. Kim, Optimum conditions for production and purification of human papillomavirus type 16 L1 protein from *Saccharomyces cerevisiae*, Protein Expr. Purif. 59 (1) (2008) 175–181.
[116] M.-K. Woo, J.-M. An, J.-D. Kim, S.-N. Park, H.-J. Kim, Expression and purification of human papillomavirus 18 L1 virus-like particle from *Saccharomyces cerevisiae*, Arch. Pharm. Res. 31 (2) (2008) 205–209.
[117] S.K. Gaik, N.S. Pujar, N.J. Titchener-Hooker, Study of detergent-mediated liberation of hepatitis B virus-like particles from *S. Cerevisiae* homogenate: identifying a framework for the design of future-generation lipoprotein vaccine processes, Biotechnol. Prog. 24 (3) (2008) 623–631.
[118] E.-J. Kim, Y.-K. Park, H.-K. Lim, Y.-C. Park, J.-H. Seo, Expression of hepatitis B surface antigen S domain in recombinant *Saccharomyces cerevisiae* using GAL1 promoter, J. Biotechnol. 141 (3–4) (2009) 155–159.
[119] R.N. Vellanki, N. Komaravelli, R. Tatineni, L.N. Mangamoori, Expression of hepatitis B surface antigen in *Saccharomyces cerevisiae* utilizing glyceraldeyhyde-3-phosphate dehydrogenase promoter of Pichia pastoris, Biotechnol. Lett. 29 (2) (2007) 313–318.
[120] H. Chen, J.H. Lü, W.Q. Liang, Y.H. Huang, W.J. Zhang, D.B. Zhang, Purification of the recombinant hepatitis B virus core antigen (rHBcAg) produced in the yeast *Saccharomyces cerevisiae* and comparative observation of its particles by transmission electron microscopy (TEM) and atomic force microscopy (AFM), Micron 35 (5) (2004) 311–318.
[121] A.A. Haller, G.M. Lauer, T.H. King, C. Kemmler, V. Fiolkoski, Y. Lu, D. Bellgrau, T. C. Rodell, D. Apelian, A. Franzusoff, R.C. Duke, Whole recombinant yeast-based immunotherapy induces potent T cell responses targeting HCV NS3 and Core proteins, Vaccine 25 (8) (2007) 1452–1463.
[122] G. Gupta, R. Glueck, N. Rishi, Physicochemical characterization and immunological properties of *Pichia pastoris* based HPV16L1 and 18L1 virus like particles, Biologicals 46 (2017) 11–22.
[123] N. Hanumantha Rao, P. Baji Babu, L. Rajendra, R. Sriraman, Y.Y.S. Pang, J. T. Schiller, V.A. Srinivasan, Expression of codon optimized major capsid protein (L1) of human papillomavirus type 16 and 18 in *Pichia pastoris*; purification and characterization of the virus-like particles, Vaccine 29 (43) (2011) 7326–7334.
[124] A. Sanchooli, K. Aghayipour, S.K. Naghlani, Z. Samiee, B.A. Kiasari, M. Makvandi, Production of human papillomavirus Type-16 L1 VLP in Pichia pastoris, Appl. Biochem. Microbiol. 56 (1) (2020) 51–57.
[125] S.B. Bazan, A. de Alencar Muniz Chaves, K.A. Aires, A.M. Cianciarullo, R. L. Garcea, P.L. Ho, Expression and characterization of HPV-16 L1 capsid protein in *Pichia pastoris*, Arch. Virol. 154 (10) (2009) 1609–1617.
[126] H. Bredell, J.J. Smith, J.F. Görgens, W.H. van Zyl, Expression of unique chimeric human papilloma virus type 16 (HPV-16) L1-L2 proteins in *Pichia pastoris* and *Hansenula polymorpha*, Yeast 35 (9) (2018) 519–529.
[127] Z. Jiang, G. Tong, B. Cai, Y. Xu, J. Lou, Purification and immunogenicity study of human papillomavirus 58 virus-like particles expressed in *Pichia pastoris*, Protein Expr. Purif. 80 (2) (2011) 203–210.
[128] C. Gurramkonda, M. Zahid, S.K. Nemani, A. Adnan, S.K. Gudi, N. Khanna, T. Ebensen, H. Lünsdorf, C.A. Guzmán, U. Rinas, Purification of hepatitis B surface antigen virus-like particles from recombinant *Pichia pastoris* and *in vivo* analysis of their immunogenic properties, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 940 (2013) 104–111.
[129] H. Lünsdorf, C. Gurramkonda, A. Adnan, N. Khanna, U. Rinas, Virus-like particle production with yeast: ultrastructural and immunocytochemical insights into *Pichia pastoris* producing high levels of the Hepatitis B surface antigen, Microb. Cell Fact. 10 (1) (2011), 48-48.
[130] A. Rahimi, S.N. Hosseini, A. Karimi, H. Aghdasinia, R. Arabi Mianroodi, Enhancing the efficiency of recombinant hepatitis B surface antigen production in *Pichia pastoris* by employing continuous fermentation, Biochem. Eng. J. 141 (2019) 112–119.
[131] E. Hardy, E. Martínez, D. Diago, R. Díaz, D. González, L. Herrera, Large-scale production of recombinant hepatitis B surface antigen from *Pichia pastoris*, J. Biotechnol. 77 (2–3) (2000) 157–167.
[132] S.N. Hosseini, A. Javidanbardan, B.S. Alizadeh Salim, M. Khatami, Large-scale purification of recombinant hepatitis B surface antigen from *Pichia pastoris* with non-affinity chromatographic methods as a substitute to immunoaffinity chromatography, Prep. Biochem. Biotechnol. 48 (8) (2018) 683–692.
[133] R. Liu, Q. Lin, Y. Sun, X. Lu, Y. Qiu, Y. Li, X. Guo, Expression, purification, and characterization of hepatitis B virus surface antigens (HBsAg) in yeast *Pichia pastoris*, Appl. Biochem. Biotechnol. 158 (2) (2009) 432–444.
[134] S. Ottone, X. Nguyen, J. Bazin, C. Bérard, S. Jimenez, O. Letourneur, Expression of hepatitis B surface antigen major subtypes in *Pichia pastoris* and purification for *in vitro* diagnosis, Protein Expr. Purif. 56 (2) (2007) 177–188.
[135] A. Vassileva, D.A. Chugh, S. Swaminathan, N. Khanna, Expression of hepatitis B surface antigen in the methylotrophic yeast *Pichia pastoris* using the GAP promoter, J. Biotechnol. 88 (1) (2001) 21–35.
[136] J. Freivalds, A. Dislers, V. Ose, P. Pumpens, K. Tars, A. Kazaks, Highly efficient production of phosphorylated hepatitis B core particles in yeast *Pichia pastoris*, Protein Expr. Purif. 75 (2) (2011) 218–224.
[137] A.J. Spice, R. Aw, D.G. Bracewell, K.M. Polizzi, Synthesis and assembly of hepatitis B virus-like particles in a *Pichia pastoris* cell-free system, Front. Bioeng. Biotechnol. 8 (2020), 72-72.
[138] N. Acosta-Rivero, J.C. Aguilar, A. Musacchio, V. Falcón, A. Viña, M.C. De La Rosa, J. Morales, Characterization of the HCV core virus-like particles produced in the methylotrophic yeast *Pichia pastoris*, Biochem. Biophys. Res. Commun. 287 (1) (2001) 122–125.
[139] G. Martinez-Donato, N. Acosta-Rivero, J. Morales-Grillo, A. Musacchio, A. Vina, C. Alvarez, N. Figueroa, I. Guerra, J. Garcia, L. Varas, V. Muzio, S. Dueñas-Carrera, Expression and processing of hepatitis C virus structural proteins in *Pichia pastoris* yeast, Biochem. Biophys. Res. Commun. 342 (2) (2006) 625–631.
[140] W. Cai, L. Su, Q. Liao, L. Ye, Y. Wu, Z. Wu, Y. She, Expression, purification and immunogenic characterization of hepatitis C virus recombinant E1E2 protein expressed by *Pichia pastoris* yeast, Antiviral Res. 88 (1) (2010) 80–85.
[141] W. Li, X. He, X. Guo, Z. Zhang, B. Zhang, Optimized expression of the L1 protein of human papillomavirus in *Hansenula polymorpha*, Shengwu Gongcheng Xuebao/ Chin. J. Biotechnol. 25 (10) (2009) 1516–1523.
[142] C. Liu, X. Yang, Y. Yao, W. Huang, W. Sun, Y. Ma, Diverse expression levels of two codon-optimized genes that encode human papilloma virus type 16 major protein L1 in *Hansenula polymorpha*, Biotechnol. Lett. 36 (5) (2014) 937–945.
[143] C. Liu, Y. Yao, X. Yang, H. Bai, W. Huang, Y. Xia, Y. Ma, Production of recombinant human papillomavirus type 52 L1 protein in *Hansenula polymorpha* formed virus-like particles, J. Microbiol. Biotechnol. 25 (6) (2015) 936–940.
[144] Y. Huang, J. Bi, Y. Zhang, W. Zhou, Y. Li, L. Zhao, Z. Su, A highly efficient integrated chromatographic procedure for the purification of recombinant hepatitis B surface antigen from *Hansenula polymorpha*, Protein Expr. Purif. 56 (2) (2007) 301–310.
[145] G. Bian, Y. Cheng, Z. Wang, Y. Hu, X. Zhang, M. Wu, Z. Chen, B. Shi, S. Sun, Y. Shen, Ej. Chen, X. Yao, Y. Wen, Z. Yuan, Whole recombinant *Hansenula polymorpha* expressing hepatitis B virus surface antigen (yeast-HBsAg) induces potent HBsAg-specific Th1 and Th2 immune responses, Vaccine 28 (1) (2009) 187–194.
[146] D.H. Hamilton, M.T. Litzinger, A. Jales, B. Huang, R.I. Fernando, J.W. Hodge, A. Ardiani, D. Apelian, J. Schlom, C. Palena, Immunological targeting of tumor cells undergoing an epithelialmesenchymal transition via a recombinant brachyury-yeast vaccine, Oncotarget 4 (10) (2013) 1777–1790.
[147] C.R. Heery, B.H. Singh, M. Rauckhorst, J.L. Marté, R.N. Donahue, I. Grenga, T. C. Rodell, W. Dahut, P.M. Arlen, R.A. Madan, J. Schlom, J.L. Gulley, Phase I trial of a yeast-based therapeutic Cancer vaccine (GI-6301) targeting the transcription factor brachyury, Cancer Immunol. Res. 3 (11) (2015) 1248–1256.
[148] H. Riemann, J. Takao, Y.G. Shellman, W.A. Hines, C.K. Edwards, D.A. Norris, M. Fujita, Generation of a prophylactic melanoma vaccine using whole recombinant yeast expressing MART-1, Exp. Dermatol. 16 (10) (2007) 814–822.
[149] M.B. Bernstein, M. Chakraborty, E.K. Wansley, Z. Guo, A. Franzusoff, S. Mostböck, H. Sabzevari, J. Schlom, J.W. Hodge, Recombinant Saccharomyces cerevisiae (yeast-CEA) as a potent activator of murine dendritic cells, Vaccine 26 (4) (2008) 509–521.
[150] E.K. Wansley, M. Chakraborty, K.W. Hance, M.B. Bernstein, A.L. Boehm, Z. Guo, D. Quick, A. Franzusoff, J.W. Greiner, J. Schlom, J.W. Hodge, Vaccination with a recombinant *Saccharomyces cerevisiae* expressing a tumor antigen breaks immune tolerance and elicits therapeutic antitumor responses, Clin. Cancer Res. 14 (13) (2008) 4316–4325.
[151] S. Hua, M.B.C. de Matos, J.M. Metselaar, G. Storm, Current trends and challenges in the clinical translation of nanoparticulate nanomedicines: pathways for translational development and commercialization, Front. Pharmacol. 9 (2018), 790-790.
[152] L.E. Markowitz, E.F. Dunne, M. Saraiya, H.W. Chesson, C.R. Curtis, J. Gee, J. A. Bocchini Jr., E.R. Unger, Human papillomavirus vaccination: recommendations of the Advisory Committee on Immunization Practices (ACIP), MMWR Recomm. Rep. 63 (Rr-05) (2014) 1–30.
[153] F. Habersetzer, T.F. Baumert, F. Stoll-Keller, GI-5005, a yeast vector vaccine expressing an NS3-core fusion protein for chronic HCV infection, Curr. Opin. Mol. Ther. 11 (4) (2009) 456–462.
[154] A.S. Lok, C.Q. Pan, S.H. Han, H.N. Trinh, W.J. Fessel, T. Rodell, B. Massetto, L. Lin, A. Gaggar, G.M. Subramanian, J.G. McHutchison, C. Ferrari, H. Lee, S. C. Gordon, E.J. Gane, Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B, J. Hepatol. 65 (3) (2016) 509–516.
[155] J.E. Chaft, A. Litvak, M.E. Arcila, P. Patel, S.P. D'Angelo, L.M. Krug, V. Rusch, A. Mattson, C. Coeshott, B. Park, Phase II study of the GI-4000 KRAS vaccine after curative therapy in patients with stage I-III lung adenocarcinoma harboring a KRAS G12C, G12D, or G12V mutation, Clin. Lung Cancer 15 (6) (2014) 405–410.
[156] P.J. DeMaria, M. Bilusic, D. Park, C.R. Heery, R.A. Madan, J. Strauss, R. N. Donahue, J. Marte, M.R. Gilbert, S.M. Steinberg, A randomized, double-blind, phase II clinical trial of GI-6301 (yeast-brachyury vaccine) versus placebo in combination with standard of care definitive radiotherapy in locally advanced, unresectable, chordoma, American Society of Clinical Oncology (2020).
[157] M. Bilusic, C.R. Heery, P.M. Arlen, M. Rauckhorst, D. Apelian, K.Y. Tsang, J. A. Tucker, C. Jochems, J. Schlom, J.L. Gulley, R.A. Madan, Phase I trial of a recombinant yeast-CEA vaccine (GI-6207) in adults with metastatic CEA-expressing carcinoma, Cancer Immunol. Immunother. 63 (3) (2014) 225–234.
[158] Y. Lobaina, M.-L. Michel, Chronic hepatitis B: immunological profile and current therapeutic vaccines in clinical trials, Vaccine 35 (18) (2017) 2308–2314.