Difficult but Not Impossible: in Search of an Anti-*Candida* Vaccine

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Abstract

Purpose of Review Pervasive fungal infection among the immunocompromised population, in conjunction with a lack of effective treatment options, has demanded further scrutiny. Millions of people are still dying annually from fungal infections. While existing treatment for these fungal infections exists, it is difficult to administer without adverse effects in the immunocompromised and is slowly becoming obsolete due to varying mutation rates and rising resistance in multiple species. Thus, vaccines may be a viable target for preventing and treating fungal infections and addressing the critical challenge of such infections.

Recent Findings *Candida albicans*, along with other non-albicans *Candida* species, is among the more virulent class of fungal specimens considered for vaccine development. *C. albicans* is responsible for a large percentage of invasive fungal infections among immunocompromised and immunocompetent populations and carries a relatively high mortality rate. In the last decade, a recent increase in infective capacity among *Candida* species has shed light on the lack of adequate fungal vaccine choices. While roadblocks still exist in the development of antifungal vaccines, several novel targets have been examined and proposed as candidates.

Summary Success in vaccine development has universal appeal; an anti-*Candida* vaccine formulation could be modified to work against other fungal infections and thus bolster the antifungal pipeline.

Keywords *Candida* · Vaccine · Antifungal · Therapy

Introduction

In observing the thousands of fungal species studied, only roughly 600 are associated with fungal infections in humans. These fungal species can have devastating effects on immunocompromised patients, including patients afflicted with HIV and cancer. Nevertheless, there have been cases of outbreaks with fungal diseases in otherwise healthy individuals. There is a family of six dimorphic fungi, which are responsible for roughly one million infections per year [1]. Of these, *Cryptococcus* and *Histoplasma* have mortality rates between 20% and 70%, while *Candida* is slightly higher at 46–75% [2]. *Candida* species are the fourth most common cause of hospital-acquired infections, and invasive infections often carry a 50% mortality rate despite the use of antifungals. *Candida*

bloodstream infections are estimated to afflict roughly 400,000 patients per year [3].

Vaccines against invasive pathogens represent a major step forward in combating illnesses. Much of the knowledge used to combat bacterial and viral infections has transferred over for use against fungal infections. Regardless of the progress made so far, fungal infections and the treatment used to eliminate them continue to be an area of concern for providers worldwide. The greatest evidence for this can be summarized in the development schedule for effective vaccines. The smallpox vaccine was developed as far back as 1796. The tetanus vaccine followed in 1923, and more vaccines were steadily developed between 1950 and 1998. With the exception of the smallpox vaccine, that roughly correlates to a new vaccine against bacteria or viruses every 10 years. To date, there have been no fungal vaccines cleared for prescription use; however, several experimental models exist.

The Burden and Challenge of Candidiasis

Candida albicans and non-*albicans Candida* (NAC) species, *Candida glabrata*, *Candida parapsilosis*, and *Candida*

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tropicalis are natural commensals and less common as pathogens. However, all species can become opportunistic pathogens in immunocompromised populations, including transplant recipients, or individuals receiving chemotherapy and other cancer medications. Patients with central lines are exceptionally at risk, as *Candida* species are responsible for roughly 12% of all central line infections [4]. Likewise, neonatal patients are susceptible to infections due to their immature immune system. According to a data set from a National Inpatient Survey in 2000, candidemia correlated to an additional 10.1 days of stay, resulting in an additional $40,000 in costs per patient. Hospitalization in infants proved even worse with an extra 21-day stay correlating to an extra $92,000+ in extra costs [4].

Roadblocks in the development of more modern treatments are common and involve a sharp decline in research, as well as a lack of incentive towards antifungal development. Development of antifungals, in comparison to medications that treat chronic conditions, often requires a high cost for a low rate of return. In the last 13 years, the FDA has approved only five antifungal agents, which can be categorized between the two classes of echinocandins (caspofungin, micafungin, and anidulafungin) and triazoles (voriconazole and posaconazole) [4]. Posaconazole is a newly approved generation of azoles that has a higher proven efficacy compared to the early-generation azoles, such as fluconazole and itraconazole. Caspofungin, micafungin, and anidulafungin are progressive generations of echinocandins.

An additional challenge is posed by the widespread distribution and emergence of new *Candida* strains. Population-based studies suggest that the distribution of *C. albicans* to NAC species varies by region. Blood samples taken have shown that *C. albicans* is mostly prevalent in the USA and Northern and Central Europe. The NAC species discussed in this review include *C. glabrata* found mostly in Northern and Central Europe [3], *C. parapsilosis* in Slovakia, Southern Europe, South America, and Asia [5], and *C. tropicalis* in the Asia-Pacific region [3]. Importantly, emerging multidrug resistant NAC species, such as *Candida auris*, are found in India, South Africa, South America, North America, and Europe [6]. Geographical distribution of other NAC species is available [7] but not discussed.

To resolve the burden and challenges posed by candidiasis and similar mycoses, current and promising antifungal therapies must be developed to be used in immunocompromised populations, who often show the most adverse effects and whose immune systems do not cooperate with chemotherapy. Due to their status, the immunocompromised may become gravely ill even from innocuous vaccines. It is because of this caveat that development of an anti-*Candida* vaccine seems less feasible. In this patient population, passive immunization has now emerged as a promising alternative to treat disseminated mycosis [8]. Also of interest is the use of antibodies to neutralize infections without induction of a memory response. What follows is a discussion of ongoing and potential efforts in the development of an anti-*Candida* therapy.

Current *Candida* Immunogenic Targets and Vaccination Efforts

A summary of current and potential targets discussed below to various *Candida* species (Table 1) and pathogenic fungi in general (Table 2) is also provided.

C. albicans

Antifungal therapies involve targeting specific molecular entities, such as the cell wall or ribosomal transcription, in pathogenic fungi. Current treatment for *C. albicans* includes the medication fluconazole, which inhibits lanosterol 14α-demethylase, leading to the inhibition of ergosterol synthesis in the endoplasmic reticulum [4]. Live samples of the infective agent have been used as an alternative to the conventional antifungal treatment. Several live attenuated samples of *C. albicans* have been tested, including genetically engineered CNC13 and PCA-2 strains. While it has been found that these strains offer some protection against lethal infection in the laboratory, none of have progressed to clinical trials [3].

Agglutin-like sequence (Als) proteins are an area of study to inhibit growth of *C. albicans* by means of blocking fungal cell adhesion, invasion, and biofilm formation. One study involving recombinant Als1 demonstrated a 50–57% increased survival in mice models [9]. Als proteins have also served as a target for successful monoclonal antibody binding. Growth of immunolabeled *C. albicans* strains shows a vast presence of Als3. The antibodies, 113 and 3-A5, were able to block *C. albicans* from adhering to endothelial and buccal cell surfaces at specific concentrations. Likewise, these antibodies studied showed specificity for *C. albicans* against other fungal species [10]. Other protein targets tested include the heat shock protein 90 (Hsp90). Heat shock proteins are released in the cell to protect against extremely high temperatures. Mice models treated with a recombinant Hsp90 vaccine (r-hsp90-CA) showed a 118% survival rate compared to the control when infected with *C. albicans* [9].

Progression towards antifungal vaccines has not been limited to protein targets. Certain carbohydrate entities have shown varied success in suppressing *C. albicans* growth. Laminarin (Lam) is a β-glucan entity isolated from brown algae. When conjugated to the diphtheria toxoid, CRM197, it has been shown to suppress *C. albicans* growth. β-glucan is a vital component of fungal cell membranes. Studies have shown that Lam-CRM binds non-uniformly to *C. albicans* hyphae as well as some septal regions. This binding had led to a decrease in fungal burden [12•].

Table 1 Summary of current and potential vaccine targets to *Candida* species discussed in the review

Species	Current or potential therapy targets	Reference(s)
C. albicans, *C. tropicalis*	Genetically engineered strains, CNC13 and PCA-2	[4]
C. albicans, *C. glabrata*	Agglutinin-like sequence (Als) proteins, 113, 3A-5	[9, 10]
C. albicans	Recombinant heat shock protein, r-hsp90-CA	[9, 11]
C. albicans	Laminarin, CRM197	[12]
C. albicans, *C. glabrata*	*HYR1*	[3, 13]
C. glabrata	Cobalt complexes	[14]
C. glabrata	Mannose-binding lectin	[15]
C. parapsilosis	Histadin-5	[16]
C. parapsilosis	Experimental vaccine (NDV-3)	[17, 18]
C. parapsilosis, *C. tropicalis*	Plant based: *Eugenia dysenterica*, *Pouteria ramiflora*, *Ocotea odorifera*	[16]
C. tropicalis	Plant based: *Lonicera japonica*, thymol	[19]
C. albicans	PKC inhibitors, cercosporamide, staurosporine	[20]
C. albicans	Human lactoferrin, hLF1-11	[16, 21]
C. albicans	Phage vaccine	[22]

Much like antibacterial therapy, gene targets have also been used to suppress *C. albicans* growth. The hyphae gene, *HYR1*, has been implicated in reduced phagocytosis of *C. albicans*. Studies involving this gene include a recombinant protein product, similar to the aforementioned heat shock proteins. Immunization with this protein has shown increased survival against *C. albicans* infections, with the effect greater when both alleles of *HYR1* were silenced [13•]. Lastly, the enzyme enolase (Eno1p) has been implicated as a viable vaccine option. Subcutaneous and intramuscular injections of the Eno1p vaccine in mice infected with a lethal dose of *C. albicans* showed survival rates of 12.5–25% [29].

C. glabrata

C. glabrata, while not a leading pathogen in humans, is nonetheless prevalent in adults [3]. Much like *C. albicans*, it can be found in our oral and GI tract. Upon immunosuppression, it can cause widespread infection. Echinocandins, most commonly used to treat infection with *C. glabrata*, inhibit β-D-glucan synthetase, an enzyme that functions in cell wall synthesis. In vitro and in vivo studies with the investigational drug, isavuconazole, have suggested efficacy against several *Candida* species, including *C. glabrata*.

More novel vaccination options for *C. glabrata* include the use of cobalt 3 (Co III) coordination complexes. Cobalt complexes function as chelating agents, drawing away vital cofactor ions that fungal enzymes need to function. In a study involving several *Candida* species, *C. glabrata* had the most sensitive MIC and MHC ranges for cobalt treatment. The study concluded that this was due to its inability to form hyphae [14].

Mannoproteins in the cell wall of *C. glabrata* and *C. albicans* have similarly been considered as targets. The molecule used against them is mannose-binding lectin (MBL), a protein that is synthesized in the liver and involved in complement cascade activation. Investigations of its function have found MBL-induced agglutination in the yeast

Table 2 Summary of potential cross protective targets to various pathogenic fungi discussed in the review

Potential therapy targets	Reference(s)
Genetically engineered strains: CNC13 and PCA-2	[4]
Agglutinin-like sequence (Als) proteins: 113, 3A-5	[9, 10]
Recombinant heat shock proteins: r-hsp90-CA	[9, 11]
Laminarin: CRM197	[12]
Replicative aging	[23–25]
Respiratory pathways: Mir1	[26]
Sphingolipid metabolism: BHBM, D13	[27]
Micafungin activated metacaspase complexes	[28]
Tricyclic antidepressants	[16]
Genetically engineered organisms: *Lactobacillus casei* containing the Eno1p antigen	[29]

phase to treat both *C. glabrata* and *C. albicans*. This was achieved through binding to the mannoproteins covering the fungal cell surface, inducing complement activation [15•]. *C. glabrata* possesses the *HYR1* mannoprotein on the surface of its hyphae. By using a recombinant version of its N terminus, the vaccine was found effective in animal models challenged against a lethal dose of *C. glabrata* [3]. The Als class of proteins were found to be viable targets for *C. albicans* vaccine efforts. An intriguing result is that recombinant Als1 has been found to be similarly effective against *C. glabrata*. In fact, this species lacks Als proteins and does not typically form hyphae unless incorporated into the aggregates initiated by *C. albicans* [30]. The interactions of *C. glabrata* and *C. albicans*, which lead to cell-to-cell aggregation, may be important in understanding the pathogenesis of *C. glabrata*. This may also be a valid explanation for the effectiveness of recombinant Als1 in this species incapable of hyphal formation. This also begs further investigation into this class of proteins against other non-hyphae-forming fungal species as an alternative treatment option.

C. parapsilosis

C. parapsilosis is an organism implicated in many nosocomial infections. The current preferred pharmacological treatment of *C. parapsilosis* targets ergosterol inhibition, such as fluconazole. Patients who cannot tolerate this medication are given amphotericin B in either a regular or lipid formulation [4]. Another commonly used drug is flucytosine, which inhibits thymidylate synthetase, an enzyme associated with nucleic acid synthesis. Metabolism of this drug may subsequently interfere with translation via accumulation of 5-fluorodeoxyuridine monophosphate [16].

Because of differences in its cell wall structure, *C. parapsilosis* produces high amounts of inflammatory cytokines. Even if further studies are required to know more about the cell wall composition of *C. parapsilosis*, by observing lectin staining, chitin exposure can be analyzed. Peripheral blood mononuclear cells (PBMCs) are responsible for the underlying mechanism for producing higher levels of pro-inflammatory cytokines due to the deletion of the α1,6-mannosyltransferase. Blocking of cell surface receptors, such as TLR4 and β-glucans, has been shown to decrease its virulence [31].

An alternative mode of antifungal treatment against *C. parapsilosis* involves the use of antifungal peptides. Histadin-5, which is found in saliva and interferes with ATP efflux in fungal cells, has been shown to produce high fungicidal activity at low concentrations [16]. Several investigated plant models, including *Eugenia dysenterica* and *Pouteria ramiflora*, have exhibited comparable potent activity against *C. parapsilosis* with low MIC values. Additionally, ellagitannins isolated from *Ocotea odorifera*, a plant

commonly used in Brazilian folk medicine, provide excellent activity against *C. parapsilosis* [16].

Als sequences are once again implicated as viable targets when treating infections with *C. parapsilosis*. Als3 has been used to develop an experimental vaccine, NDV-3. Phase I studies have found that NDV-3 induces strong B and T cell responses to candidiasis and is generally well tolerated. Furthermore, B and T cell responses increased when exposed to additional NDV-3 doses [17•].

C. tropicalis

C. tropicalis infections are commonly associated in patients with neutropenia and malignancy [3]. Current treatment options include polyenes, triazoles, and echinocandins. Resistance to flucytosine has been noted in this species and is not considered for treatment [32•]. This limits targets to its cell wall and cell membrane. Unfortunately, *C. tropicalis* has developed multiple resistance mechanisms. Mutations in the *ERG11* gene have conferred azole resistance and in the *FKS1* gene to echinocandins.

Given the already limited scope of prescription therapy, the search for alternative treatments is expected. Extracts from the Chinese plant, *Lonicera japonica*, exhibited strong antifungal activity, anti-inflammatory cytokines, and potent wound healing capacity [19]. Likewise, the plants *E. dysenterica* and *P. ramiflora* also showed strong activity. Thymol is a constituent of essential oils from thyme that has been shown to possess antifungal properties. It measured an MIC value of 78 µg/mL against *C. tropicalis* [19].

Live attenuated *C. albicans* has been shown to provide non-specific cross protection against subsequent challenge with virulent *C. albicans* in mice. Specifically, the protection was observed by injecting mice with an avirulent strain and by macrophage-like cells, whereby adoptive transfer of "plastic adherent" cells also provided protection. Cross protection against *C. tropicalis* and *Staphylococcus aureus* was comparably observed (studies summarized in [3]). Further, isavuconazole has been shown to be active in vitro against NAC species, including *C. tropicalis*. This drug is currently in Phase III testing stage. Albaconazole was equally effective; however, it is only in Phase II [4].

C. auris

Due to an increase in antimicrobial use, strains of *Candida* have emerged that are resistant to most conventional treatments. One such species, *C. auris*, was identified in 2009. A major concern with *C. auris* for specific patient populations is biofilm formation on implanted devices, such as peripherally inserted central catheter (PICC) lines or central arterial lines. Removal of these devices is of utmost importance. Patient resistance to at least one antifungal often helps identify

C. auris. However, it presents additional barriers to treatment because it is often misidentified as other yeasts. It is unique from other strains of *Candida* in that it can be more readily transmitted between patients in hospitals and the environment. Its rapid identification of the organism is often one of the most important aspects of proper treatment.

Most cases in the USA have been treated with echinocandins since most of identified strains have been susceptible to them. Patients are sometimes be followed up with broad-spectrum antibiotics for treatment [33•]. Combination therapy, while widely used in treating resistant bacterial infections, has been less explored in treating resistant antifungal infections. Regardless, certain combination antifungal therapies have been developed along with new formulations, such as liposomal Ambisome [16].

Alternative strategies may target fungal stress response regulators. Hsp90 protects the cell from extreme temperatures, while calcineurin acts as a protein phosphatase. Evolutionary studies suggest that altering these targets enhances the effects of azoles and makes them more fungicidal [11•]. Further investigation into cell stress targets revealed that these stress signals function as part of a protein kinase C (PKC) signaling pathway. PKC has been shown to regulate cell wall integrity in *Candida* spp. Cercosporamide is a selective Pkc1 inhibitor and can be purified from the fungus *Cercosporidium henningsii*. Additional PKC inhibitors include staurosporine. Furthermore, combination therapy of cercospiramide with micafungin displayed synergistic activity against a previously resistant strain of *C. albicans* [20•].

Human lactoferrin is a peptide found in humans that functions to hydrolyze RNA molecules and may be a potential solution for emergent species. It has been shown to assist in clearing infections, as well as stimulating dendritic cells and macrophages. Studies show that human lactoferrin 1-11 (hLF1-11) also inhibited *C. albicans* biofilm formation at early stages through the Ras-cAMP-Efg1 pathway, interfering with biofilm cellular density and metabolic activity [16]. The potential of this protein as a stand-alone treatment is evident but more interesting in conjunction with existing antifungal therapy. In fact, this synergistic approach may enable a lowering of the standard antimycotic intake required by existing therapy [21]. Further studies will need to be done to establish efficacy of the combination therapy, especially in a clinical setting. However, this approach seems worthy of consideration.

Potential *Candida* Immunogenic Targets

Increasing incidence of resistance to antifungals, in conjunction with parallel evolutionary development with humans, has prompted the search for vaccine targets. The NDV-3A vaccine has recently announced positive Phase II results and uses the Als3 surface protein as a vaccine target [18].

Fungal aging, a more novel area of research that looks into the microevolution of the pathogen, has also demonstrated potential for an antifungal vaccine. Studies in *Cryptococcus neoformans* and *C. glabrata* have demonstrated that the cell wall undergoes changes with the age of the cell [23, 24]. Additional observations revealed that phenotypic switching can lower the number of replications that daughter cells undergo compared to the parent cell. Hypothetically, a vaccine that induces increased phenotypic switching rates could increase the effectiveness of existing antifungals. This difference in aging has even been documented in the emergent species, *C. auris* [25].

Respiratory pathways in fungal pathogens are an emerging field of experimentation when it comes to vaccine development. Mir1 is a protein required for respiration in *Saccharomyces cerevisiae*. Its requirement for *Candida* species has not been extensively studied; however, the ML316 probe used against mitochondrial phosphate transport exhibited more potent fungicidal activity against azole resistant *Candida* [26•]. Nevertheless, many fungi are facultative aerobes and may still persist in anaerobic environments if needed.

Another novel approach that has gained traction is sphingolipid metabolism. In a study conducted with the synthetic drug, BHBM, an assay showed dose-dependent killing of *C. neoformans*. Mice subjects treated with BHBM and its derivative D13 survived after infection with live *C. albicans* [27]. Research into this avenue of fungal vaccination is one of the first to clear Phase II trials.

Medications and vaccines that cause direct death of the cell run the risk of increasing mutations to favor additional resistance mechanisms in the pathogen. *Candida* species can evade the humoral immune response by secreting Sap9 and Sap10 to inactivate antimicrobial peptides. They have also developed ways to attach phase inhibitors of the complement cascade to their outer wall and avoid complement activation [34]. In addition, they can proliferate and form hyphae within macrophages and lyse the cells [35].

Potentially, a well-designed vaccine would cause the cell to die of its own volition, perhaps by altering its apoptotic pathway. In this scenario, the vaccine would signal the cell that it is non-functional when, in reality, it is viable. The delivery method of this vaccine would require evasion of host immune response, likely by encasement in a lipid endosome. In a study involving micafungin-activated metacaspase complexes, the antifungal increased reactive oxygen species, lowered mitochondrial membrane potential, and activated metacaspase activity in caspofungin susceptible and non-susceptible *C. albicans* and *C. parapsilosis* [28]. The major drawback to this therapy is that micafungin is a foreign molecule, and thus the fungal cell may recognize this and develop resistance mechanisms, such as efflux pumps or inactivating enzymes.

Unconventional treatment options may need to be considered. Studies suggest that tricyclic antidepressants can not only kill *C. albicans* hyphae and inhibit biofilm formation but also kill mature *Candida* residing in biofilms [16]. Additionally, using genetically engineered organisms, which are benign to the host but lethal to the invading pathogen, is another potential avenue. *Lactobacillus casei* modified with a HPV16 E7 molecular display and a *Streptococcus pyogenes* M6 anchor protein have been shown to confer immunity to human papillomavirus in mice models. When tested with *C. albicans*, *L. casei* containing the Eno1p antigen generated seven times more antibodies compared to a control [29].

The aforementioned design can be applied to using phages against fungal infection. Mice inoculated with a recombinant phage containing a peptide from fructose biphosphate aldolase induced humoral and cellular immune responses and relieved kidney damage in mice with candidiasis [22]. Similar phage models are used in gene therapy to treat patients with genetic diseases, such as cystic fibrosis. While this may provide some relief of symptoms, constant administration is needed.

Critical Challenges and Future Directions

C. albicans has coevolved with humans for at least the past 2000 years. Not surprisingly, this has led to the development of several host immune evasion mechanisms, including high genetic, phenotypic, and morphological plasticity [3]. Since *C. albicans* can switch phenotypically, identification of the organism during infection may be delayed. Gross variations of *Candida* species colonization can be observed across different geographical regions, making a targeted vaccine approach difficult. Within the last decade, *C. auris* has also identified itself as a threat, specifically because it is multidrug resistant and difficult to rapidly identify. Both *C. auris* and *C. albicans* also form biofilms, which further increase resistance to antifungal treatment. NAC species, such as *C. glabrata*, show extensive azole resistance, have been identified in candidemia infections, and would therefore benefit from vaccine development.

A viable vaccine for *Candida* has the potential to contribute to the antifungal pipeline [36], specifically by widening the search for vaccines against other fungal infections responsible for high mortality. *Aspergillus fumigatus* causes chronic pulmonary disease in about three million people per year and can carry a wide range of mortality [2]. It can also form Rod A, a resistance mechanism that diminishes the ability of neutrophils to phagocytose fungal cells [34]. *C. neoformans*, much like *C. albicans*, can form biofilms. *C. neoformans* affects patients worldwide and has mortality rates as high as 70%. It has a capsule and can form large bodies called titan cells, which allows it to evade key immune cells and prevents it from being phagocytosed easily [34]. Ultimately,

C. neoformans would similarly benefit from the development of a functional vaccine.

Of particular interest to the antifungal pipeline is the development of antibodies to neutralize an infection without inducing an active immune response. The human recombinant antibody against Hsp90, Mycograb®, already shows synergistic activity in combination with amphotericin B [37]; it would be worth investigating its application to other fungal pathogens, including *Aspergillus* spp. and *C. neoformans*, both of which possess *Hsp90*. With the same logic, the monoclonal antibodies IgM(B6.1) and IgG3(C3.1) made against the β1,2-mannotriose epitope of *C. albicans* could be used for passive immunotherapy [38]. These have an established protective role in a murine infection model. The killer toxin discussed in this review, among others [39], is also an option for passive immunotherapy [40]. Lastly, the IgG2a WGA-Fc, which binds to chitin, is among a line of universal vaccine candidates [41].

Clearly, there are several areas being investigated in the race to develop a viable FDA-approved antifungal vaccine. Despite this, vaccine efforts are costly and require years of research and multiple phase testing to be cleared. Further, vaccine development for *Candida* species comes with the challenge of dealing with an immunosuppressed patient population, as well as showing efficacy against constantly evolving and multidrug-resistant organisms. While this review showcases several developments towards an anti-*Candida* vaccine, ideally the most promising candidates will (1) include multivalent fungal antigens that divert potential risk, (2) protect against several fungal pathogens, and (3) combine antifungal drugs and immunostimulants. Ultimately, fungal infections pose a serious illness and require further novelty and persistence in the race to vaccine development. But because the finish line can now be seen, it is time to prioritize and bolster these developments.

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