frontiers | Frontiers in Tropical Diseases

TYPE Review
PUBLISHED 08 August 2024
DOI 10.3389/fitd.2024.1446477

OPEN ACCESS

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RECEIVED 09 June 2024
ACCEPTED 12 July 2024
PUBLISHED 08 August 2024

CITATION
Alapan D, Bisweswar O, Prasenjit S, Prasanjit D and Arkapal B (2024) Recent advances in the clinical development of antifungal vaccines: a narrative review. *Front. Trop. Dis* 5:1446477. doi: 10.3389/fitd.2024.1446477

Recent advances in the clinical development of antifungal vaccines: a narrative review

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Vaccine development tools for fungal infections are undergoing transformation where newer technologies like nanotechnology and bioinformatics are used to create new and improved vaccine candidates. Immunocompromised individuals and those with multiple chronic conditions are especially vulnerable to invasive fungal infections. These patients are at increased risk of developing widespread infections and experiencing poor health outcomes. Current management of fungal infections is associated with diagnostic challenges, side effects, and resistance. Vaccination is an effective strategy to prevent infections and boost immunity. Despite the significant burden of fungal disease, there are currently no licensed fungal vaccines available. This review is focused on various vaccine development strategies, including whole-cell, subunit, and nucleic acid-based vaccines. Various challenges like safety concerns, weak and nonspecific immune response, ideal adjuvants, and the need for improved drug delivery systems are also highlighted in this review. Sustained antigenic response, addressing host immune response variability, and eliciting persistent predictable immune response are crucial for vaccine development. Standardized protocols and robust preclinical studies are essential for the clinical development of potential vaccine candidates. Exploring novel targets using advanced technologies like bioinformatics, nanotechnology, and reverse vaccinology are being rapidly explored.

KEYWORDS

invasive fungal infections, vaccine development, nanotechnology, bioinformatics, *Candida* vaccine, cryptococcal vaccine, pan fungal vaccine

1 Introduction

In the realm of public health, invasive fungal infections (IFIs) have been identified as one of the major global health concerns, especially in people with impaired immunity due to chemotherapy and organ transplants, or individuals with immunodeficiency such as HIV/AIDS, as well as those with poor hygiene or belonging to financially less privileged

social classes. Though imprecise, studies have suggested that IFIs can have an annual incidence of 6.5 million and an annual mortality rate of 3.8 million people globally (1). Thus, there arises an urgent need to prioritize the development of antifungal vaccines, which can empower comprehensive efforts to overcome this formidable community health crisis (2).

Fungi are pervasive in the environment, and many of them are pathogenic in nature. The most common fungi are *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis* (3). They can cause a wide variety of infections ranging from a minor infection to severe disseminated diseases like invasive candidiasis, aspergillosis, and cryptococcal meningitis (4).

Increased use of immunosuppressive therapies, misuse of broad-spectrum antibiotics, and the booming population of immunocompromised people are increasing the risk of infection to great extent (5). The limited number of antifungal drugs and the emergence of resistant strains have further complicated the condition (6). Traditional antifungal therapies (azoles, polyenes, and echinocandins) are effective but are often associated with unwanted side effects, drug interactions, and the gradual development of resistance (7). Moreover, delays in diagnosis and the low concentration of the drug at the infection site are other contributing factors that enhance the seriousness of the condition (8).

Antifungal vaccines have a potential for disease prophylaxis and active control of such infections. Vaccines can be helpful in boosting immunity, amplifying the ability to identify a virulent pathogen and generate an effective response in such pre-emptive efforts, which can suppress the risk of infection, cut down the extra burden on healthcare delivery systems, and ultimately ensure patient safety (9).

Multiple platforms have been used for the development of antifungal vaccines. These include whole-cell vaccines, subunit vaccines, and nucleic acid-based vaccines. Whole-cell vaccines, composed of inactivated or attenuated fungal cells, have good prospects in murine models but have issues of safety and reproducibility (10). Subunit vaccines are preferred due to their increased safety and specificity but immune response is often weak (11). Nucleic acid-based vaccines (DNA and RNA vaccines) are an upcoming novel approach offering better protection and improved safety profile.

However, optimization of delivery systems, robust immunogenicity, and the risk of adverse effect are some concerns to be addressed (12).

Diversity among pathogens and variability in host immune responses are some areas that need attention to make broad-spectrum vaccines (13). Owing to the lack of clarity between correlates of protection, ill-understood mechanisms regarding immunogenicity, and complicated regulatory criteria, clinical studies on fungal vaccines have been impacted heavily. To facilitate the transformation of promising vaccine candidates from animal experiments to clinical practice, standardized protocols and guidelines for the development of fungal vaccines, evaluation, and approval are imperative (14).

IFIs are usually defined as systemic infections resulting from yeasts or molds in deep-seated tissues. In contrast to superficial fungal infections, they are usually fatal conditions with high rates of morbidity and mortality (15).

Mycosis in humans can have a wide range, from minor dermal infection, superinfection followed by bacterial infection, and even severe lethal conditions involving multiple organs and systems. We can also classify fungal infections according to sites and severity of the infections, superficial, subcutaneous, and invasive. Invasive is a term that generally denotes introduction of the infection to the circulation and reaching the tissues supplied by the blood. Immune status of the host, site of infection, and virulence capability of the fungi play a vital role in the severity of the disease and the prognosis of the patient (16–18).

The development of mild fungal infection into IFI is influenced by several factors. These include the type of fungus, the host's immune system, duration and severity of the infection, and the presence of underlying medical conditions. Major risk factors include neutropenia, bone marrow suppression, hematological malignancies, bone marrow transplantation, prolonged (>4 weeks) treatment with corticosteroids, prolonged (>7 days) stays in intensive care, chemotherapy, and HIV infection (16, 17).

The most common invasive infections are associated with *Candida* sp., *Aspergillus* sp., *Cryptococcus* sp., *Pneumocystis* sp., etc. In addition, *Blastomyces*, *Histoplasma*, *Paracoccidioides*, and *Coccidioides* are endemic fungal strains that have also been implicated in causing severe systemic infections in immunocompromised patients (18, 19) (Table 1).

2 Platforms for fungal vaccine development

Despite the diverse array of platforms for fungal vaccine development, the majority remain in preclinical stages. Few have progressed to clinical phases, and none of the fungal vaccines have been approved by regulatory agencies. The following section of this article will delve into the current landscape of fungal vaccine development platforms (Figure 1).

TABLE 1 WHO fungal pathogen priority list (20).

Critical group	High group	Medium group
Cryptococcus neoformans	*Candida glabrata*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	*Eumycetoma causative agents*	*Coccidioides* spp.
Candida albicans	*Mucorales*	*Candida krusei*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

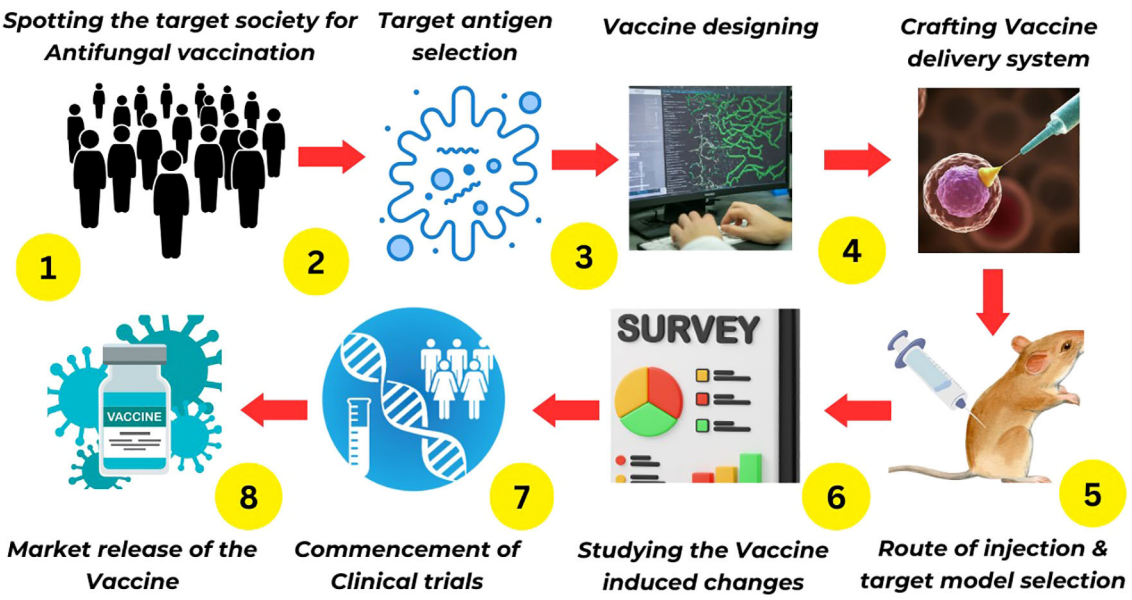

FIGURE 1
Antifungal vaccine development milestones.

2.1 Live attenuated fungal vaccines

Live attenuated fungal vaccines operate on the fundamental principle of eliciting an immune response through exposure to a modified or weakened form of the pathogenic fungi. This attenuated strain, while incapable of eliciting overt disease, stimulates robust protective immunity within the host against subsequent exposure to the virulent wild-type pathogen.

A genetically modified *Blastomyces dermatitidis* strain deficient in *WI-1* adhesin has exhibited promising vaccine efficacy against lethal pulmonary infections induced by various strains. The attenuated fungal strain triggers a delayed-type hypersensitivity reaction and a predominantly type-1 cytokine response, with interferon-gamma playing a key role. Additionally, its cell wall/membrane antigens contribute to protective immunity, highlighting the potential of targeting virulence factors for the development of novel antifungal vaccines (21). In a murine model, a genetically engineered *Candida albicans* strain with a tetracycline-regulatable *NRG1* gene, rendered avirulent through controlled morphology, elicited protective immunity against candidiasis. This protection was found to be dependent on T cell-mediated adaptive immune responses, as evidenced by the absence of protection in T cell-deficient mice (22). In a murine model of systemic candidiasis, a live attenuated *C. albicans* strain deficient in the *HOG1* gene (CNC13) demonstrated protective efficacy, predominantly mediated by the elicitation of robust cellular and humoral immune responses. Proteomic analysis identified eight antigenic proteins eliciting protective IgG_{2a} antibodies, including two novel antigens (Imh3p and Acs2p), which can be potential candidates for future fungal vaccine development (23).

Live attenuated fungal vaccines present a compelling approach for conferring durable protection against fungal pathogens by eliciting potent and comprehensive humoral and cell-mediated immunity, mirroring the complexity of natural infection responses. However, concerns regarding potential reversion to virulence in immunocompromised individuals, stringent storage requirements, and limited clinical data pose challenges to their widespread implementation.

2.2 Killed whole-cell vaccine

Killed whole-cell fungal vaccines employ inactivated (killed by heat, chemicals, or radiation) whole fungal cells to elicit a protective immune response against fungal infections. This approach offers a safer alternative to live attenuated vaccines, particularly for immunocompromised individuals, as they can eliminate the risk of reversion to virulence.

Inactivated *C. neoformans* fbp1Δ cells elicit a robust Th1-mediated immune response, conferring protection against a broad spectrum of fungal pathogens, like *Cryptococcus* and *Aspergillus* strains, and partial protection against *C. albicans*, in both immunocompetent and CD4+ T cell-deficient murine models (24). Inactivated *Saccharomyces cerevisiae* cells by heat have exhibited prophylactic efficacy against *Candida, Aspergillus, Coccidioides*, and *Rhizopus*, in preclinical murine studies, suggesting their potential as a broad-spectrum antifungal vaccine candidate. While it may not be suitable for commercialization because the model is not a purified preparation, it serves as a valuable positive control in evaluating novel molecular vaccine candidates and elucidating key components of effective pan-fungal vaccines (25).

Killed whole-cell fungal vaccines offer a safer alternative for immunocompromised individuals due to their non-replicating nature, potentially providing broad-spectrum and pan-fungal

protection. However, their efficacy may be limited by weaker immune responses and potential for adverse reactions, necessitating further research to optimize their formulation and adjuvant selection.

2.3 Recombinant (subunit) vaccine

Recombinant fungal vaccines, composed of purified antigenic components such as proteins or polysaccharides, offer a safe and effective alternative to live attenuated vaccines for immunocompromised individuals. While their efficacy often requires co-administration with potent adjuvants to elicit robust immune responses, these subunit vaccines present a promising approach for prophylactic interventions against fungal pathogens.

In a randomized phase 2, placebo-controlled, double-blind clinical trial, the recombinant subunit vaccine NDV-3, targeting the *C. albicans* adhesin/invasin protein, exhibited a favorable safety profile and elicited immune responses in women with recurrent vulvovaginal candidiasis (26). In preclinical studies, the innovative vaccine candidate PEV7, a fusion of truncated *C. albicans* Sap2 protein and influenza virosomes, demonstrated potent induction of both systemic and mucosal antibodies. Intravaginal administration of PEV7 conferred significant, durable protection against candidal vaginitis in a rat model (27).

Recombinant subunit fungal vaccines offer targeted immune responses and enhanced safety, particularly for immunocompromised individuals, but may exhibit reduced immunogenicity and require adjuvant co-administration to elicit robust protection.

2.4 Conjugate vaccine

Conjugate fungal vaccines are composed of a polysaccharide antigen covalently linked to a carrier protein, enhancing immunogenicity and inducing robust, long-lasting antibody responses against the target fungus. This approach circumvents the weak immunogenicity of polysaccharide antigens alone and offers a promising avenue for developing effective prophylactic interventions against invasive fungal diseases.

The conjugation of *Cryptococcus neoformans* capsular glucuronoxylomannan (GXM) to tetanus toxoid elicits antibody-mediated protection against experimental cryptococcal infection in mice, marking a significant milestone in the development of immunotherapies for systemic fungal diseases (28). The covalent linkage of galactoxylomannan (GalXM) from *C. neoformans* to either bovine serum albumin or *Bacillus anthracis* protective antigen, facilitated by the cyanylating agent CDAP, elicited potent and durable antibody responses in murine models (29). A novel vaccine candidate, Lam-CRM, consisting of laminarin (derived from *Laminaria digitata*) conjugated to diphtheria toxoid CRM197, has exhibited protective effects in preclinical murine models against both disseminated and mucosal candidiasis caused by *C. albicans*, as well as lethal challenge with *Aspergillus fumigatus*. The protective mechanism is likely mediated by anti-β-glucan antibodies, which exhibit direct antifungal properties *in vitro* by binding to and inhibiting fungal hyphal growth (30).

Conjugate fungal vaccines elicit robust, T cell-dependent antibody responses against target fungal polysaccharides, offering broad-spectrum protection. However, challenges in production complexity and potential for carrier-induced epitopic suppression warrant further optimization.

2.5 DNA vaccines

DNA vaccines, comprising plasmids encoding target antigens and potentially co-stimulatory molecules or cytokines, offer a promising approach for eliciting robust immune responses through direct antigen presentation by host antigen-presenting cells (APCs). Despite theoretical advantages, their translation to human use has been hindered by challenges related to safety, immunogenicity, and efficacy, necessitating further research and optimization (31).

In a murine model of paracoccidioidomycosis, a DNA vaccine encoding the immune-protective peptide 10 (P10) elicited significant and durable protection against *Paracoccidioides brasiliensis* infection, concurrent with an expansion of memory CD4+ T cells and Foxp3+ regulatory T cells, crucial for balancing effective fungal clearance with mitigation of immunopathology (32). Another DNA vaccine encoding tandem variants of the Pneumocystis p55 antigen (p55-TAG) significantly reduced pathogen burden and lung inflammation, and elicited cellular and humoral immune responses in immunosuppressed rats, demonstrating its potential as a prophylactic intervention against *Pneumocystis* pneumonia (PCP) (33).

DNA fungal vaccines offer targeted antigen expression and cellular immunity while being inherently safer than live attenuated vaccines. However, limited clinical data, delivery challenges, and potential for unintended genomic integration necessitate further investigation.

2.6 Pan-fungal vaccine

Leveraging the conserved nature of fungal cell wall polysaccharides, a pan-fungal vaccine strategy has been proposed involving the conjugation of these polysaccharides to immunogenic peptides. This approach aims to elicit broad-spectrum protective immune responses against diverse fungal pathogens by targeting shared epitopes, potentially addressing the growing threat of invasive fungal diseases.

A highly conserved 13-amino-acid sequence within the fungal chaperone protein calnexin, identified across various ascomycete species, elicits protective CD4+ T-cell responses and demonstrates potential as a broad-spectrum vaccine candidate. Immunization with calnexin encapsulated within glucan particles (GPs) induces robust antigen-specific CD4+ T-cell expansion, conferring protection against virulent strains in preclinical models (34). As discussed, earlier heat-killed yeast (HKY) *S. cerevisiae* exhibits broad-spectrum prophylactic efficacy against diverse fungal pathogens in murine models, including *Aspergillus*, *Coccidioides*, *Candida*, *Cryptococcus*, and *Rhizopus*, highlighting its potential as a

pan-fungal vaccine candidate (25). Pan-fungal vaccines, targeting conserved fungal antigens, offer the potential for broad-spectrum protection against diverse pathogens and simplified vaccination strategies, particularly in immunocompromised individuals. However, challenges remain in identifying suitable antigens, eliciting comprehensive immune responses, and navigating regulatory hurdles due to the complexity of demonstrating efficacy against multiple pathogens.

2.7 Nanotechnology in fungal vaccine development

In vaccine development, nanoparticles act as potent carriers, enhancing antigen stability, improving immunogenicity, and potentially serving as immunostimulatory adjuvants. Diverse nanostructures, including polymeric nanoparticles, nanostructured lipid carriers, phospholipid-based vesicles, dendrimers, nano-emulsions, and metallic/magnetic nanoparticles, are currently being explored as vaccine adjuvants.

Liposomal nanoparticles incorporating fatty acyl analogs of muramyldipeptide (MDP) demonstrate promising adjuvant potential for fungal vaccine development, eliciting Th1-biased immune response and stimulating cellular proliferation, crucial for effective antifungal immunity (35).

In preclinical studies, both liposomal and PLGA nanoparticle platforms have demonstrated successful delivery of the DNAhsp65 vaccine, eliciting protective immunity and mitigating pulmonary fungal burden in a murine model of paracoccidioidomycosis. Notably, the intranasal administration of the liposomal formulation presents a potentially more accessible and convenient route for adjuvant vaccine therapy against systemic fungal infections in clinical settings (36).

Nanotechnology-based fungal vaccines offer enhanced antigen stability, targeted delivery, and improved immunogenicity, but their development is hindered by potential biocompatibility concerns, regulatory hurdles, and cost-effectiveness challenges.

3 Current vaccine strategies against various invasive fungal infections

3.1 Invasive candidiasis

It is a severe systemic infection (global prevalence: 100,000 to 400,000 per year) due to *Candida* sp. (*C. albicans* 50%–60%) that can disseminate to the circulation and deep organs from mucosal surfaces (37). Risk factors include prolonged hospitalization, broad-spectrum antibiotics, central venous catheters, immunosuppression, recent surgery with high morbidity (38), and 20% to 50% mortality rate (37).

3.1.1 Pathophysiology

Deep-seated infection due to *Candida* occurs after disrupting host mucosal protection or indwelling medical devices and invasion into the bloodstream (39). As phagocyte-dependent innate immunity and adaptive T-cell responses are critical for infection control, impaired neutrophil and T-cell defects cause enhanced susceptibility (40). The interaction between candidal virulence factors (e.g., hydrolytic enzymes, adhesins, morphological and phenotypic switching, and formation of biofilm) and the host's immune status determines disease progression and severity (41).

3.1.2 Vaccine development strategies

3.1.2.1 Live attenuated vaccines

Genetically engineered strains of *Candida* have been developed by researchers to protect animals against systemic infections in preclinical studies. In rodent models, the tet-NRG1 strain of *C. albicans*, which can be treated by doxycycline, has demonstrated an effective response (22). Immunogenicity is also seen in strains like CNC13, PCA-2, and gpi7 (23, 42–44). As vaccine vehicles, avirulent strains of *S. cerevisiae* have been tried due to their flexible genetic makeup and other adjuvant-like properties (45).

3.1.2.2 Recombinant subunit vaccines

Owing to the inherent complexities in the production of live vaccines and potential adverse effects, researchers are focusing on recombinant protein vaccines that have specific immunogenicity and better safety profile due to the absence of the pathogen (46).

Key recombinant protein vaccines for candidiasis include the following:

- *Als1p and Als3p proteins:* Because of their pivotal role in adhesion as well as infection, these protein-derived vaccines have shown protection against various preclinical models (47, 48).
- *NDV-3:* Recombinant protein like Als3p using alum for the adjuvant generates immunogenicity as strong B- and T-cell responses and provides ample safety against mucosal and disseminated candidiasis (49, 50).
- *PEV-7:* In rodents, a genetically engineered version of aspartyl proteinase-2 in influenza virosomes offers immunogenicity as well as protection (27).
- *Mdh1p:* Malate dehydrogenase has also been found to be a promising antigen (51).

Potent and specific adjuvants (e.g., inulin combined with TLR agonists) have been found to be vital in amplifying immune response, causing heightened protection in preclinical animal models.

3.1.2.3 Conjugate vaccines

Polysaccharides can stimulate B cells, and with the help of polysaccharide-bound peptides, they play a key role in the presentation of antigens to T cells (52).

Common polysaccharide epitopes (e.g., β-glucans) demonstrate promising opportunities for developing pan-vaccines, which can be helpful against variable fungal infections. Vaccines against *C. neoformans* and other *Candida* sp., using a similar concept and conjugating shared polysaccharides with proteins like TT or diphtheria toxin that can invoke immune response, have been tried in the recent past as well (46, 47).

3.1.2.4 Killed whole-cell vaccines

These approaches are safe and stable due to the low risk of pathogenic transformation, a simpler manufacturing process, and low cost. However, they also come with several problems including incomplete protection against all kinds of candida infection.

- *Heat-killed C. albicans:* Intranasal immunization of heat-killed *Candida* and a genetically engineered heat-labile toxin of *Escherichia coli* (R192G) have been found to be effective in animal models except mucosal infections (53).
- *Heat-killed S. cerevisiae:* Heat-killed subcutaneous vaccines from *S. cerevisiae* provide cross-protection against fungi including *C. albicans, A. fumigatus*, and *Coccidioides posadasii*. The immune response is due to antibody against common cell wall glycans and activation of Th1 and Th17 cellular responses (25, 54, 55). *S. cerevisiae* is used due to its simplicity regarding culturability and manipulation, with well-understood physiology and molecular biology. β-1,3-D-glucan and mannan, proteins found in yeast cell walls, tend to generate an immune response and make it a good adjuvant.

Challenges and potential: The challenge remains a comprehensive protection in all forms of candidiasis including mucosal infections. Heat-killed vaccines have a unique cross-protective nature, and they can be of great help in yeast display, recombinant yeast cells, and virus-like particle preparations. These vaccines have better patient compliance and ease of administration via the oral route (54–56).

3.1.2.5 Oral vaccines

They are convenient, cost-effective approaches especially for mass vaccination to protect at-risk populations. Novel antigenic candidates and adjuvant targets are imperative for developing such vaccines. Key developments in this field are as follows:

- *Antigen display:* Eno1p antigen has been effectively displayed on the surfaces of *E. coli* and *S. cerevisiae* cells, indicating them to be promising oral vaccines for the future.
- *Mice immunized with Eno1p (oral, intranasal, and subcutaneous):* They were shown to enhance anti-Eno1p antibody levels and protection against *C. albicans* [Eno1p-expressing *S. cerevisiae* (oral) → 60% and *L. casei* cells 20% protection] (51, 57).

Challenges with adjuvants: Conventional adjuvants used for injectable routes of administration are mostly ineffective in the environment of enteric systems.

Future directions: Proteomic analysis is a promising technology in drug target identification. Several ongoing research into new antigenic candidates through proteomics aim to further enhance the effectiveness of oral vaccines against candidiasis (58).

3.1.2.6 Bacterial ghost vaccines

Hollow bacterial cells with core components like immunogens or drugs retain the ability of the organism to induce strong immune responses. This approach could pave the way for highly effective anti-*Candida* vaccines (59). Intraperitoneal administration of *Candida* ghosts with nanoparticles of silver and gold offers enhanced humoral and cellular immunity, faster wound healing, and controlled inflammation in Sprague–Dawley albino rats. They also stimulated white blood cell proliferation and systemic interferon (IFN)-γ levels and did not trigger hyperallergic responses (60). Bacterial ghosts inherently aggravate the innate immune response of the host and provides better efficacy of the vaccine without disrupting the pathogen's structural features (59).

3.2 Invasive aspergillosis

Invasive aspergillosis (IA) is a fatal fungal infection caused by *Aspergillus* sp., primarily *A. fumigatus*. The global prevalence of this infection is approximately 200,000 cases annually, with a high mortality rate (30% to 90% in high-risk populations) (61).

3.2.1 Pathophysiology

The infectivity of the fungus is due to accidental inhalation of spores followed by germination, hyphae formation, and bloodstream invasion leading to widespread organ dissemination (62). Impaired phagocytic and cell-mediated immunity in special populations (i.e., immunodeficient people) facilitates fungal proliferation (63).

3.2.2 Vaccine development strategies

3.2.2.1 Crude vaccines

Whole or fractionated *Aspergillus* can protect the rodents, but the efficacy differs with heat-killed and live conidia. Live conidia show aggravated immune response due to tissue growth and filamentous forms. Challenges in this type of vaccine include autoimmune response and reactogenicity (64, 65).

3.2.2.2 Pan-fungal vaccines

They are known to target several fungal species because of the presence of common antigens among different fungi. β-1,3-glucan with diptheria toxoid provides protection against multiple fungal infections (aspergillosis and candidiasis) (66). NXT-2 peptide binds to fungal conidia and hyphae, enhancing immune response and resulting in mortality reduction in preclinical studies (67).

3.2.2.3 Dendritic cell-based vaccines

Dendritic cells (DCs) pulsed with *Aspergillus* conidia and RNA have shown efficacy in infections after haploidentical transplants in preclinical and small clinical trials (68). Owing to the increased cost and non-specific adaptive immune response, no vaccine has been tried in large clinical trials (66, 68–70). Because of environmental interruption in the form of exposure to fungal conidia, specific and immunogenic antigens need to be identified for vaccine production in order to surmount non-specific antibody responses after immunization (71).

3.3 Invasive cryptococcosis

Invasive cryptococcosis, a serious life-threatening fungal infection, is caused by inhalation of infectious spores or desiccated yeast cells of

encapsulated yeast *C. neoformans* and *C. gattii* (72). Over 180,000 cases are registered annually (mortality rate ranging from 20%–70%) with a high burden in Sub-Saharan Africa and Southeast Asia (73). High-risk groups include immunocompromised patients, those who underwent organ transplantation, and those with an advanced age (74).

3.3.1 Pathophysiology

Inhalation of infectious particles, followed by dissemination to the central nervous system and other organs through circulation, is the usual route of infection. Cell-mediated immunity, including response of T-helper cells and activated macrophages, has a vital role in infection control. Capsular polysaccharide diminishes phagocytosis and the host immune response, contributing to its infectivity, mainly affecting the respiratory and nervous systems, but can also include skin, bones, and prostate (75).

3.3.2 Vaccine development strategies

The capsule is composed of GXM, a major virulence factor that acts as the cause for both challenges and advantages for vaccine development (76).

3.3.2.1 Whole yeast approaches

It includes designing new strains of *Cryptococcus* that provide immunity via DC stimulation (77, 78). A study has reported that heat-inactivated acapsular *C. gattii* stimulates Th17 cells and thus decreases the infection (79).

3.3.2.2 Polysaccharide-based vaccines

GXM was the first experimental vaccine to induce immunogenicity without the involvement of T cells. Conjugation with the GXM-tetanus toxoid has shown improvement in antigenicity, inducing partially protective murine antibodies (28, 80). The GXM-conjugated mimetic peptide helps in the generation of protective antibodies (81).

3.3.2.3 Mutant strains

It includes engineered strains that lack fungal proteins like chitosan and provides immunity via Th-1 cells (82). Other strains like Fbox protein knockout mutants and the morphogenesis regulator protein Znf2 over expressive strains can be of use too (83, 84).

3.3.2.4 Protein antigen vaccines

To create strains with decreased virulence for ideal vaccine candidate, Chitosan, GXM, F-box protein and Steryl glycosides like key factors are used as adjuvant (24, 82, 83, 85–87).

3.3.2.5 Subunit vaccines

Nonhomologous proteins of chitosan deacetylase class are included (88).

3.3.2.6 Innovative formulations

Tetraethyl orthosilicate (TEOS) helps in the encapsulation of Cda2 antigen within GPs and has played a role in providing heat stability and protection against fatal respiratory infections (89, 90).

3.4 Invasive mucormycosis

Multiple fungi (*Rhizopus*, *Mucor*, and *Lichtheimia*) of the order Mucorales cause severe and life-threatening infection, with invasive mucormycosis mostly affecting patients with impaired immune systems (91). Though global incidence has been 0.2 to 1.7 cases per million annually, during the COVID-19 pandemic, there was a massive surge of morbidity and mortality (77). Risk factors include defective phagocytic activity and innate immune system, prolonged neutropenia, diabetes mellitus, use of corticosteroids, organ transplantation, and iron overload.

3.4.1 Pathophysiology

Virulence includes iron entrapment abilities of the host, causing extensive angioinvasion and tissue death. Proteolytic enzymes facilitate tissue invasion and dissemination. The fungi can escape the host's immune response through suppression of oxidative burst and cytokine production (78).

3.4.2 Vaccine development strategies

3.4.2.1 Proteome studies

- Identification of two protein candidates can be effective in various mucormycetes (88).
- A vaccine that has multi-epitopes can be the ideal candidate, which can have a prominent immunological and physicochemical nature (79).

3.4.2.2 Immunoinformatics approach

- For mucormycosis due to different fungi, newer epitopes with activity against *Mucor circinelloides* and *Rhizopus oryzae* provide better population protection and immune response.
- Molecular docking analyses demonstrated a stable and effective vaccine in minimal disordered regions (92).

3.5 Invasive histoplasmosis

It is a potentially life-threatening fungal infection due to a dimorphic fungus (93). Immunocompromised individuals like those with HIV/AIDS, organ transplant recipients, and patients treated with immunosuppressives drugs are at higher risk for developing invasive disease (94).

3.5.1 Pathophysiology

Inhalation of *Histoplasma* spores are subsequently taken up by alveolar macrophages. They are transformed to the yeast phase, after which they replicate, leading to a disseminated infection (95). While primarily being a respiratory infection, it can spread to the liver, spleen, bone marrow, and central nervous system, leading to

complications such as pulmonary fibrosis, adrenal insufficiency, and neurological deficits, impacting their quality of life (96, 97).

3.5.2 Vaccine development strategies

- Antigens derived from cell wall and membrane extracts recognized by T cells triggered protective immunity against lethal *Histoplasma* challenge in rodents.
- *Specific antigen vaccines:*
  - rHsp60 glycoprotein vaccine activates specific CD4+ or CD8+ T cells and provides protection in animal models. However, because of the similarity with its human counterpart, this vaccine shows a higher risk of autoimmune reaction and restricted its wide use (98, 99).
  - A specific IgG1 isotype of monoclonal antibodies against Hsp60 and surface M antigen demonstrated immunogenicity in murine models (100, 101).
- *Reverse vaccinology:* Using knowledge of pharmacogenetics, comprehensive genome sequencing and computational analysis indicated that β-1,3-glucanosyltransferase and proteins similar across strains can be included in future immunotherapy (102).

3.6 Invasive coccidioidomycosis

Dimorphic fungi *Coccidioides immitis* and *C. posadasii* cause this disseminated fatal infection that is endemic in North and South America and Mexico. The risk is higher for patients with impaired immunity.

3.6.1 Pathophysiology

Spores of *Coccidioides* are inhaled, and within alveolar macrophages, they undergo endosporulation, leading to severe complications in bones, joints, and the central nervous system, leading, in turn, to severe manifestations (103). There is less risk of interpersonal transmission, which can only take place in cases of organ transplantation or laboratory accidents (104).

3.6.2 Vaccine development strategies

3.6.2.1 Formalin-killed spherules

- A 1967 study found that vaccinated rodents with formalin-killed spherules have shown protection (105).
- A double blind study in 3,000 subjects showed limited success due to risk of toxicity and lesser disease incidence (106). However, soluble spherule models have shown to be helpful in murine models (107).

3.6.2.2 Targeted antigen approaches

- Pmp1 and Cs-Ag (108) have shown good immunogenicity and identified as promising vaccine candidates.
- Subcutaneous injection with antigen Ag2 for prime as well as booster doses causes improved cell-mediated responses. Increased Th1 and Th17 responses indicated potential benefits (109).

3.6.2.3 Cellular vaccination

Intranasal DC transfection with Ag2 DNA generates specific IgG responses, though further studies are needed to understand the immune response better (110, 111).

3.6.2.4 Other strategies

Intervention of genes like *CTS2, CTS3*, and *ARD1* in HLA-DR4 transgenic mice displayed Th1 and Th17 cell expansion and engagement of innate immunity, proving to be a useful tool for future therapeutics (112).

3.7 Invasive paracoccidioidomycosis

Invasive paracoccidioidomycosis is a similar life-threatening condition due to dimorphic fungus *Paracoccidioides* infection. It is endemic in Latin America (highest prevalence in Brazil, Colombia, Venezuela, and Argentina) (113). Risk factors include immunocompromised state, male gender, smoking habits, and workers with exposure to soil or dust (114).

3.7.1 Pathophysiology

Inhaled spores engulfed by macrophages in the alveoli and converted to the yeast phase and following multiplication cause severe disseminated infection to other organs (115). Person-to-person transmission rarely occurs by accident or through organ transplantation.

3.7.2 Vaccine development strategies

- *Therapeutic DNA vaccines:* gp43 encoded DNA vaccines using plasmids displayed mixed T helper responses that can be amplified and specified using plasmids encoding the P10 minigene. IL-12 added to these vaccines improves the immune response and suppresses the fungal loads significantly in long-term infections (116).
- *Immunotherapy with peptide P10:* Antifungal treatment consisting of chemotherapy and P10 peptide immunization increases IL-12 and IFN-γ production while reducing IL-4 and IL-10, enhancing the efficacy of antifungal treatment (117). P10-based immunotherapy, even without chemotherapy, has shown good response with the addition of adjuvants, nanoparticles, and DC priming (88).
- The P10 segment of the gp43 antigen of *P. brasiliensis* has the ability to generate robust T-helper immune response through IFN-γ, leading to fungal elimination (32, 118).
- *Fungal whole cells:* Yeast cells attenuated by methods like gamma-irradiation evoke strong immune reaction with T-

helper cells and prove to be another option for research and trials (88).

- *Protective antibodies*: gp43 targeting monoclonal antibodies showed promise in immunotherapy (119–121).
- A newer drug delivery system and the implementation of gene therapy can be a good prospect for the future (88).

3.8 Invasive blastomycosis

Dimorphic fungus *B. dermatitidis* causes serious fungal infections in certain regions of North America (122, 123). Patients with impaired immunity or occupational or accidental exposure to soil or decaying vegetation are mostly at risk of getting infected (38).

3.8.1 Pathophysiology

Inhaled spores from aerosol is the main infective form that has the ability to cause both pulmonary and extrapulmonary infection that can disseminate and cause severe infections (38).

3.8.2 Vaccine development strategies

- *Recombinant BAD1:* Though this vaccine could induce immunogenicity, it has a modest role in enhancing survival or the significant lowering of fungal burden. When IL-12 was added to improve its efficacy, alteration of the antibody response and aggravation of the delayed-type hypersensitivity reactions are observed (13). Monoclonal antibodies targeting *BAD1* did not raise killing power or protection. However, B-cell knockout mice demonstrated better protection. *BAD1*-null mutant displayed notable survival and attenuated murine fungal load through the generation of robust IFN-γ production and T-cell activation (124).
- *Vaccination strategy:* People with increased risk of exposure like construction workers, agricultural workers, and those engaging in activities like spelunking in endemic areas can benefit from this vaccination. The recommended age for vaccination would be approximately 2–3 years (13).

3.9 Invasive *Pneumocystis* pneumonia

Invasive PCP is caused by *Pneumocystis jirovecii*. It causes severe and fatal opportunistic infection primarily in immunodeficient individuals worldwide. The primary risk factor for developing PCP is impaired immunity, especially CD4+ T-cell deficiency (125).

3.9.1 Pathophysiology

It is transmitted mainly through the airborne route. The trophic forms attach and infiltrate the epithelial cells and then undergo asexual reproduction, causing the accumulation of cysts and trophic forms in the alveoli (126). Pathological hallmarks include diffuse alveolar damage and alveolar casts, interstitial inflammation, and fibrosis in severe cases.

3.9.2 Vaccine development strategies

3.9.2.1 Whole organism vaccines

In past decades, inactivated whole organisms have been tested to provide strong protection in animal models (127–129). Intranasal vaccination with non-viable fungus with cholera toxin B induced prominent immunogenicity through IgA and IgG antibody responses (128). *Pneumocystis* cultivation in laboratories has halted the viability and mass production of this approach (130).

3.9.2.2 Subunit vaccines

- *Pneumocystis cross-reactive antigen 1 (Pca1):* This antigen was known as A12 antigen in the past. Pca1 provides protective response through the suppression of organism burden and lung inflammation. It has also displayed cross-reactivity with several *Pneumocystis* species (131).
- *SPD1:* It has similarity with *P. jirovecii* and induces IgG antibody responses and enhances memory B-cell development, ultimately eliminating organism burden in chronic infection (132).

3.9.2.3 Other antigen candidates

Major surface glycoprotein (MSG) and recombinant p55 antigen have been targeted for both therapeutic and prophylactic vaccine development (130, 133, 134).

3.10 Invasive sporotrichosis

It is a form of subacute or chronic subcutaneous mycosis due to dimorphic *Sporothrix schenckii* and its related species, targeting immunocompromised individuals as well as people with occupational exposure to infected vegetation or soil (135). The disease has a global distribution, especially in tropical and subtropical regions. Apart from immunosuppression or immunodeficiency, occupational or accidental contact (e.g., gardeners, farmers, and florists) and inoculation through injury with contaminated plant materials or soil are common sources of infection (136).

3.10.1 Pathophysiology

Pathogenesis: Infection is primarily acquired through percutaneous inoculation due to traumatic implantation. Inhalation and zoonotic transmission (from infected animals) are less common routes (137).

3.10.2 Vaccine development strategies

- *Whole organism and glycoprotein-based vaccines: S. schenckii*-infected mice showed high titers of anti gp70 antibodies and reduced colony-forming units (CFUs) (138). Monoclonal antibodies targeting gp70 (mAbP6E7)

are seen to induce phagocytosis and reduce fungal burdens and are potential agents for prophylaxis and therapeutics (139).

- *Cell wall protein vaccines:* Passive transfer of serum from mice vaccinated with ssCWP (*S. schenckii* cell wall proteins) and adjuvants showed immunogenicity after *S. schenckii* challenge (140).
- *Recombinant phage vaccines:* Chen et al. developed a vaccine that used a recombinant phage displaying a gp70 peptide, and it can reduce the fungal burden in *S. globosa* infections through heightened Th1 cell responses and through a robust humoral response (139).
- *Antibody-based therapies:* Antibodies against phage-KR extracted from vaccinated mice also displayed protection against *S. globosa* infection through suppressed colonization of the fungi, increased survival rates, and inhibited inflammatory changes in mice (141).
- *Immunoproteomic approaches:* Using immunoproteomic methods, newer targets in the form of antigenic peptides from *S. brasiliensis* were identified. Peptides like ZR3, ZR4, and ZR8 helped in increasing cytokine levels and CD4+ T-cell proliferation and reduced lesion sizes in sporotrichosis (142).

4 Fungal vaccines in pipeline (2018 onwards)

There are several ongoing studies on exploring new horizons in the field of fungal vaccines. Most of these are in preclinical phases. The latest vaccines for individual fungal infection that have been examined since 2018 are described here (Table 2; Figures 2, 3).

4.1 Invasive candidiasis

4.1.1 Cell-based vaccine

4.1.1.1 EV

Extracellular vesicles (EVs) from *C. albicans* were used as vaccine against murine candidiasis. They augmented specific serum IgG antibodies production, producing robust humoral immune response. It also decreased fungal load in various organs like liver, kidney, and spleen in a candidal murine model. The vaccine with or without an adjuvant can protect lethally challenged mice entirely, according to a study (143).

4.1.2 Heat-killed vaccine

4.1.2.1 V132

Sublingual heat-killed vaccine trained immunity in peripheral blood mononuclear cells and monocytes, resulting in amplified oxidative phosphorylation and glycolysis. These changes occurred by enhanced pro-inflammatory responses through robust Th1/Th17 responses. Specifically, stimulation with adjuvant (MV140) leads to stronger Th17 responses in immunized mice, eliciting both innate and adaptive immunity (144).

4.1.3 Live attenuated vaccine

- FLO8: The yeast form of the *FLO8*-deficient *C. albicans* strain conferred protection against lethal infection through inducing IL-10 induced by the Dectin-2/CARD9 pathway in macrophages and DCs. IL-10 inhibits T-cell apoptosis, preventing thymus atrophy. This facilitates the constant release of T cells from thymus, enhancing strong Th1-based immunity responses that produce protection from polymicrobial infection (145).
- **Low-virulence *Candida species*:** Myeloid-derived suppressor cells' (MDSCs') defense is facilitated by Gr-11 leukocytes against intra-abdominal infection. Ly6G1 G-MDSC enhanced trained innate immunity (TII), which is produced after inoculation (IP/IV) of the live attenuated strain, which decreases organ-specific fungal load in the murine model (146).
- **gpi7 strain:** Mutant gpi7 strain produces long-lived plasma cells (LLPCs) that induce Dectin-1-facilitated innate immunity response regulating adaptive humoral immunity, facilitating the production of candidacidal antibodies. Specifically, it activates Th2 cell-mediated immune response by increasing IL-4 and IL-13 levels. This pathway could also initiate RelB induction to stimulate IL-18, thus generating a novel horizon for adaptive humoral immunity as well as innate immunity mediated by Dectin-1 (44).

4.1.4 Subunit vaccine

- **rHyr1p-N:** Hyphal-regulated protein-1 (Hyr1) helps in the growth of fungal hyphae, which evades phagocytolysis, an important host defense mechanism, thus contributing to virulence, in which recombinant Hyr1 produces immunity mediated by high levels of IgG and IgA antibodies. These antibodies promote effective opsonic activity, cytotoxicity, complement activation, and improved neutrophilic chemotaxis. rHyr1p-N, formulated with Freund's adjuvant (FA), effectively shields neonatal mice from systemic candidiasis by inducing a vertically transmitted strong immune response (147).
- **Hsp90-CTD:** The heat shock protein 90 C-terminal domain, which is also known as Hsp90-CTD, mixed with polyethyleneimine (PEI) formed the nano-vaccine. The vaccine induces a rapid and long-lasting humoral and cellular antibody response. It also significantly decreased kidney damage by candidal infection in a murine model (148).
- **rSap2**: Secretory aspartate protease (Sap) helps in bond formation, hyphal development, biofilm establishment, and immune response facilitating penetration of host cells. The vaccine produces both humoral and cellular immune responses by Sap2-specific antibodies (IgG and IgM) and neutrophil-mediated fungicidal activity and boosts Th1/Th2/Th17 cytokine levels, indicating an immunomodulatory role (149).

TABLE 2 Overview of recent antifungal vaccines (2018 onwards).

Target pathogen	Type	Vaccine name	Antigen/ Component	Adjuvant/ solvent	Animal/ Human	ROA	Development stage	Efficacy	Reference
Candida	Cell based	Extracellular vesicles (EVs)	EV	No	Immunosuppressed mice	Not specified	Preclinical	IL-12p70, TNFα, and IFN-γ were detected	(143)
	HK	V132	Heat-killed *C. albicans*	MV140	Balb/c mice	SL/IP	Preclinical	Robust Th1/Th17 and regulatory T-cell immunity	(144)
	LA	FLO8	(*flo8*) mutant *C. albicans*	No	C57BL/6, Balb/c, and nude mice	IV	Preclinical	Th1-biased antifungal immune responses.	(145)
		Low-virulence *Candida* species	Low-virulence *Candida* species	No	Mice	IP/IV	Preclinical	Ly6G1 G-MDSC-mediated trained innate immunity	(146)
		Gpi7	gpi7 mutant strain	No	C57BL/6 mice	IV	Preclinical	Th2 immune response	(44)
	Subunit	rHyr1p-N	Recombinant forms of the cell wall proteins Hyr1	Freund's adjuvant	Balb/c mice	SC	Preclinical	Primarily characterized by high titers of IgG2 antibodies, alongside IgG1 and IgA.	(147)
		Hsp90-CTD nanovaccine	Heat shock protein 90 (Hsp90-CTD) from *C. albicans*	Polyethyleneimine (PEI)	C57BL/6 mice.	SC	Preclinical	Both humoral and cellular immunity	(148)
		rSap2	Sap2 protein	Alum	Balb/c mice	IN	Preclinical	Th1/Th2/Th17 mixed response	(149)
		NDV-3A	Als3	VLP	Human	IM	Phase 2	Production of IFN-γ and IL-17A	(150)
		CaS1	Eno1	Freund's adjuvant	White leghorn (*Gallus domesticus*) chickens	IM	Preclinical	IgG antibodies	(151)
Aspergillus	HK	HK-sgl1	Heat-killed sgl1 *Aspergillus* conidia (HKAC)	Sterylglucosides (SGs)	CBA/J mice	IN	Preclinical	Proinflammatory immune response characterized by the activating neutrophils. canonical CD4+T/CD8+ T cells and noncanonical γδ T cells	(152)
	Subunit	AF. KEX1	Endoprotease Kexin	Squalene adjuvant TiterMax	Balb/c mice	SC	Preclinical	IgG titer achieved following vaccination	(153)
		VesiVax® Af3/9	Liposome formulations of Asp-f3/f9	Lipidated Tucaresol (LT1), Monophosphoryl Lipid A (MPL), PAM-3-CAG (P3C)	Swiss Webster mice	SC	Preclinical	CD4+ T-cell responses	(154)

(Continued)

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TABLE 2 Continued

Target pathogen	Type	Vaccine name	Antigen/ Component	Adjuvant/ solvent	Animal/ Human	ROA	Development stage	Efficacy	Reference
Cryptococcus	Cell-based	Dendritic cell (DC) vaccine	Dendritic cell (DC) vaccine	No	C57BL/6 mice	IV	Preclinical	Long-term Th17 immune response	(155)
	HK	H99γ	Cn strain H99γ	No	BALB/c mice	IN	Preclinical	STAT1-mediated classical macrophage (MΦ) activation	(156, 157)
	HK/LA	Znf2	*Cryptococcus neoformans* ZNF2-brf1Δ mutant vaccine	No	A/J mice	IN	Preclinical	Th1 and Th17 type of immune response	(158)
		cda1Δcda2Δ.	Mutant strain cda1Δcda2Δ	No	BALB/c, C57BL/6, and CBA/J mice	IN/orotracheal	Preclinical	Th1, Th2, antibody-mediated	(159)
	LA	GXM-SG	*Cryptococcus neoformans* Δsgl1 mutant vaccine	No	CBA/JC and C57BL/6 mice	IP	Preclinical	Th1 and Th17 type of immune response	(87, 160)
		sgl1	Cn Δsgl1 mutant strain	No	CBA/J (Envigo) female mice	IN	Preclinical	Th1, Th2, antibody-mediated	(161)
		fbp1	fbp1 Mutant strain (HK-fbp1)	No	BALB/c mice and CBA/J	IN	Preclinical	Th1 and Th17 type of immune response	(24, 162)
	Subunit	Cda2-Pep1	*Cryptococcus neoformans* ZNF2-brf1Δ mutant vaccine.	GP	BALB/c and C57BL/6 mice	SC	Preclinical	Mixed Th1/Th17 type of immune response	(163)
		Glucan particle (GP) vaccine	Recombinant *Cryptococcus* proteins	Mouse serum albumin (MSA)	BALB/c and C57BL/6 mice.	SC	Preclinical	Humoral (antibody-mediated) and cellular (T-cell-mediated) immunity	(164)
		EV	Nanoprotein-based extracellular vesicle	No	BALB/c and C57BL/6 mice	IP	Preclinical	Strong IgG antibody response	(82)
Mucorales	Multivalent peptide vaccine	BF vaccine (BFV)	FTR1	RS09 and human beta-defensin-3	Mice	Not specified	Preclinical	Innate and adaptive immunity [Th cells, cytotoxic T cells, B cells, natural killer (NK) cells, and macrophages]	(84)
Histoplasma	Subunit	Alkaline extract of *Histoplasma capsulatum*	Encapsulated alkaline extract	GP	C57BL/6 mice	SC	Preclinical	Th1 and Th17 response	(165)
Coccidioides	Cell-based	Ag2/PRA-DC	Ag2/PRA	DC	BALB/c mice.	IN	Preclinical	Increased levels of serum IgG	(111)

(Continued)

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TABLE 2 Continued

Target pathogen	Type	Vaccine name	Antigen/ Component	Adjuvant/ solvent	Animal/ Human	ROA	Development stage	Efficacy	Reference
	LA	Cps1	Avirulent Δcps1	Not specified	Beagle dogs	SC	Preclinical	CD4+ T cells secreting IFN-γ	(166)
	Subunit	GCP-rCpa1	Recombinant coccidioidal antigen (rCpa1)	Glucan–chitin particles (GCPs)	C57BL/6	IN	Preclinical	Th1 and Th17 type of immune response	(167)
Paracoccidioides	Subunit	P10	Peptide P10	Cationic liposome (CAF01)	C57BL/6 mice	SC	Preclinical	Th1 and Th17 type of immune response	(168)
		P10	Peptide P10	Chitosan nanoparticles	Balb/c mice	IN	Preclinical	Th1 and Th2 immune response	(169)
Blastomyces	Subunit	Bl-Eng2	Endonuclease2	Advax3, TLR agonist	C57BL/6	SC	Preclinical	IFN-g- and IL-17-releasing antigen-specific T cells	(170)
Pneumocystis	Subunit	Recombinant $A12_{1-85}$	A121–85 protein segment	Not specified	Balb/c mice	SC	Preclinical	Increasing serum IgG	(171)
		Recombinant KEX1	Kexin 1 (KEX1)	Aluminum hydroxide	Rhesus macaques	IM	Preclinical	Humoral response	(172)
		KEX1/(α-GC)	Kexin 1 (KEX1)	α-Galactosylceramide (α-GC)	Rhesus macaques	IM	Preclinical	Stimulating invariant natural killer T (iNKT) cells—humoral response	(173)
Sporothrix	LA	Δcps1	Sporothrix mutant strain Δcps1	Not specified	Mice (BALB/c strain)	SC	Preclinical	Releasing Th1, IFN-γ, Th17, and IL-17A	(142)
	Subunit	SsEno/PGA	Enolase	Montanide™ PetGel A (PGA)	Balb/c mice	SC	Preclinical	Strong Th1 immune response	(174)

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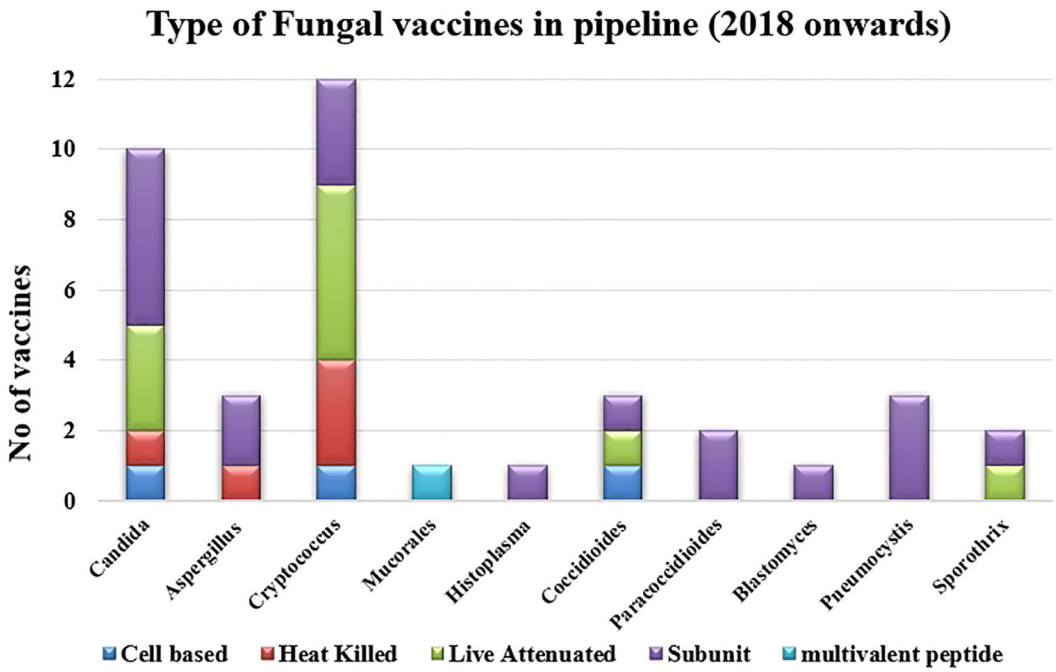

FIGURE 2
Recent advances in antifungal vaccine development based on targets.

- **NDV-3A:** A phase 2 trial was conducted to check the effectiveness of the NDV-3A vaccine. The preparation was based on recombinant hypha-precise surface antigen (Als3) fortified with alum. It included 188 women with recurrent vulvovaginal candidiasis (RVVC). The study demonstrated safety and effectiveness by producing strong rapid B- and T-cell immune response. Results of the trial facilitated the effectiveness of NDV-3A in treating vulvovaginal candidiasis. Disease recurrence rates were also reduced (150).
- **CaS1:** It targets fungal enolase (Eno1) and disrupts the binding of CaEno1 to plasminogen. Based on an *in vitro* experiment, scFv CaS1 (a single-chain variable antibody)

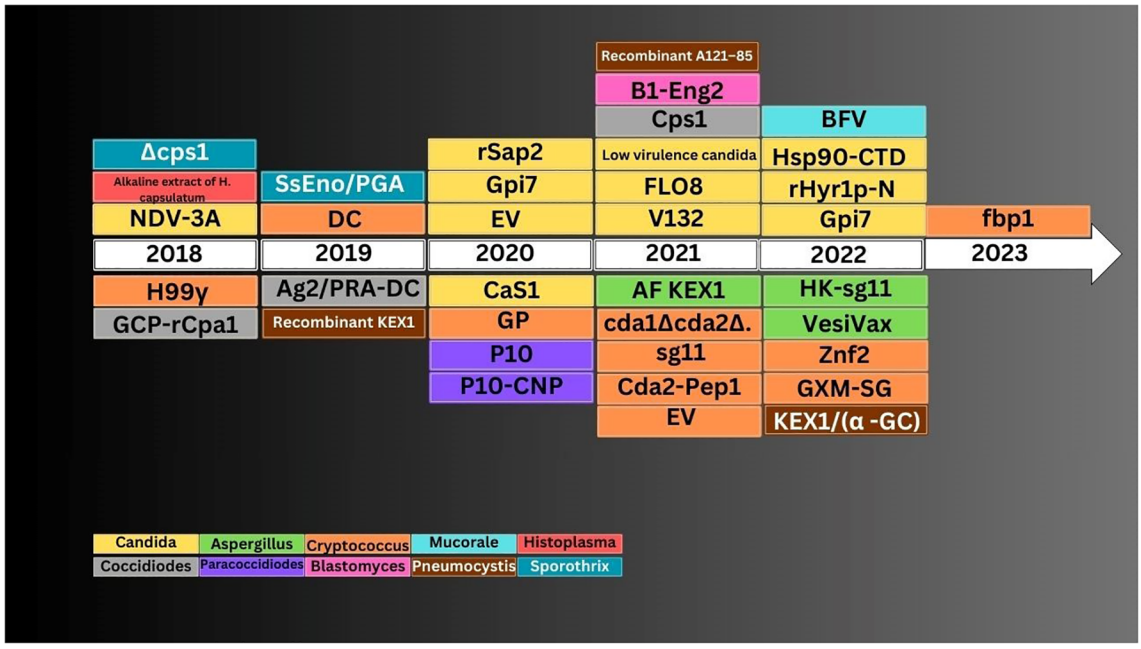

FIGURE 3
Antifungal vaccine development timeline.

has anti-fungal properties comparable to fluconazole. Furthermore, the intramuscular administration of CaS1 in white leghorn chicken leads to a distinguished reduction in fungal load and cytokine levels by producing specific IgG antibodies (151).

4.2 Invasive aspergillosis

4.2.1 Heat-killed vaccine

4.2.1.1 HK-sgl1

The sterylglucosidase-encoding gene (*SGL1*)-deficient, avirulent *A. fumigatus* strain (Δsgl1) demonstrated whole host protection in the mouse model. SGs are immunomodulatory glycolipids added with Δsgl1 as adjuvants. Mice with intranasal inoculation with Δsgl1 conidia survived without any visible morbidity signs until day 75 post-test while all control group mice succumbed before day 15 (152).

4.2.2 Subunit vaccine

- **AF. KEX1:** This vaccine targets recombinant endoprotease Kexin. AF. KEX1-vaccinated murine produced reduced mortality rate and significantly inhibited fungal load in lung compared to non-vaccinated mice. This is inversely associated with the anti-AF. KEX1 IgG antibody levels were determined following subcutaneous injection (153).
- **VesiVax® Af3/9:** Two liposomal formulations developed as VesiVax® Af3 and VesiVax® were conjugated to form this vaccine. Added with Lipidated Tucaresol, Monophosphoryl Lipid A, and PAM-3-CAG as adjuvant, the vaccine produced protective CD4+ T-cell immunity. It provided protection to the immunized mice (154).

4.3 Invasive cryptococcosis

4.3.1 Cell-based vaccine

4.3.1.1 DC vaccine

The vaccine produces protective pro-inflammatory responses including neutrophil stimulation and granuloma formation in cryptococcosis by promoting long-term memory Th17 cells, which can be demonstrated at least 6 months post-vaccination in the lungs. It also induces IL-17A upon antigen re-challenge (155).

4.3.2 Heat-killed vaccine

- **H99γ:** An epigenetically engineered *C. neoformans* strain induced immunity generated in immunocompromised T cell-deficient mice by expressing IFN-γ. This protection is directed by STAT1-mediated classical macrophage (MΦ) activation, which is associated with TII against cryptococcosis (156, 157).
- **Znf2**: It is a regulatory protein that helps in the transition of dimorphic fungi also interacting with the host. Vaccines with ZNF2 overexpression, both heat-killed (HK) or live attenuated (LA) formulations, reduce virulence and promote protective immunity in mice from lethal fungal strain. These cells with overexpressed ZNF2 help the host to distinguish protective Th cells, induced Th1/Th17 immune response of CD41 T cells in murine lungs at post-immunization day 7 (158).
- **HK/LA-*cda1Δcda2Δ*:** The mutant strain lacks two key enzymes (Cda1 and Cda2) that help in converting chitin to chitosan in the cell wall. This modification is vital for the virulence. Without sufficient chitosan, the fungus is more vulnerable to host defenses and is cleared more effectively. The vaccine incorporating the *cda1Δcda2Δ* mutant strain induces a Th1- and Th2-mediated strong protective adaptive immune response in mice (159).

4.3.3 Live attenuated vaccine

- **GXM-SG:** The vaccine accumulates sterylglucosides (SGs) and retains capsular polysaccharide GXM. Fungal γδ T cells, by activating TLR2 signaling pathway, induce IFN-γ and IL-17A. The vaccine generates a strong γδ T-cell response indicating a mixed Th1 and Th17 type of immunity, which is crucial for host protection in immunocompromised animals (87, 160).
- **Sgl1:** Ergosterol 3β-D-glucoside is accumulated by live attenuated Sgl1 deficit *C. neoformans(Cn)* strain (***Δ Sgl1***), which converts Cn avirulent and protects immunocompromised mice (161).
- **Fbp1:** E3 ligase lacking SCF subunit protein (Fbp1) helps in proteolysis and fungal virulence. HK-fbp1 revealed increased Th1 and Th17 responses, demonstrated by augmented IFN-γ and IL-17A titer, which is protective against virulent strain, even in immunocompromised mice. Early-treated animals also exhibited decreased Th2 cytokines (IL-13). This result is significant in the context of the HIV/AIDS-induced immunocompromised state, which is a profound risk factor in humans (24, 162).).

4.3.4 Subunit vaccine

- **Cda2-Pep1:** The vaccine based on peptides Cda2 produced from the *C. neoformans* ZNF2-*brf1Δ* mutant strain helps in stimulating robust $CD4^+$ T-cell responses. Animals vaccinated with Cda2-Pep1 were significantly protected compared to the unvaccinated group. GP adjuvant promotes protective Th1 and Th17 responses (163).
- **Glucan particle vaccine**: This was loaded with recombinant Cryptococcal proteins. The vaccine employs GPs to deliver recombinant proteins, which stimulate the immune system to recognize and combat Cryptococcus infections. The immune

response involves both humoral and cellular type. GPs added with mouse serum albumin (MSA) engaging Dectin-1 and complement receptors promote a strong activation of innate immune responses in a murine model (164).

- **EV:** The lipid bilayer of EVs is coated with a mannoprotein-embedded fibrillar embellishment, with the capsular polysaccharide forming the outermost covering. BALB/c mice immunized with two different doses (1 and 10 μg) of EV-protein derived from an avirulent Cn mutant strain (*cap59Δ*) showed a robust IgG antibody production that extend the survival time in a murine model. These results point to the fact that cryptococcal EVs can induce an adaptive immunity (82).

4.4 Invasive mucormycosis

4.4.1 Multivalent peptide vaccine

4.4.1.1 BFV

The vaccine targets the ferrous permease (FTR1) protein, necessary for iron accumulation by fungi, important for their virulence. This epitope (CTL, HTL, and LBL)-based vaccine produces a strong immunogenic response due to CTL, HTL, and LBL response. Added adjuvants RS09 and human β-defensin-3 help induce innate as well as adaptive immunity by activating B cells, Th cells, macrophages, cytotoxic T cells, and natural killer (NK) cells in a mouse model of mucormycoses (84).

4.5 Invasive histoplasmosis

4.5.1 Subunit vaccine

4.5.1.1 Alkaline extract of *Histoplasma capsulatum*

The alkaline extract, composed of GPs, induces robust Th1/Th17 cell base immunity by producing both IFN-γ and IL-17 in organs such as the lungs. The vaccine also decreased approximately 80% of fungal load and significantly improved survival in an animal model (165).

4.6 Invasive coccidioidomycosis

4.6.1 Cell-based vaccine

4.6.1.1 Ag2/pRA-DC

The vaccine involves the use of DCs transfected with Ag2/PRA plasmid DNA to induce immunity. DC activates T cell-mediated immune response. The vaccinated mice showed no visible signs of morbidity or no noteworthy deviations in lung injury indicators like lactate, albumin, and other lung proteins. The vaccine induced cytokine-secreting cells (IL-17, IL-4, and IFN-γ) in murine organs. It also resulted in augmented serum IgG levels (111).

4.6.2 Live attenuated vaccine

4.6.2.1 Cps1

The Δcps1 vaccine induces adaptive immunity. Subcutaneous immunization with live Δ*cps1* arthroconidia of beagle dogs alleviated pulmonary coccidioidomycosis by elevated CD4+ T cells releasing IFN-γ in organs such as the lungs and splenocyte of immunized dogs. No animal demonstrated sickness, thus not warranting clinical veterinary consultation. Every dog was immune from clinically relevant coccidioidomycosis (166).

4.6.3 Subunit vaccine

4.6.3.1 GCP-rCpa1

The vaccine consists of a recombinant Coccidioides antigen (rCpa1) packaged with adjuvant Glucan Chitin Particle (GCP). This vaccine produces a strong Th17 response via CARD9-mediated Dectin-1/2 signaling pathway activation (175). GCP-rCpa1 stirred greater macrophage penetration at injection sites. It demonstrated a meaningfully greater decrease of fungal load in the HLA-DR4 transgenic murine model, which also showed increased IL-17 production by Th1/Th17 cells. They also exhibited increased survival rates compared to animals immunized with GCPs alone (167).

4.7 Invasive paracoccidioidomycosis

4.7.1 Subunit vaccine

4.7.1.1 Peptide P10

The vaccine encompasses *P. brasiliensis* glycoprotein-derived peptide P10, absorbing with cationic liposomes (CAF01). The vaccine induces Th1 response, leading to cellular immunity. P10 and CAF01 significantly decreased fungal load in the lungs of infected mice and was associated with protective granulomatous tissue responses, indicating effective therapeutic potential against Para-coccidioidomycosis (168). This peptide encapsulated with chitosan nanoparticles improved bioavailability and stability and demonstrated immunomodulatory effect enhancement. It caused mixed Th1 and Th2 immune responses. The intranasal inoculation of the P10-chitosan nanoparticle vaccine reduced the fungal burden (CFUs) in the lungs of vaccinated mice. Notably, the conjugated P10-chitosan nanoparticles permitted a maximum of 20-fold vaccine dose reduction compared to previous studies (169).

4.8 Invasive blastomycoses

4.8.1 Subunit vaccine

4.8.1.1 Bl-Eng2

δ-inulin-based adjuvants (Advax) mixed with agonists of Toll-like receptor (TLR) boost endoglucanase2 (Bl-Eng2)-mediated immune response, producing proinflammatory antigen and dectin-2 pathway agonist. Bl-Eng2/Advax3 comprising TLR9 agonist and Bl-Eng2/Advax8 comprising TLR4 agonist are more effective in providing the best immunity against pulmonary Blastomycoses rather than complete FA. Overall, 50% of mice vaccinated with Bl-Eng2/Advax3 had survived up to 24 days, whereas animal vaccinated with only Bl-Eng2 succumbed 14 days post-vaccination. Among the adjuvants, Advax3 was better in producing IFN-γ and IL-17, which releases Bl-Eng2-specific T cells (170).

4.9 Invasive *Pneumocystis* pneumonia

4.9.1 Subunit vaccine

- **Recombinant $A12_{1-85}$:** This is derived from the B-cell epitope region of the A12 surface protein of *P. carinii*. Recombinant $A12_{1-85}$ protein induced both types of immune responses (humoral and cellular) by increasing serum IgG titer, promoting IFN-γ secretion by CD4+ and CD8+ T cells, and elevating the titer of pro-inflammatory cytokines like IL-17, IFN-γ, and IL-12 in the lungs. Vaccination with recombinant $A12_{1-85}$ meaningfully reduced the fungal load in an immunocompromised murine model (171).
- **Recombinant KEX1:** This vaccine induced robust and durable humoral immunity responses generated by memory B-cell responses against KEX1. Antibody titer could be maintained during tacrolimus- and methylprednisolone-induced immunosuppression in non-human primates (172).
- **KEX1/(α -GC):** The therapeutic immunization strategy boosts the immune response through adjuvant α-galactosylceramide. α-GC works by inducing invariant natural killer T (iNKT) cells. The KEX1/(α-GC) vaccine showed significant anti-KEX1 IgG titer elevation and produced long-lasting humoral memory responses without the presence of CD4+ T cells, vital for HIV-infected immunocompromised patients with diminished CD4+ T-cell counts (173).

4.10 Invasive sporotrichosis

4.10.1 Live attenuated vaccine

4.10.1.1 Δcps1

The Δcps1 mutant vaccine works by removing the *CPS1* gene, which is vital for the survival and virulence. This deletion results in a strain that can stimulate an immune response without causing diseases. The Δcps1 vaccine induces a protective immune response characterized by the augmented release of Th1, Th17, IFN-γ, and IL-17A. This response helps in the activation of neutrophils and macrophages, leading to enhanced fungal clearance and reduced lesion size (142).

4.10.2 Subunit vaccine

4.10.2.1 SsEno/PGA

The vaccine contains recombinant enolase (rSsEno) from *S. schenckii*, mixed with Montanide™ PetGel A (PGA), a polymeric adjuvant containing dispersed sodium polyacrylate microparticles in water. Increased IFN-γ levels with Th1 cells (IFN-γ/CD4+ T cells) and elevated rSsEno-specific IgG titers produce a robust Th1 immune response. However, it did not induce a significant Th17 response (174).

4.11 Pan-fungal vaccine

- **NXT-2:** It is a recombinant peptide with vaccine-like features used in vaccinated mice and nonhuman primates against *Aspergillus*, *Candida*, and *Pneumocystis*. It generates antibodies that bind to the fungal surface inducing opsonophagocytic killing. It also induces strong and long-lasting plasma IgG titers leading to inhibited biofilm development, which is a vital virulence factor of *Candida*. In animal models of aspergillosis, NXT-2 associated with an important decline in mortality and lung fungal load and had a twofold mean survival period. In an HIV-infected primate model of *Pneumocystis* infection, reduced incidence of opportunistic infection and chronic colonization was reported. Overall, the NXT-2 vaccine shows promising broad-spectrum antifungal efficacy across multiple fungal pathogens in various animal models (67).
- **CRM197:** The β-glucan-based vaccine CRM197 can harvest protective immunity in mice diseased with *C. albicans* and *A. fumigatus*. β-Glucans are also a strong activator of TII (176).

5 Challenges and the way forward

Fungal infections pose a significant threat, particularly to immunocompromised individuals and those with multiple comorbidities as they often experience disseminated disease and have poor clinical outcomes. Despite the high disease burden, there are no licensed fungal vaccine preparations. Appropriate adjuvants and drug delivery systems that generate good immune response are currently limited (83).

Fungal immune response is highly variable, complex, and an unpredictable immunological phenomenon. As intraspecies and interspecies antigenic variation exists among the fungi, targeting multiple epitopes seems to be a pertinent solution. Translation response from animal models to humans in vaccine development is variable. Hence, reactogenicity and safety evaluation of the preclinical models must be robust. Experiments in multiple animal models, *ex vivo* studies in human cell lines, and the use of advanced bioinformatics can be a promising solution (177).

Pan-fungal vaccines to confer immunity against multiple systemic fungal infections can be a potent solution to the current problem. β-Glucan targets have shown to stimulate trained immunity, i.e., activation of innate immunity leading to epigenetic changes that enhance response to subsequent infections by a wide variety of fungal species. Common T- and B-cell epitope targets could be identified and combined to yield new candidates (178).

Nanotechnology-based drug designing can be an effective technique for a better outcome. Reverse vaccinology principles can be utilized to study the proteome of fungal species in *in silico* models. Computational strategies can be used to identify novel

targets. EV-based fungal platforms (like *Cryptococcus*) are a promising way forward. Protective antigens of such fungal cell wall can act as antigenic targets for future development (10).

Exploring novel targets will hold the key to successful clinical development of fungal vaccines. Research efforts addressing key scientific and logistical barriers can pave the way for future availability of clinically approved effective fungal vaccines.

Author contributions

DA: Formal Analysis, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Investigation, Supervision, Validation, Software. OB: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SP: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Software. DP: Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing, Formal analysis, Supervision, Investigation, Software. BA: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

Author SP was employed by the company Wipro Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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