Existing and emerging therapies for the treatment of Invasive Candidiasis and Candidemia

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Word count excluding abstract : 6987 (5000-7000) References: 109 (<100)

Abstract: (200 w) 196

Introduction

Invasive candidiasis or candidemia is a severe infection affecting more than 250,000 people worldwide every year. It is present in up to 16% of ICU patients. The prognosis of these infections is unfavorable, with global death estimated around 50,000 per year, which corresponds to up to 40% depending on patient severity and comorbidities. Therapeutic failure is not rare due to the emergence of multiresistant strains and of new species poorly responsive to current therapies like *Candida auris.*

Areas Covered

We first review the positioning of antifungal drugs used to treat candidiasis, namely polyenes, azoles, echinocandins and pyrimidine analogues. We then discuss the progresses brought by new formulations, new derivatives within these classes, compounds acting on new targets or repurposed drugs in terms of pharmacokinetic profile, spectrum of activity, potency, safety or risk of drug-drug interactions.

Expert opinion

While new formulations (amphotericin B cochleate) improve oral bioavailability of the corresponding drugs, new azoles or echinocandins offer higher potency including against strains resistant to former generations of drugs. Repurposed drugs show

synergism with current therapies in vitro. Results from ongoing and future clinical trials will be decisive to establish the interest for these drugs in our arsenal.

Keywords

Antifungals

Invasive candidiasis

Candidemia

Cell wall

Fungi cell

Plasma membrane

Severe infection

1. **Background** (500-700 w) 573

1.1. Epidemiology

Candida infection is a frequent healthcare-associated disease. It comprizes invasive candidiasis and non-invasive candidiasis including cutaneous, oropharyngeal, and vulvovaginal infections [1]. Invasive candidiasis covers two subsets of infections: deep-seated candidiasis and candidemia. Deep-seated candidiasis mainly originates from direct inoculation or hematogenous dissemination and is probably the most common fungal disease in our hospitals. Candidemia is considered as the fourth most frequent cause of blood stream infections in the United States [2] and the seventh one in Europe [3,4]. A recent epidemiological survey estimated the global number of patients with invasive candidiasis to 750,000 [5]. This infection remains associated with a high morbidity and even mortality from 15% to 40% [1]. The incidence rate of invasive candidiasis is about 15 cases per 100,000 patient-years [6]. Risk factors for invasive candidiasis are shown in Table 1.

1.2. Clinical syndromes

Clinical manifestations of candidemia or invasive candidiasis are not specific. Systemic inflammatory response is the main presentation of invasive candidiasis ranging from isolated fever to septic shock [7], making it difficult to distinguish from bacterial infection. Cutaneous [8], cardiac [9], neurological [10], abdominal [11], and ocular [12] manifestations exist and should be searched upon.

1.3. Candida species

Candida species are commensals of the skin and the gut and are present without causing disease in 30 to 70% of healthy human beings [13]. More than 30 species of *Candida* have been reported in human infections. Invasive disease has for long been

considered as caused by five species, namely *C. albicans, C. krusei, C. glabrata, C. parapsilosis,* and *C. tropicalis* but *C. auris* has recently been added to the list and associated with severe problems that will be specifically addressed in the section on "Medical Needs". The five first Candida species represent around 95% of all invasive diseases [14]. *C. albicans* is the most preeminent etiologic agent accounting for 50% of Candida infections [15]. Non albicans species are rising, probably due to an increase in treatments based on azole drugs [7,14,16].

1.4. Diagnosis

The gold standard for the diagnosis of invasive candidiasis and candidemia consists in culture from blood or other sterile fluids or in histopathological demonstration of tissular invasion. Yet, it remains difficult, with a large proportion of false negative results. The sensitivity of blood cultures is estimated around 50% for invasive candidiasis but to only 42% for cultures from infected tissues [17]. Microbiological data also need to be interpreted in the light of the characteristics of the disease. Primary candidemia often comes from the gastrointestinal tract, from which the fungi translocate to the blood, or from intravenous catheters. Deep-seated candidiasis can also result from a non-hematogenous introduction of *Candida* into sterile sites, most commonly the abdominal cavity [11]. On this basis, diagnostic tests must identify three situations, namely candidemia without deep-seated candidiasis, deep-seated candidiasis in the absence of candidemia and, lastly, candidemia associated with deep-seated candidiasis [17]. Culture-based methods being not sensitive enough and taking time, culture-independent diagnostic methods have been developed including detection of 1,3-β-D-glucan [18], mannan antigen and anti-mannan antibodies [19], or *C. albicans* germ tube antibody [20], (multiplex) PCR [21], and T2 Magnetic Resonance assay (T2MR) detecting 5 Candida species [22]. Non-culture

diagnostics for candidiasis may help to improve patients' care, but are currently far from being available in all hospitals [23].

The purpose of this paper is to review the existing therapeutic options, to discuss the current therapeutic needs, and to give an overview of the emerging innovative therapies in 2022.

2. Medical need (200-350 w) 695

The main medical needs are related to the problems clinician are facing today when dealing with these infections, namely the development of multidrug resistance in the most spread species [24] and the emergence of *C. auris,* which also often harbors acquired resistance to currently available antifungals [25].

2.1. Multidrug Resistance

Anti-fungal resistance is one of the main causes of therapeutic failure. Polyene resistance is principally linked to mutations in genes of the ergosterol biosynthetic pathways, leading to the replacement of ergosterol by other sterols in the membrane, or to the production of a catalase reducing the oxidative stress induced by the antifungal [26]. Although still rare, amphotericin B resistance has been anecdotally reported in *C. albicans*, *C. krusei* or *C. tropicalis* but in up to 1/3 of the *C. auris* [26], Polyene resistance is frequent in *C. lusitaniae* and even higher in *C. haemulonii* spp complex, suggesting it is intrinsic [26].

Resistance to azole antifungals is much more common and can proceed from five main mechanisms: overexpression of efflux transporters from the ABC and MFS superfamilies or altered azole import that reduces the drug concentration inside the

cell, altered sterol import or mutations/overexpression of *ERG11* encoding the 14-α-sterol-demethylase targeted by these drugs, and aneuploidy and other chromosomal alterations [27]. The prevalence of azole resistance is highly dependent of the species, ranging from less than 1 or 5 % in *C. albicans* depending on the study [28,29] to more than 15% in *C. glabrata* [29].

Echinocandin resistance is mainly due to mutations in *CaFKS-1 and 2*, encoding the 2 subunits of the echinocandin pharmacological target 1,3-β glucan synthase [30]. It remains uncommon, reaching less than 0.1% in most species but at least 2% in *C. glabrata* and < 1 to 5 % in *C. auris* [29,31,32]. These figures may quickly evolve because of clonal spread of *C. auris* in institutions with limited infections control capacities [31].

5-fluorocytosine is the only pyridine used in humans. Resistance is essentially mediated by mutations in enzymes involved in its transport into the cell and its metabolism [33].

Multidrug resistance (i.e. coresistance to azoles and echinocandins) has been essentially reported in *C. glabrata*, the second most prevalent species. It is much more frequent in bloodstream isolates from the US (10% resistance) than from Europe or Asia (less than 1% resistance) [34] .

2.2. C. auris

C. auris has been first isolated from a patient's external ear canal in Japan more than 10 years ago [35] and a few years later in blood cultures [36]. Since then, it has been seen in outbreaks around the world. A European survey reported 349 cases between January 2018 and May 2019: three fourths were colonizations and, among infections, one fourth were bloodstream infections [37]. C. *auris* seems easy to

spread and can therefore cause epidemics. It can survive on surfaces for a long time, facilitating cross-contamination in the hospitals, and its antifungal drug resistance is of significant concern [38]. Early and correct identification of patients colonized with C. *auris* is critical to limit its spreading. However, *C. auris* can be misidentified as other related pathogens [39] and it is not present in the panel of species detected by T2MR.

Resistance rates are alarming in *C. auris.* The majority of the strains show elevated MICs of fluconazole (MIC_{90} > 64 mg/L). In the US, values as high as 65% resistance to amphotericin B and almost 4 % resistance to echinocandins have been reported [40], but a review of worldwide cases reported lower values (44% resistance to fluconazole, 15%, to amphotericin B, and 3.5%, to caspofungin) [41]. An epidemiological survey including isolates from Pakistan, India, South Africa, and Venezuela reported resistance rates reaching up to 35% for amphotericin B, 93% for fluconazole, , 41% of resistance to two antifungal classes, and 4% of resistance to three classes [42].

Echinocandins are the drugs of choice for the initial therapy of invasive infections by *C. auris* [31]. Nevertheless, new drugs are probably necessary to adequately treat these patients.

3. **Existing Treatment** (1000-1500 w) 1658

The mode of action of the four families of existing drugs active against Candida is shown in Figure 1. Polyenes, azoles and echinocandins are the three main classes of antifungal drugs proposed for the treatment of invasive candidiasis. Flucytosine is anecdotally used and never in monotherapy. The allylamine terbinafine is considered

inadequate for treating Candida infections. The choice of the best therapeutic option for each individual patient is critical because invasive Candida infections are associated with a non-negligible mortality. Retrospective studies showed that the early treatment of invasive candidiasis and candidemia by anti-fungal drugs and the control of the source of the infection are essential as they seem to contribute to decrease mortality. In a first retrospective study including 230 patients, mortality was 15% when fluconazole was started the day of the first positive blood culture but rose to 41% if started 3 days later ($p \leq 0.0009$) [43]. A second study showed a 2-fold increase in mortality when patients received anti-fungal treatment more than 12 hours after sampling [44]. These data highlight the interest of non-culture diagnoses. Prediction tools based on clinical risk factors, the presence of candida colonization, and the β-D-glucan screening test have been proposed, but their clinical usefulness is doubtful due to the low prevalence of invasive candidiasis. To date no studies have permitted to show their interest for reducing mortality or length of stay.

The IDSA recommends an echinocandin as first-line treatment for candidemia (caspofungin: loading dose 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily), based on the fact echinocandins are fungicidal and azoles only fungistatic against *Candida* [45]. ESCMID guidelines strongly recommend echinocandins for the targeted initial treatment of candidaemia, while liposomal amphotericin B and voriconazole are supported with moderate, and fluconazole with marginal strength [46].

3.1. Polyenes

Polyenes are fungicidal drugs that interact with ergosterol in the plasma membrane to form ion-leaking pores leading to the death of fungal cells. Alternatively, it has been proposed that they could extract or sequester ergosterol and also induce oxidative

stress [26]. Polyene drugs include amphotericin B (AmB) and nystatin, but only amphotericin B is used for systemic treatment. Polyenes bind with much higher affinity to ergosterol than to cholesterol [26], due to differences in the three-dimensional structure of both sterols, which explains their relatively specific toxicity for fungal cells. The toxicity is mainly renal [47] and infusion-related reactions have also been observed [48]. Renal toxicity, related to the weak interaction with cholesterol, is partially relieved if using adequate formulations. These include a cholesteryl sulfate complex, a lipid complex, or a liposomal formulation [49]. Compared to conventional amphotericin B, these formulations show different pharmacokinetic characteristics. Liposomal AmB, the only one currently on the market, reaches a higher blood trough due to slower clearance by the reticuloendothelial system. The doses for conventional amphotericin B are ranging from 0.7 to 1 mg/kg/day [50], while those of the lipid formulation are usually 3-5 mg/kg/day. Amphotericin B is rarely required for the treatment of invasive candidiasis, except for neutropenic patients (because of its fungicidal activity), or in case of resistance to other drug classes or of inadequate penetration of other drugs in the relevant niche. C. *auris* remains in general susceptible to amphotericin B, which may probably increase its use in the near future [51]. Amphotericin B has been recommended as a monotherapy for the treatment of candidemia in non-neutropenic patients or in disseminated hepatosplenic candidiasis, or in association with flucytosine for native valve and prosthetic valve endocarditis or central nervous system infections [45].

3.2. Azoles

Triazoles have been used for almost 30 years with the two first drugs including fluconazole and itraconazole. Most recent drugs in the class include voriconazole,

posaconazole, and isavuconazole; they have a more extended spectrum than the older ones. In general, *C. albicans* resistance to azoles is less than 1-2% [52]. Itraconazole, posaconazole, and isavuconazole are not indicated, however, for the treatment of invasive candidiasis or candidemia and will not be described here. Chemically, all azoles are weak bases containing aromatic rings, and generally not soluble in water. Triazoles inhibit CYP-dependent C-14 -demethylase necessary for the conversion of lanosterol to ergosterol, causing a loss of cell membrane integrity [53]. Fluconazole is water-soluble and can be administered by oral or IV routes. Its binding to serum proteins is low (~10%) [54], explaining why it shows a large distribution in the organs and tissues, including in the cerebrospinal fluid. The current recommended doses in invasive candidiasis range from 400 to 800 mg daily but dose adaptation is required in case of renal failure (creatinine clearance < 30mL/min). Voriconazole has a broad spectrum of activity including most pathogenic yeasts. It has a limited water solubility but is available for oral and IV administrations, with a high oral bioavailability of around 90% [55]. It moderately binds to serum proteins (58%). Voriconazole as fluconazole distributes in the epithelial lining fluid, the central nervous system and cerebrospinal fluid, but voriconazole does not accumulate in urine. The therapeutic scheme consists in 2 loading doses of 6mg/kg at an interval of 12 hours and a maintenance dose of 4mg/kg twice daily. In contrast to fluconazole, the dose does not need adjustment in case of renal failure. Isavuconazole shows a broad spectrum against yeasts in general and Candida in particular. It is available for oral or IV administrations. It is highly bound to serum proteins (99%). The oral bioavailability is very high (98%) [56]. The recommended daily dose of isavuconazole is 200 mg but after 6 loading doses of 200 mg every 8 hours during 48 hours. There

is no need to adjust the dose in case of renal failure, neither in case of liver failure with child Pugh A and B scores.

A major limitation of azoles drugs is that there are excellent substrates and inhibitors of hepatic cytochromes, which share homology with their target enzyme in fungi (fluconazole: inhibitor of CYP2C2, 2C19, 3A4; voriconazole: substrate of CYP 2C19, inhibitor of CYP 2B6, 2C9, 2C19, 3A4;). Accordingly, they cause a wide variety of clinically-significant drug interactions that may justify dose adjustment or change of medication for the co-administered therapies. In addition, there is a need for pharmacokinetic monitoring of azoles [57], notably trough levels should be measured for voriconazole.

3.3. Echinocandins

The most recent class of antifungal drugs to be introduced on the marked are echinocandins, with caspofungin being the first approved by the FDA in 2001. These cyclic lipopeptides inhibit the activity of 1-3-β-D-glucan synthase, involved in the synthesis of 1-3-β-D-glucan, one of the main polysaccharidic structural components of the fungal cell walls. They are highly fungicidal, especially against yeasts. There is no cross resistance between echinocandins Three drugs in this class are available, namely caspofungin, micafungin and anidulafungin. Caspofungin has a wide activity against Candida, especially *C. albicans*, *C. tropicalis* and *C. glabrata*. Its potency decreases against *C. krusei*, *C. guilliermondii* and *C. lusitaniae* [58]. It also shows activity against *C. auris* but resistance has already been described in this species [31]. The recommended daily dose of caspofungin is 50 mg after one loading dose of 70 mg [59]. No dose adjustment is required in case of mild liver or renal failure, but

in case of severe liver failure, no loading dose should be given, and the maintenance dose daily dose should be reduced to 35 mg. Anidulafungin is also active against most yeast, including C. *krusei* but is less active against C. *parapsilosis* and C. *guilliermondii* [60]. After one loading dose of 200 mg, the recommended daily dose of anidulafungin is 100 mg. There is no need to adjust this dose in case of renal or hepatic failure. Micafungin has a good fungicidal activity against yeasts, which is nevertheless reduced against *C. krusei* and *C. lusitaniae*. Micafungin is rapidly distributed to tissues and has a high protein binding (99%). Its daily recommended dose varies from 100 to 200 mg. Its AUC decreases in case of moderate hepatic failure, probably related to a decreased volume of distribution and protein binding, but dose adjustment is not recommended; there is a lack of data in severe liver failure [61].

Serum protein binding is high for all three drugs (>98%). They do not penetrate in the central nervous system neither in the mesothelial cavities; they do not concentrate in urine [62].

Drug interactions are much less frequent with echinocandins as compared to azoles. Anidulafungin is not subject to CYP-mediated interactions. Caspofungin concentrations are increased in the presence of inhibitors of the OATP-1B1 transporters like ciclosporin, but reduced by inducers of hepatic metabolism like rifampin, justifying an increase of its maintenance dose to the level of the loading dose. Conversely, caspofungin reduces the concentrations of tacrolimus by a still unknown mechanism. Micafungin reduces the clearance of tacrolimus and sirolimus, so that therapeutic monitoring is recommended for these immunosuppressive drugs.

3.4. Pyrimidine

306 Flucytosine is metabolized in 5-flourouracil and 5-fluorodeoxyuridinemonophosphate,
307 interfering respectively in the synthesis of fungal RNA and DNA. Its use is limited by
308 frequent resistance, imposing drug combinations [63] and adverse effects
309 (hepatotoxicity and hematological toxicity). Its posology is 25 mg/kg 4 times a day in
310 association with lipid amphotericin B in central nervous system infections [45].

311 *3.5. Duration of therapy*

312 Few studies have been conducted allowing to set recommendations regarding the
313 total duration of therapy. Although echinocandins are associated with better survival
314 rates and clinical success, a step-down procedure to intravenous or oral azoles [64]
315 should be considered based on clinical stabilization of the patient rather than on
316 identification of the infecting species and its susceptibility to azoles [1]. A strategy to
317 step-down to an oral azole as early as 5 days after the start of intravenous treatment
318 with an echinocandin has been evaluated in phase 4 studies. The purpose, after
319 candida was cleared from the blood stream and no resistance to azole was seen,
320 was to show no difference in outcome when compared to a 10-days parenteral
321 echinocandin. Anidulafungin was effective, safe and well tolerated for the treatment
322 of candidosis/invasive candidosis in selected groups of ICU patients [64].

323

324 **4. Market review (350-500 w)** 246

325 Fungal diseases range from relatively-minor superficial and mucosal infections, which
326 represent a large and persistent market, to severe, life-threatening systemic
327 infections, which will probably become more common in a near future. Moreover,
328 delayed diagnosis and treatment can lead to poor patient outcomes and high medical
329 costs. For these reasons, the estimation of Direct Healthcare Costs of Fungal

Diseases in the United States are around 7.2 billion dollars per year [65]. The financial evolution of pharmaceutical companies involved in the development of antifungal drugs over the last 5 years as well as the R&D budget will be determinant to bring new drugs on the market. Many promising antifungals never reach the market due to poor recruitment or trial failures, but also because of lack of funding [66]. Antifungal R&D is a dynamic market. Appili Therapeutics Inc. bought ATI-2307 from FUJIFILM Toyama Chemical Co. Ltd. in 2019. Pfizer has just bought in April 2021 Amplyx Pharmaceuticals, Inc. which was developing the first-in-class drug Fosmanogepix. Mycovia Pharmaceuticals was created in 2018 when NovaQuest bought Viamet ~~Viamed~~ Pharmaceuticals involved in the search of new tetrazoles like quilseconazole or VT-1129, oteseconazole or VT-1161, and VT-1598. Table 2 summarizes the evolution of the financial status of companies having important research on antifungal drugs currently in clinical development. When available, these financial data show an increase in financial over the last five years especially when clinical trials demonstrate an efficacy of the new drugs.

5. Current research goals (150-200 w) 295

Research efforts are made in two directions. First, it remains useful to try identifying new drugs in the already existing classes such as polyenes, azoles and echinocandins, but which show improved properties in terms of antimicrobial activity, pharmacokinetics, or safety. Second, it is even more important but also challenging to search for drugs acting on still unexploited targets. Fungi are eukaryotes, making it more difficult to identify specific targets than against bacteria or viruses. Cell wall is inexistent in human cells and membrane sterols are different, which explains why these targets were the first to be exploited for the currently available drugs [67].

Inhibition of metabolic pathways such as pyrimidine biosynthesis, cytochrome P450 enzymes, iron or acetate metabolism or heme biosynthesis is an interesting alternative as these processes are essential for the virulence and the viability of fungal cells [68]. A third option could consist in trying to inhibit signal transduction pathways. Environmental and nutritional signaling cascades involve mitogen activated protein kinase, phosphoinositide-dependent kinase 1 or calcium signaling regulate mating, growth of filaments and cell differentiation in fungi [69]. Lastly, modulation of gene expression could be achieved by targeting transcription factors or epigenic mechanisms. Transcription factors evolved differently in humans and fungi whereas epigenic therapy has been used by interrupting the modification of nucleic acids. Fungerps, manogepix, arylamidines and polyoxins are successful examples of novel antifungal drugs illustrating the new directions offered by biochemistry research. Other molecules are still in the preclinical stages of investigation, like mohangamides A and B (perturbing glyoxylate cycle), APX879 (inhibitor of fungal calcineurin), efungumab (antibody against HP90), ambutricins and phenylpyrroles (disturbing the high-osmolarity glycerol (HOG) pathway in *C. albicans* and *C. neoformans*) or cercosporamide (inhibitor of Pkc1, an enzyme playing a central role in cell wall biosynthesis and remodeling) [70].

6. Scientific rationale (200-350 w) 233

Considering the dynamics of resistance development is critical in the area of anti-infective pharmacology. In fungi, resistance involve genetic (mutations) and physiological (genetic instability) changes [71]. Developing new drugs in existing classes offers the advantage of less risky investments as the global profile of activity and of safety is already known, but it also limits the added value that could be expected from a new compound. Taking triazoles as an example, we see that the

more recent compounds show a broader spectrum of activity or improved potency, or even maintain activity against strains resistant to first generation molecules (for posaconazole, e.g.). Yet, they globally keep the properties that are intrinsically linked to their pharmacophore, and thus a risk of cross-resistance with the other drugs in the class, which can be temporary masked by their higher potency, or of CYP-mediated drug interactions. Conversely, drugs directed towards new targets offer the possibility of fully avoiding the risk of cross-resistance and possibly also of showing a different spectrum of activity, as they can be initially screened against the most problematic pathogens (see Figure 2). The drawback is the lack of background information regarding their pharmacokinetics, pharmacodynamics, or safety, which may slow down their development or even lead to premature interruption of their development or event withdrawal soon after their marketing in case of detection of rate adverse events once the drug starts to be widely used.

7. Competitive environment (1500-2000 w) 2299

As explained above, the first new drugs in clinical development belong to the already existing classes of antifungals, namely polyenes, azoles and echinocandins.

7.1. Polyenes

A major limitation of amphotericin B is its intravenous route of administration, associated with infusion-related adverse reactions and dose-dependent renal toxicity. Amphotericin B cochleate (CAmB) has been designed for oral administration. It protects the drug from gastrointestinal degradation while maintaining the advantage of lipidic formulations to reduce nephrotoxicity. The formulation consists in a multilayered structure of negatively-charged phosphatidylserine and divalent cations

404 (Ca^{2+}) encapsulating the hydrophobic drug with no aqueous space in a structure
405 resembling a cigar roll [72]. In a phase I trial published in 2009, single doses of 200-
406 400 mg CAmB were well tolerated by healthy volunteers, but gastro-intestinal
407 symptoms were frequent at 800 mg [72]. Results from a first phase II trial were
408 published only 10 years later. Patients with moderate to severe candidiasis were
409 enrolled and showed no signs of liver, kidney, or hematologic disorders were
410 observed [73]. No difference was seen in terms of clinical and microbiological
411 outcome or in safety between CAmB and fluconazole in another trial, with 57%
412 success by day 12 [72].

413 *7.2. Azoles*

414 Tetrazoles as glucans inhibit the synthesis of ergosterol but show a better specificity
415 towards fungal Cyp51 than mammalian CYP450 enzymes than triazoles [74], which
416 can reduce the risk of drug interactions. Three molecules, quilseconazole or VT-
417 1129, oteseconazole or VT-1161, and VT-1598 are currently under investigation.
418 Quilseconazole has been studied mainly on cryptococcal meningitis but has also
419 been tested against Candida spp. All *C. glabrata* and *C. krusei* isolates were inhibited
420 by quilseconazole after 24 h of incubation at concentrations below 2 µg/ml, with
421 geometric mean MICs of 0.22 µg/ml and 0.34 µg/ml for *C. glabrata* and *C. krusei*,
422 respectively, and MIC_{90} of 1 µg/ml for both species [75]. Furthermore,
423 quilseconazole has a long half-life of approximatively 6 days. These data suggest
424 that quilseconazole may offer a useful alternative for infections caused by *C. glabrata*
425 and *C. krusei,* two Candida species showing high levels of intrinsic or acquired
426 resistance to current therapies. Phase I trials are being prepared. In vitro, VT-1598 is
427 as or more potent against yeasts and molds than amphotericin B, fluconazole,
428 voriconazole, posaconazole, or caspofungin [76]. In animal models of aspergillosis,

PK/PD indices for VT-1598 were comparable as those measured for other azoles, i.e. an AUC-free/MIC for 1-log of 5.1 and 1.6 h, against two strains. An ongoing phase 1 study examines the pharmacokinetics and safety of VT-1598 for doses ranging between 40 to 640 mg daily. Oteseconazole or VT-1161 is the most advanced compound in this class. It exhibits potent in vitro activity against most but not all fluconazole-resistant *C. albicans* and *C. krusei* isolates (mean geometric MIC ≤ 0.15 µg/mL) as well as echinocandin-resistant *C. glabrata* [75,77]. In phase 1 trials, oteseconazole has been administered at doses between 150 mg once daily and 600 mg twice daily for candidiasis. In a phase 2 trial aiming at evaluating its efficacy and safety for recurrent vulvovaginitis, oteseconazole has been administered to the long term after treatment of the acute infection by fluconazole at 5 different doses: (1) 150 mg once daily for 7 days, then 150 mg once weekly for 11 weeks, followed by a once-weekly dose of placebo for 12 weeks; (2) 300 mg once daily for 7 days, then 300 mg once weekly for 11 weeks, followed by a once-weekly dose of placebo for 12 weeks; (3) 150 mg once daily for 7 days, then 150 mg once weekly for 23 weeks; (4) 300 mg once daily for 7 days, then 300 mg once weekly for 23 weeks; or (5) a matching placebo regimen for 24 weeks [78]. Among the 215 patients included in the study, the number of subjects presenting ≥1 acute candidiasis episode ranged from 0-7% across the 4 oteseconazole arms vs 52% in the placebo arm, with all arms achieving statistical significance vs placebo. The drug was well tolerated. The safety profile was favorable, and the incidence of adverse events was lower in all oteseconazole arms compared with placebo [78]. These results suggest that oteseconazole may be a promising agent to treat recurrent candidiasis, which is a condition associated with a very high burden of disease and for which there are

actually no approved therapies. In total four phase 2 trials have been completed and 3 phase 3 trials are under recruitment.

In parallel, topical formulations of triazole compounds are developed for specific indications. Efinaconazole topical solution (10%) is evaluated for the treatment of onychomycosis in adult and pediatric patients, which is out of the scope of this review. PC945 is active on *Aspergillus fumigatus* but also *C. auris,* with low MIC (geometric mean value, 0.058 µg/ml) including against most of the strains resistant to other azoles [79]. It is developed for topical administration by inhalation and currently in phase 3 for the treatment of invasive pulmonary aspergillosis [66].

7.3. Echinocandins

Rezafungin or CD101 is a derivative of anidulafungin showing improved stability and solubility and prolonged half-life (133 h). It shows an activity comparable to the other echinocandins against most clinically relevant Candida species, including *C. albicans*, *C. glabrata, C. krusei,* and *C. tropicalis,* and also cross-resistance with caspofungin and anidulafungin. Interestingly, it is more potent than the other echinocandins against *C. auris* with MIC_{90} of 0.5 µg/ml [80] Available clinical data show a quite robust safety. In clinical trials in phases 1 and 2, rezafungin was administered IV with a loading dose of 400 mg and then a weekly dose of 200 mg. In the STRIVE phase 2 study, adults with systemic signs and mycological confirmation of candidemia and/or invasive candidiasis were randomized to rezafungin 400 mg QWk (400 mg), rezafungin 400 mg on week 1 then 200 mg QWk (400/200 mg), or caspofungin (70 mg loading dose followed by 50 mg daily for at most 4 weeks). Candidemia was cleared in 19.5 and 22.8 hours in rezafungin and caspofungin

patients, respectively. Cure rates were 60.5% for rezafungin 400 mg, 76.1% for rezafungin 400/200 mg, and 67.2% for caspofungin [81]. A phase 3 trial has recently been completed comparing rezafungin with caspofungin in invasive candidiasis and candidemia and showed non-inferiority regarding all-Cause Mortality at Day 30; and an adequate global cure at Day 14. Another phase 3 trial is ongoing to evaluate rezafungin once-weekly as a monotherapy for the prophylaxis of fungal infections. .

7.4. New drugs

Four novel classes of drugs are currently in clinical development for the treatment of invasive candidiasis and/or candidemia. In addition, orotomides are an interesting class of IV and oral drugs but mainly active against *Aspergillus* spp. and therefore out of the scope of this review. Ibrexafungerp (formerly SCY-078) and SCY-247 are the two drugs under investigation in the fungerp family. Ibrexafungerp has received FDA approval for the treatment of vulvovaginal candidiasis (VVC). These drugs inhibit 1-3-β-D-glucan synthesis like echinocandins but their chemical structure is different (triterpenoid derivative of is a enfumafungin) [82]. Like echinocandins, their spectrum of activity includes a broad range of clinically significant Candida spp., including *C. glabrata* and *C. auris* [83]. Despite similar mechanisms of action, ibrexafungerp maintains in vitro activity against echinocandin-resistant Candida, with 80% of the resistant strains showing MIC similar to those of wild-type strains [84], This suggests a difference in the affinity for the target site [85]. Ibrexafungerp is highly bioavailable and can be administered orally or intravenously. A phase 2 study showed that a single 1250 mg loading dose followed by subsequent 750 mg daily doses allowed to reach the target exposure in 80% of the population and was safe [86]. A phase 3 multicenter, open-label, non-comparator, single-arm study is ongoing, to evaluate the efficacy, safety, tolerability, and pharmacokinetics of oral ibrexafungerp as an

emergency use treatment for patients with a documented *C. auris* infection. The treatment is initiated by a loading dose of 750 mg PO BID for the first two days, followed by 750 mg PO daily for subsequent doses. A complete response was reported for the two first patients after 17 and 22 days, respectively ([83] and abstract cited therein). The second-generation fungerp SCY-247 demonstrated an activity similar to that of ibrexafungerp against all of the organisms tested. Phase 1 trials will start soon.

Fosmanogepix (formerly APX001) is a prodrug of manogepix, the first-in-class inhibitor of the fungal Gwt-1 protein [87]. This conserved enzyme catalyzes inositol acylation, an early step in the GPI-anchor biosynthesis pathway Inhibition of this enzyme affects maturation and localization of GPI-anchored mannoproteins to the cell membrane or the cell wall [88]. It has demonstrated activity against numerous pathogenic fungi, including *C. auris* [88-90]. Clinical trials have demonstrated high oral bioavailability (above 90%), allowing for an easy switch from the IV to the oral formulation. A favorable pharmacokinetic profile (once daily administration and wide tissue distribution), and a lack of drug interactions were reported in phase 1 trials, encouraging further clinical development [88]. A first phase 2 clinical trial has evaluated the efficacy of fosmanogepix (1000 mg IV twice a day for one day, followed by 600 mg IV once daily for at least two days, then by either 600 mg IV once daily or 700 mg orally once daily for a total treatment duration up to 14 days) against in invasive fungal infections caused by Candida. Clearance of the infection was achieved in 80% of the patients with no detection of serious adverse events [88]. Another study has recruited patients infected by *C. auris* (ClinicalTrials.gov NCT04148287) but the results have not yet been released.

Arylamidines are the next novel class of antifungal drugs. Among them, ATI-2307 exhibits broad-spectrum in vitro and in vivo antifungal activities against clinically significant pathogens including Candida spp., Cryptococcus spp., and Aspergillus spp. Interestingly, it shows low MICs against *C. auris* [91]. It has an original mode of action: after being selectively transported into fungal cells through a polyamine transporter, it inhibits mitochondrial respiratory chain complexes III and IV, reducing ATP synthesis and leading to a fungicidal effect [92,93]. It shows higher affinity for the mitochondria of yeast than for those of mammalian cells. Phase 1 clinical trial to determine safety, efficacy and human dosing are just finishing but their results have not yet been released [94]. Phase 2 studies are coming in 2022 (ClinicalTrials.gov).

The last class of antifungals acting on a new target in Candida are polyoxins, with nikkomycin being the only drug under clinical development since more than 15 years, asking question about its potential future. It is a competitive inhibitor of chitin synthase, an essential structural component of fungal cell walls [95]. It shows fungicidal activity against endemic dimorphic fungi, including Coccidiosis's, Histoplasma, and Blastomyces spp, but inconstant activity against other fungi, with MIC ranging from 0.125 to >64 mg/L. MIC_{50} and MIC_{90} are respectively 2 and 32 mg/L for *C. auris* [96]. Nikkomycin it thus mainly used in combination with amphotericin B, azoles, or echinocandins, allowing to observe synergistic effects against a range of medically important fungi [97,98]. The first three phase 1 trials have been only recently completed. Drug dosage were tested between 50 mg BID to 750 mg TID or ones orally between 250 mg to 2,000 mg [99].

7.5. Repurposing of non antifungal drugs

In view of the medical need and of the paucity of existing alternatives, repurposing of existing drugs for an antifungal activity is also an active field of research. In vitro data demonstrated an antifungal activity for tamoxifen, sertraline and auranofin. Tamoxifen is a selective estrogen receptor modulator frequently used for the treatment of breast cancer. It inhibits Ccr1 NADPH-cytochrome P450 reductase activity in yeast, which alters cell wall integrity [100]. Yet, the active concentrations (10-20 µg/ml) are orders of magnitude above the therapeutic concentrations in patients treated for breast cancer (100-200 ng/ml), making this drug unusable as a monotherapy. As it shows synergistic activity with azoles and polyenes at clinically-achievable concentrations [101], a first clinical study has been launched to evaluate the efficacy of a combination with amphotericin B and fluconazole in the treatment of fungal CNS infection. Tamoxifen is being given orally at a daily dose of 300 mg for the first 14 days following diagnosis of infection together with amphotericin (1mg/kg/day) and fluconazole (800mg/day) [102]. The primary efficacy endpoint will be the rate of clearance of yeast cells from cerebrospinal fluid. Inhibitors of serotonin reuptake like sertraline also display activity against fungi, like Candida, at supratherapeutic concentrations of 10-20 µg/ml [103]. It has been studied in the same setting as tamoxifen, in combination with fluconazole in the treatment of fungal CNS infection at a daily dose of 400 mg. All 11 treated patients survived at 6 months in the sertraline and fluconazole group. Interestingly, sertraline is fungicidal against *C. auris* and prevents biofilm formation, but again at supratherapeutic concentrations [104]. Phase 1 clinical trials are coming soon in this particularly interesting indication, as *C. auris* is quite resistant to current antifungals. The last non antifungal drug investigated is auranofin. This gold thiol compound used to treat rheumatoid arthritis has shown activity in vitro against *C. albicans* [105] and prevents *S. aureus and C. albicans*

mono- and dual biofilm formation [106]. These effects are however observed at concentrations at least 10 times higher than those measured in the serum of patients receiving a dose of 6 mg for rheumatoid arthritis [107]. Moreover, its immunosuppressive effects need to be taken into consideration as patients infected by fungi are often immunosuppressed [108]. Phase 1 trial are performed with a 6 mg oral dose of auranofin once every 24 hours for 7 days. A lot of other drugs are being evaluated but additional research is needed to determine their specific efficacy against resistant Candida species, especially *C auris* [109]. Table 3 shows the new antifungal drugs under clinical trials.

8. Potential development issues (350-500 w) 179

Big pharmaceutical companies have decreased their research pipeline in anti-infective therapy in general, and antifungals in particular. Their focus is mainly on high-profit drugs for the treatment of chronic diseases, especially those associated with our sedentary lifestyle, which concern a lot of patients. As a result, smaller biotech companies with more limited resources are working to develop drugs with smaller market, like the next generation of novel antifungal drugs, but their financial investment in R&D is limited, so that they need to develop partnerships with big companies once they have identified a promising compound that can enter phase II or III clinical trials. A series of initiatives like Generating Antibiotic Incentives Now (GAIN), Orphan Drug Acts, as well as the Fast Track designation by the Food and Drug Administration in the United States, all apply to new antifungal agents, which has contributed to renew the interest in antifungal drug development [63]. Among the drugs discussed here, we may hope that rezafungin, ibrexafungerp, and

fosmanogepix will soon successfully achieve their development plan and be part of our future therapeutic arsenal.

9. Conclusion (150-200 w) 209

Today, four out of five classes of antifungals are active on yeast and used in the treatment of invasive candidiasis or candidemia. These are polyenes, azoles, echinocandins and pyrimidine analogues. Amphotericin B remains interesting, but its use is limited by renal toxicity even for the liposomal formulation. Azoles cause important drug-drug interactions due to their capacity to inhibit hepatic cytochromes. Echinocandins are now the initial drugs of choice for invasive candidiasis and candidemia due to increasing resistance to other classes of drugs and to better clinical outcomes. Their IV administration imposes to maintain the patients in the hospital, with associated costs. Ibrexafungerp may offer an interest against echinocandin-resistant strains in the future; as it binds to another site of the same target. ~~Yet, resistance to this class (echinocandin) is also increasing and.~~ Flucytosine may be underused, as it is highly effective when given in combination with synergy proven in vitro, in animal models, and in clinical trials. New drugs in these classes show a series of advantages over currently available compounds and their development is facilitated by the fact the general properties of previous generation molecules are already well known. Drugs acting on novel targets offer more innovative perspectives, but their development is riskier especially regarding possible safety issues.

10. Expert opinion (500-700 w) 404

In spite of the difficulties evoked here above, we see that research remains active in the field of antifungals agents, with some promising compounds arriving in clinical trials. Yet, we know that failures are not rare during this development phase, due to unexpected toxicity, difficulty to recruit patients for infrequent infections, or lack of funding.

Depending on the axis of research, the difficulties or needs may markedly differ. Optimizing existing drug formulation is the less risky strategy, but the added value is limited to the advantage brought by the new formulation, as illustrated by amphotericin B cochleate. Looking for new drugs in existing classes offers the benefit of capitalizing on a deep knowledge of the pharmacology of previous generations of molecules to improve their potency or spectrum of activity, cope with some resistance mechanisms, or improve safety profile. The limitation of this approach is that, in most of the cases, cross-resistance with other members of the class is inevitable, even if it is not seen in the clinics immediately due to the higher intrinsic potency of optimized derivatives. Searching for for totally new drugs is clearly the most promising strategy to fight against strains that have developed resistance to all existing classes or against new species like *C. auris* that are poorly responsive to conventional antifungals. But it is also the most challenging, as preclinical research should first explore in details their pharmacological properties and determine their potential clinical interest or risk of toxicity, which are largely unknown, before starting more risky clinical trials. The future will tell us more about the real potential of fungerps, manogepix, arylamidines and polyoxins. Finally repurposing existing drugs as antifungal agents may appear appealing as their pharmacological profile is known, but this strategy remains controversial because off-target effects are often observed at high, and thus probably toxic concentrations, and adverse effects related to the

main pharmacological activity of these compounds cannot be avoided. Yet, this approach may give the opportunity of discovering new pharmacophores that may thereafter serve as a template to develop more active compounds in which the antifungal activity could be dissociated from the original pharmacological effect.

In the coming years, in view of our current needs, the emphasis should be put on the search for compounds active against multiresistant strains and against emerging species like *C. auris.* Both types of microbes should thus be included in the early stages of development for new antifungal agents.

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Author's contributions

PMH, DDB designed the paper.

DDB, FVB and PMH participated in drafting and reviewing.

DDB, EM, FVB, SR and PMH read and approved the final version of the manuscript.

Acknowledgements

Our thoughts are with Prof David de Bels (first author) who passed away at the age of 56 years before the revision of this manuscript.

We would like to acknowledge the tremendous input of Dr Ibrahim Bousbiat from CHU Brugmann and ULB university.

Funding

None.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors declare to have no competing interests.

1006 **Tables and figure legends**

1007 Table 1: Risk factors for invasive candidiasis

Categories	Factors
Severity	Critically ill Neutropenia Drugs (IV) use
Age	Neonates Elderly
Drugs	Corticoids Chemotherapy Broad Spectrum antibiotics Parenteral nutrition
Sickness	Acute necrotizing pancreatitis Diabetes mellitus Cancer (solid or hematologic)
Strategy	Long term ICU Stay Hemodialysis Vascular catheters Abdominal surgery Organ transplantation Mechanical ventilation

1008 IV: intravenous; ICU: Intensive Care Unit

1009 Table 2: Financial status of companies having important research on antifungal drugs

Company	R&D 2016	R&D 2020	Finance 2016	Finance 2020	Increase rate
Matinas	3.9	3,3	9,8	74,8	7,6
Amplyx	4.4	Pfizer	118	Pfizer	NA
Scynexis	20	36.5	58.6	93.0	1.6
Valley Fever Solutions	NA	7.0	NA	19.0	NA
Cidara	35.7	68.0	104.6	110.1	Neutral
Pulmocide	NA	NA	19.5	53.9	2.8
Fujifilm Toyama	NA	NA	21940	23150	1.06
Appili	NA	3.2	NA	33.9	NA
Viamet	12	NA	48	NA	NA

Mycovia	NA	NA	NA	88.2	NA

Data in $ x1000000 ; R&D : Research and development ; NA: not available or not secured

References from US Securities and Exchange Commission (https://www.sec.gov/)

1016 Table 3: Competitive Environment

Compound	Company	Structure	Indication	Stage of development	Mechanism of action
Amphotericin B cochleate	Matinas	Polyene	Candida spp.	II	Binding to ergosterol
Quilseconazole	Viamet~~d~~	Tetrazole	Candida spp.	I	Cyp51
Oteseconazole	Mycovia		Candida spp.	III	Cyp51
VT-1598	Mycovia		Candida spp.	I	Cyp51
PC945	Pulmocide	Triazole	Candida spp. A Fumigatus	III	Cyp51
Rezafungin	Cidara	Echinocandin	Candida spp. Including Auris	III	Inhibition of 1,3 B-D glucan synthesis
Fosmanogepix	Amplyx	Isoquinoline	Candida spp.	II	Gwt1 inhibitor
Ibrexafungerb	Scynexis	Triterpene	Candida spp. Including Auris	III	Inhibition of glycan synthase
ATI-2307	Appili	Arylamidine	Candida	I	Mitochondrial disruption
Nikkomycin	Valley Fever Solutions	Polyoxin	Candida	II	Inhibition of chitin synthase

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1018 Figure 1: Mechanisms of action of the four families of existing drugs active on

1019 *Candida.*

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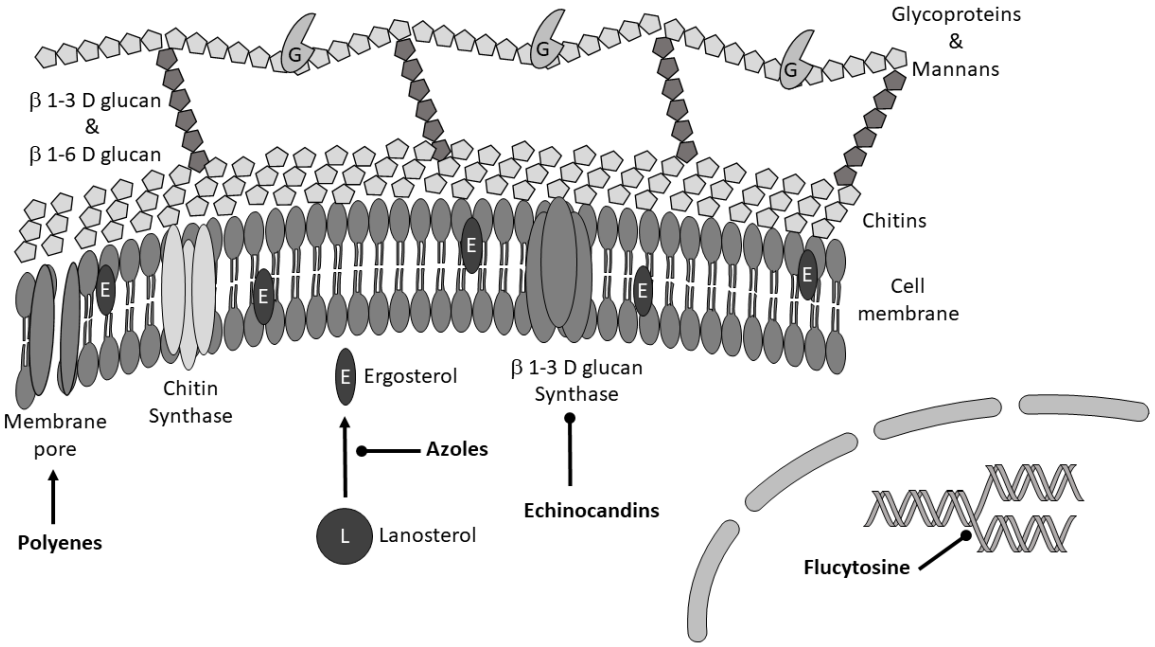

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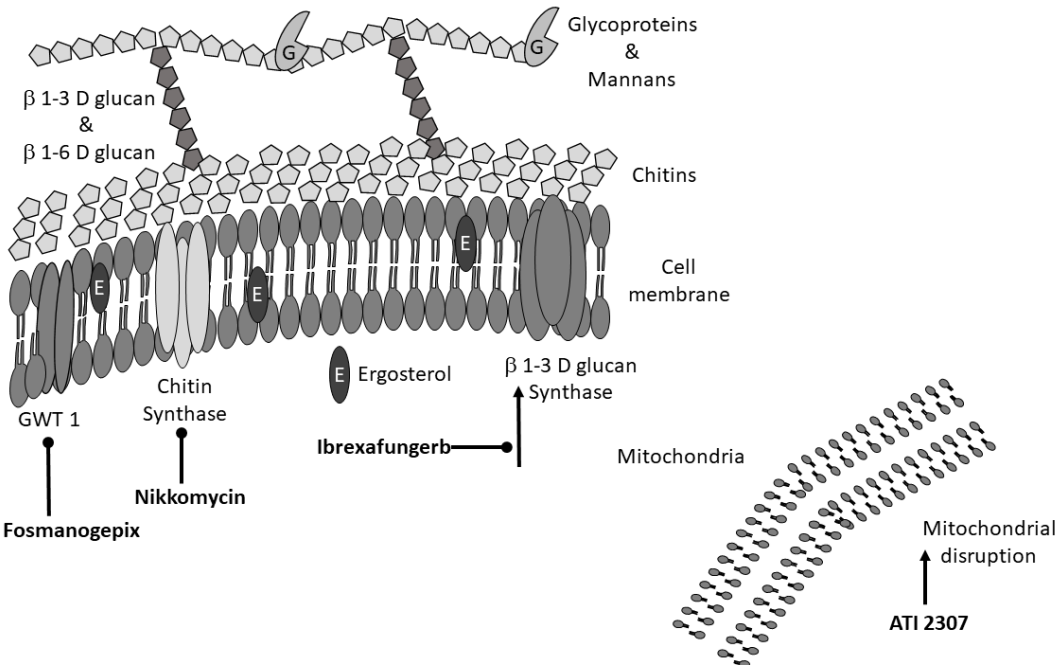
1023 Figure 2: Targets of new antifungals in early stages of clinical development



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