Immunotherapy as an Antifungal Strategy in Immune Compromised Hosts

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Abstract

Purpose of Review IFIs cause high morbidity and mortality in the immunocompromised host worldwide. Although highly effective, conventional antifungal chemotherapy faces new challenges due to late diagnosis and increasing numbers of drug-resistant fungal strains. Thus, antifungal immunotherapy represents a viable treatment option, and recent advances in the field are summarized in this review.

Recent Findings Antifungal immunotherapies include application of immune cells as well as the administration of cytokines, growth factors, and antibodies. Novel strategies to treat IFIs in the immunocompromised host target intracellular signaling pathways using SMTs such as checkpoint inhibitors.

Summary Studies using cytokines or chemokines exerted a potential adjuvant role to conventional antifungal therapy, but issues on toxicity for some agents have to be resolved. Cell-based immunotherapies are very labor-intensive and costly, but NK cell transfer and CAR T cell therapy provide exciting strategies to combat IFIs. Antibody-mediated protection and checkpoint inhibition are additional novel immunotherapeutic approaches.

Keywords Invasive fungal infections · Immunotherapy · Innate and adaptive immunity · Cell therapy · Cytokine therapy · Checkpoint inhibitors

Introduction

Opportunistic fungal infections are still a major health problem in immunocompromised patients worldwide and cause an estimated 1.6 million deaths each year [1]. This reported high morbidity and mortality of fungal infections result from the lack of reliable and fast diagnostics as well as from the limited options of antifungals especially for the treatment of resistant fungi [2]. Patients with prolonged neutropenia, allogeneic hematopoietic stem cell transplant (HSCT), solid organ transplant (SOT), inherited or acquired immunodeficiencies, i.e., AIDS, or corticosteroid use are at highest risk of life-threatening invasive fungal infections (IFIs) [3]. Although abundance of, e.g., *Aspergillus* conidia is high and hundreds

are inhaled every day by each person, immunocompetent individuals are protected due to mucociliary clearance and the presence of phagocytic cells in the lungs [4, 5]. Thus, IFIs are almost exclusively observed in immunocompromised hosts. In most cases, IFIs are caused by fungi from the genera *Candida*, *Aspergillus*, *Cryptococcus*, or *Pneumocystis*, but until today, these infections are treated with antifungal drugs, since no vaccine is currently available [4]. At the moment, there are three classes of antifungal drugs available and in clinical use: azoles (e.g., fluconazole), polyenes (e.g., amphotericin B, AmB), and echinocandins (e.g., caspofungin) [6].

The incidence of mycoses is rising due to increasing use of immunomodulatory drugs to treat autoimmune diseases, cancer, and transplant recipients [4, 7]. Additionally, reports on a global emergence of antifungal resistance especially in *Candida* spp. and *Aspergillus* spp. clearly highlight the limited treatment options and the urgent need for novel strategies to improve patient prognosis with IFIs [8]. Recently, researchers could describe new classes of drugs with promising antifungal activity, but further studies are needed to exhibit the real potential of these antifungals [6].

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Since innate and adaptive immune responses play an essential role in the protection against fungal infections, more researchers became interested in developing novel or adjunctive therapies that boost cellular and humoral functions in the host to improve patient outcome [4, 9]. Phagocytic cells such as alveolar macrophages and neutrophils are among the first cells that encounter fungal pathogens and have also been reported to play a crucial role in the eradication of invading pathogens in the lungs [9]. These innate immune cells express a variety of extra- and intracellular pattern recognition receptors (PRRs), which recognize fungal antigens and induce pro-inflammatory, but also regulatory responses. Interactions of fungi with PRRs, such as C-type lectins on dendritic cells, have been characterized, and these studies revealed induction of protective adaptive immunity [10]. These qualities make innate immune cells promising therapeutic targets.

Strategies to modulate the host immune system and to strengthen antifungal immune responses include the administration of recombinant cytokines and growth factors, granulocyte- and granulocyte-macrophage colony-stimulating factors (G-CSF, GM-CSF) and antibodies (abs). Additionally, immunocompromised patients can be treated with cell therapy approaches, which include transfusion of innate and adaptive immune cells to enhance antifungal immunity. In this review, we discuss recent data on established immunotherapeutic approaches against fungal infections, but also present novel ideas using checkpoint inhibitors as antifungal therapy.

Recombinant Cytokines and Immune Activating Compounds as Immune-Therapeutic Approach in Fungal Diseases

Administration of recombinant cytokines as host-directed therapy is an important approach to strengthen immunity and fight fungal infections (Fig. 1). In addition, increased susceptibility to fungal diseases often originates from immune defects such as primary or acquired immunodeficiencies [11, 12]. To reinforce host defense mechanisms against fungi, recombinant CSFs or IFN γ are used.

Colony-Stimulating Factors

Defects in neutrophils are a predisposing factor for opportunistic fungal infections. In order to counteract these defects, recombinant CSFs are used as an adjunct therapy to increase and activate neutrophils and enhance the host immune response to fungal pathogens (Fig. 1). Recombinant forms of GM-CSF and G-CSF that are primarily used for augmentation of neutrophils but also activation and proliferation of other myeloid cells, such as macrophages, dendritic cells (DCs),

circulating monocytes, platelets, or eosinophils are FDA-approved [13–15].

In animal studies, these two FDA-approved factors illustrated promising results with respect to treatment of severe fungal infections—GM-CSF alone or in combination with recombinant IFN γ exerted an improved fungicidal activity of innate phagocytic cells (macrophages, dendritic cells, neutrophils) [16]. Besides the fungicidal effects observed in murine models, faster renewal of neutrophils, lowering of fungal burden in the lung of immunosuppressed mice, or inhibition of macrophage suppression by the corticosteroids cortisone acetate or dexamethasone were demonstrated after GM-CSF and G-CSF administration [17, 18]. In a murine model, an improved outcome following infection with *Aspergillus (A.) fumigatus* was reported after treatment with G-CSF together with caspofungin or caspofungin/amphotericin B intralipid with respect to survival rates, fungal burden in organs, and reduced galactomannan serum levels [19].

Since both CSFs are approved by the FDA, G-CSF is often applied after stem cell transplantation to restore neutrophil counts and to reduce time of neutropenia in patients (reviewed in [20]) and data from animal studies using GM-CSF or G-CSF seemed encouraging; studies in respect to treating IFIs in humans using CSFs appear likely.

A retrospective study by Safdar et al. investigated the use of rhGM-CSF in immunosuppressed patients with IFI [21]. The study illustrated that GM-CSF administration turned out to be safe and well tolerated, but in contrast to data in mice, where improvement of IFIs due to activation of innate phagocytes was observed, adjunct GM-CSF treatment during high-dose corticosteroid therapy failed. Patients on antineoplastic therapy showed a better outcome after GM-CSF administration [15]. A prospective multicenter randomized phase IV clinical trial in 206 allogeneic stem cell patients by Wan et al. demonstrated a decrease in the combined 600-day IFI-related mortality and yeast-disease incidence, which were administered prophylactically with either GM-CSF or G-CSF alone or in combination [15]. In contrast to yeast-disease incidence, no benefit for invasive *Aspergillosis* was observed in this study. Thus, contrary to murine data, both GM-CSF and G-CSF were illustrated to not being directly related to improvement in IFI-related mortality in humans. However, GM-CSF and G-CSF treatments might also support faster recovery of immune-deficient individuals during IFI, which to date has not been confirmed in larger patient cohorts. A recent study in a patient suffering from *Aspergillus ventriculitis* pointed to a successful recovery where GM-CSF was applied in combination with voriconazole, AmB, and caspofungin [22]. In a *Scedosporium apiospermum*-infected patient, co-administration of the antifungal micafungin and GM-CSF resulted in a successful therapy [23]. Taken together, more in-human studies are needed for studying the effects of GM-CSF/G-CSF during fungal infections.

Fig. 1 Overview on humoral- and cellular-mediated immunotherapies against fungal infections. Complement receptors (CRs), cytotoxic T lymphocytes (CTLs), interferon (IFN), interleukin (IL), natural killer

(NK), pathogen recognition receptor (PRR), reactive oxygen species (ROS), T helper cell (Th), toll-like receptor (TLR), and tumor necrosis factor (TNF)

Unlike G-CSF and GM-CSF, M-CSF is not FDA-approved for use in humans, and this cytokine mainly stimulates growth of macrophages (reviewed in [24]). Improvement of fungal infection was depicted in transplant mouse models treated with M-CSF [25]. In case series from 1993 by Nemunaitis et al., positive effects regarding survival of M-CSF co-administered to conventional antifungal treatment were reported in 46 patients receiving stem cell transplantation and suffering from IFI—no follow-up study was performed, therefore this cytokine should be considered further for treatment in patients suffering from IFI after stem cell transplantation.

IFN γ

Administration of the recombinant IFN γ to patients suffering from IFI is limited to case reports and a few clinical trials [26, 27]. IFN γ promotes polarization of naïve CD4⁺ T cells to Th1 cells, a helper cell type associated with antifungal control (Fig. 1). Besides Th1 polarization, IFN γ exerted positive effects on fungistatic and fungicidal activities of neutrophils, and mediated O₂ production from PMNs in vitro after infection with *Pneumocystis carinii* [28]. Furthermore, in patients suffering from IA, an increased production of pro-inflammatory cytokines, among those IL-1 β , TNF- α , IL-17,

IL-22 and higher HLA-DR, expression was observed following co-treatment with antifungals and IFN γ [29]. All these factors are pointing to a protective role by enhancing antifungal control mechanisms, e.g., activation of Th1, Th17, Th22, improving barrier functions, or macrophage and neutrophil mobilization. A further study showed that three renal transplant patients suffering from IA were cured after combined treatment with Amb and IFN γ [30]. Recombinant IFN γ was proved safe in allogeneic stem cell transplant recipients in a single-center retrospective analysis [31]. IFN γ was used as salvage therapy in patients with cerebral cryptococcosis and non-responding to antifungal drugs—co-administration of IFN γ and antifungals resulted in enhanced clearance of *Cryptococci* [32], pointing to a supportive role by IFN γ . In vitro experiments of *Scedosporium* infection revealed positive effects of IFN γ /GM-CSF co-application [28]—no in vivo data are available so far.

TNF- α

Neutrophils or PMNs in general are, too, activated for increased fungicidal actions by TNF- α , the most potent innate cytokine during the defense against *Aspergillus* [33]. Experiments in corticosteroid-treated mice with IA revealed a decreased mortality rate if treated with TNF- α [34]. Another

study in neutro- and non-neutropenic mice highlighted important protective functions of TNF- α , since due to increased TNF- α levels more PMNs infiltrated the lungs and decreased mortality rates. Inhibition of TNF- α resulted in higher fungal burden and higher mortality [35].

In vitro analyses using human cells showed, that similar to IFN γ , TNF- α mediated production of oxygen radicals from PMNs, which resulted in hyphal damage during *Aspergillus* infection. Also, increased fungicidal effects against *Candida* were observed when PMNs were treated with TNF- α in vitro [36]. While no enhanced fungal killing was observed in alveolar macrophages, they illustrated a higher phagocytosis of *Aspergillus fumigatus* in the presence of TNF- α in vitro [37]. A major drawback on using TNF- α as antifungal immune therapeutic agent is its high toxicity after systemic administration [38].

IL-12 and IL-15

Not only IFN γ and TNF- α but also IL-12 and IL-15 have been assessed in preclinical trials as adjunct therapy during IFI. IL-12 and IL-15 are pro-inflammatory cytokines protective during IFI due to their Th1-polarizing effects [39]. Th1 cells are essential to protect against fungal pathogens such as *Candida* species, *Aspergillus* species, or *Histoplasma capsulatum*. Production of IL-12 is strongly correlated with Th1 immunity against *Candida (C.) albicans* [40] and *Cryptococcus neoformans* [41] and increased the antifungal capacity of monocytes against *A. fumigatus* in vitro [42]. Furthermore, IL-12 was depicted to restore NK cell functions in Cryptococcus/HIV-1-co-infected individuals [43]. In this context, IL-12 treatment reversed the defective NK cell's anti-cryptococcal activity and caused modulation of NK cells. However, it was also shown that in the host, IL-12 may be associated with enhanced susceptibility to fungal pathogens [44, 45], which makes IL-12 controversial to use as an immune therapeutic agent during fungal infections.

IL-15 was illustrated to increase the antifungal activity of granulocytes or monocytes against *C. albicans*, *A. fumigatus*, *Fusarium* spp., and *Scedosporium* spp. [46, 47]. IL-15 is, too, an activator of NK cells and induced IL-8 and superoxides in in vitro experiments upon infection with filamentous fungi [48]. Therefore, IL-15 exerts a potential adjuvant role to conventional antifungal therapy, but this needs further investigation.

Thymosin- α -1 (Ta1)

In kidneys of *Candida*-infected immune-compromised mice, lower fungal burden and higher intracellular killing of *C. albicans* was measured upon T α 1/fluconazole medication [49]. T α 1 is a thymus-derived immune-stimulatory compound that is approved as an immune adjuvant for viral

treatment in over 30 countries [50]. However, in vivo data are still missing with respect to T α 1 in humans suffering from IFI. Thus, there is a gap of knowledge, whether this compound in combination with fluconazole is also effective in immune-suppressed humans in respect to lowering fungal burden or improving intracellular killing during *Candida* infection.

Cell Therapy as Immune-Therapeutic Approach in Fungal Diseases

Neutrophils, macrophages, dendritic cells (DCs), NK cells, and monocytes comprise first innate cellular defense mechanisms during fungal infections by immediately recognizing PAMPs on fungi via their abundantly expressed surface PRRs (Fig. 1) [51].

Neutrophil Enrichment by Granulocyte Transfusion (GTX)

During GTX, granulocytes containing neutrophils from a donor are transferred to a neutropenic patient, who is at risk of developing IFI [52, 53]. GTX studies in humans indicated that transfusion of neutrophils from donors stimulated with G-CSF are able to restore severely neutropenic patient's blood supply and neutrophil inflammation response, thereby rescuing the host's defenses against fungi [54]. Due to improved isolation procedures and high-quality recombinant cytokines, problems in the beginning using GTX could be overcome, which resulted in a 50–90% response rate in IFI patients after GTX/G-CSF treatment [55]. It was further documented that prophylactic GTX might decrease the risk of fungal or bacterial infections [56]. In various case reports, GTX was demonstrated to improve survival rates in cancer patients with candidemia, to prevent recurrent fungal infections and to be effective during IA and mucormycosis [31, 54]. Yet, there are insufficient data in patients to recommend GTX during IFI [57].

Dendritic Cell (DC) Immune Therapy

During immunosuppression also DC functions may be affected, thus resulting in insufficient or inappropriate T cell mobilization, or cytokine production. There are several ways to restore DC functionality in immune-compromised individuals for inducing more efficient antifungal defense mechanisms. On the one hand, DCs can be activated via recombinant cytokines, but also via specific PRR activation, e.g., murine and human DCs were illustrated to up-regulate TLR2 and TLR9 upon treatment with T α 1, they functionally matured and produced pro-inflammatory cytokines [58].

In mice, T α 1 administration following *A. fumigatus* infection resulted in better survival rates, lower fungal burden, and IFN γ -producing Th1 cells on the one hand [58], and induction

of IDO-mediated immune tolerance on the other (Fig. 1) [59]. This balanced induction of immunogenic and tolerogenic DCs by T α 1- or T α 1-loaded DCs gave rise to protective immunity against *A. fumigatus* [59] and provides a promising adjunct therapeutic approach for IFI treatment, which has to be approved in humans. In a HSCT mouse model of IA, TLR9 triggering on DCs by CpG oligodeoxynucleotides as adjuvant together with Asp f 16, a major *A. fumigatus* allergen, resulted in protective Th1 responses [60]. Of course, all these observations from mice need approval in clinical trials in humans.

Transfusion of IL-12-transduced and *Aspergillus*-activated DCs and DCs pulsed ex vivo with live *A. fumigatus* or *A. fumigatus* RNA were, too, shown to mediate a protective Th1 response and protective immunity in murine models [60, 61].

Another novel DC-based strategy might be triggering of complement receptors (CRs) 3 and/or 4 (CR3, CR4) to mediate improved antifungal immune responses—as recently shown by us in vitro using single DC cultures or DC/T cell co-cultures of primary human origin, opsonization of viral and fungal pathogen resulted in significantly higher production of pro-inflammatory cytokines and enhanced polarization of Th1/Th17 cells [62–64]. This induced pattern is associated with protective functions during fungal infections, but needs to be approved in clinical trials.

If DCs are directly targeted ex vivo, they might mediate a strong, protective immune response as well as induction of fungus-specific, cytotoxic CD8⁺ T cells (CTLs) due to cross-presentation of fungal antigens [65]. Fungus-specific DC-based vaccination strategies are very labor-intensive and costly, because they require GMP (good manufacturing practice) guidelines, but the results so far emphasize the high potential of ex vivo-pulsed or modified DCs in therapeutic vaccination against invasive fungal infections [66]. Since most of the data were generated in animal studies or in vitro, approaches need to be tested during clinical trials in patients suffering from IFIs.

NK Cell Therapy

Human NK cells have been shown to be effective against a broad spectrum of fungi, among those *A. fumigatus* [67–69], *C. albicans* [70], *Rhizopus oryzae* [71], and other clinical isolates of mucormycetes [72] to name a few. Antifungal activity of NK cells is through direct mediators such as perforin and granzyme [73] and high ROS and NK cell levels correlated with improved control of IA in allo-SCT patients [74]. NK cell therapy is currently in its trial stage for cancer, but no clinical trial exists to date using NK cells for IFI treatment. However, there is a high potential of NK cell therapy as immunotherapeutic option of IFI, which has to be approved in future studies.

Adoptive T Cell Transfer

An artificial increase of fungus-specific T cells after allo-SCT might greatly support clearing of *Aspergillus* in immunosuppressed individuals—these are more prone to fungal infections, since even months after allo-SCT only few *Aspergillus*-specific T cells may be detected in vivo due to very slow recovery rates of adaptive immune cells [75]. A preliminary study in allo-SCT patients with uncontrolled IA demonstrated promising results, when in vitro-stimulated and selected *Aspergillus*-specific T cell clones were transferred to the individuals [75].

In another experimental approach, a Treg-based immunotherapy was applied [76], where co-infusion of Treg and conventional T cells improved immunity to fungal pathogens and prevented lethal graft-versus-host disease in the absence of immunosuppressants. However, the benefit of Treg-based immunotherapy in combination with fungus-specific, functionally active Th1 cells needs to be addressed in more detail and in bigger cohort studies.

To be clinically successful, sufficient fungus-specific and highly pure CD4⁺ T cells have to be transferred in allo-SCT patients within a very short time due to the rapid progression of IA; this is also the major obstacle with respect to adoptive T cell transfer, and many groups are working on optimizing the whole procedure. Currently, one GMP-compliant protocol is in a clinical trial (EudraCT Nr. 2013-002914-11) [77]. It is of utmost importance to control the quality of transferred T cells, since immunosuppressants frequently used in allo-SCT may affect them [78].

Another possibility is the application of gamma/delta ($\gamma\delta$) T cells, which recognizes fundamentally different ligands from the short peptides that are seen by alpha/beta (α/β) T cells in the context of MHC class I or class II molecules. These cells were also demonstrated to mediate anti-*Aspergillus* activity [79]. $\gamma\delta$ T cells exert potent cytotoxic activity, secrete a variety of cytokines, and link innate and adaptive immunity via DCs or complement. In vivo $\gamma\delta$ T cells were easily activated and expanded by stimulation with aminobisphosphonates [80]. Hence, adoptive transfer of $\gamma\delta$ T cells may be a promising candidate for adoptive immunotherapy to treat IFIs, since they also do not mediate alloreactivity.

Chimeric Antigen Receptor (CAR) T Cell Therapy

Currently, chimeric antigen receptor (CAR) T cell therapy is FDA-approved for several forms of aggressive, refractory non-Hodgkin lymphoma as well as for relapsed or refractory acute lymphoblastic leukemia in patients up to an age of 25. Additionally, other trials using CAR T cell therapy are ongoing at the moment (US Food and Drug Administration FDA approves axicabtagene ciloleucel for large B cell

lymphoma; available at www.fda.gov/drugs/informationondrugs/approveddrugs/ucm581296.htm). This therapy is based on re-engineered T cells with chimeric antigen receptors. Here, the extracellular ligand recognition domain is modified to express, e.g., specificity for fungal pathogens, and connected to an intracellular signaling complex consisting of CD28, 4-1BB, or OX40 as co-stimulators and CD3 ζ , to enable activation of the engineered T cells after antigen ligation (Fig. 1) [81, 82]. With respect to fungal infections, the single-chain variable region was exchanged by Kumaresan et al. by the C-type lectin Dectin-1, a fungus-specific receptor, to specifically target β -1,3-D-glucan on the fungal surface [82]. Cytotoxic Dectin-1-targeting CAR T cells were able to target *A. fumigatus* in vitro and in vivo in murine models, and this proof-of-principle study provides an exciting and novel immune-therapeutic approach in IFIs, but of course needs approval in clinical studies.

Other Immunotherapeutic Approaches

Administration of abs or passive immunization is a powerful tool to treat ongoing microbial infections, but since the development of modern day antibiotics and antifungals, its use has become less significant. In general, humoral immune responses were considered to play a minor role in protection against fungal infections, but recent data demonstrated the importance of antibody responses in protecting the host. Furthermore, the development of a fungal vaccine to protect individuals, who are anticipated to have severe immunosuppression in the future, has been advanced in the last years.

Antibody Therapy and Fungal Vaccines

The importance of humoral immunity in the defense of fungi has been investigated in individuals suffering from primary antibody deficiencies, e.g., X-linked agammaglobulinemia (XLA) or common variable immunodeficiency (CVID), since these patients have a higher incidence of fungal infections [83, 84]. Fungal antigens such as cell wall components (carbohydrates and glycoproteins), proteins, and other surface molecules (heat-shock proteins, HSPs) represent very promising targets for the development of protective monoclonal abs. Antibody-mediated protection can be achieved via direct and indirect mechanisms and were mostly studied in *C. albicans* and *Cryptococcus neoformans* [85]. Antibody-mediated protection can be mediated directly to inhibit biofilm formation, inhibition of polysaccharide release, interference with dimorphic changes, alterations in gene expression, iron starvation, and mimicking killer toxin using anti-idiotypic abs. The indirect group covers mechanisms such as phagocytosis, complement activation, antibody-directed cellular cytotoxicity (ADCC), phagosome activation, and modulation of

inflammatory responses [85]. So far, two mAbs, 2G8 and the anti-HSP90 (efungumab, Mycograb), have been tested in immunotherapy studies. Studies using the mAb 2G8 targets laminarin, which consists of β -glucans, showed effective protection against *C. albicans*, *A. fumigatus*, and *Cryptococcus neoformans* [86, 87]. The anti-HSP90 antibody is a single chain variable fragment (scFv) antibody and was tested in combination with lipid-associated amphotericin B (AmB) in a clinical trial. Mycograb together with AmB was well tolerated by patients and exhibited reduction of *Candida*-associated mortality and improved fungal clearance [87]. Novel approaches in antibody-mediated protection of fungal infection include radio-immunotherapy, which is a combination of radiation and immunotherapy mostly used for cancer patients. Here, antifungal abs is linked to radioisotopes to specifically deliver cytotoxic radiation to target structures [88]. Studies on *Cryptococcus neoformans* showed that radio-immunotherapy provides a novel mechanism for the treatment of resistant fungi irrespective of the host immune status with the potential to minimize treatment time [88].

In general, vaccination is the most effective way to prevent from infection by administration of live-attenuated and inactivated pathogens or microbial protein subunits. The goal is to induce protective cellular and humoral adaptive immunity without experiencing the disease. Only a few vaccine trials were conducted against fungal infections—mostly against *C. albicans*, *Aspergillus* spp., and *Cryptococcus* spp. [89]. Als3p, a fungal protein, and other fungal cell wall components, were tested in combination with different adjuvants or delivery systems to boost antifungal immunity [90, 91]. Vaccination approaches were yet performed using murine models—in vivo studies investigating the recombinant *Aspergillus* proteins (Aspf3, Asp f9, Asp f16, Gel1, and Pep1) revealed protection in murine *Aspergillosis* models [91]. Furthermore, panfungal approaches have been tested using glucans and galactomannans from or heat-inactivated *Saccharomyces cerevisiae* as vaccines against various fungi. Data generated in these studies demonstrated protective antifungal immunity against *Aspergillus* spp. without the help of any additional adjuvants [91, 92]. Cenci et al. investigated the impact of live versus heat-inactivated *A. fumigatus* on the induction of protective antifungal immunity in a mouse model. In comparison with heat-inactivated fungi, using live *A. fumigatus*, higher CD4⁺ T cell numbers and higher IFN γ and IL-2 levels were detected, which are associated with protection against fungal infection. [93] Vaccines against fungi are a promising tool for preventing IFIs in humans, but knowledge gaps due to missing vaccination approaches in humans exist in respect to effectivity, risks of vaccine-induced autoimmunity, or worsening the underlying disease, which need to be addressed to generate a safe antifungal vaccine.

Novel Approaches: Checkpoint Inhibitors

Small molecular therapeutics (SMTs) that regulate, block, or inhibit intracellular signaling pathways in host cells have been widely used in cancer patients. These patient cohorts receive SMTs and immunosuppressive drugs to down-regulate host immunity and are therefore at high risk of IFIs. SMTs are widely used to treat autoimmune disease such as systemic lupus erythematosus and rheumatoid arthritis placing nonconventional populations to an elevated risk of fungal diseases [94–96]. Although the use of tyrosine-protein kinase (TK) inhibitors, non-TK inhibitors, mammalian target of rapamycin (mTOR), and autophagy regulators was associated with impaired antifungal immunity, checkpoint inhibitors could show a beneficial effect in early *Aspergillus* infection [97, 98]. The PD-1/PD-L1 complex acts as an immune checkpoint by dampening the immune response and promoting tolerance [99]. In early *A. fumigatus* infection, PD-L1 was reported to induce tolerance in DCs, which resulted in up-regulation of CTLA-4 activity and increased secretion of auto-inflammatory cytokines [100]. Thus, in the future checkpoint inhibitors may represent a promising immunotherapeutic drug, not only to treat malignant or autoinflammatory diseases but also for fungal infections.

Conclusion

IFIs are a major health problem due to growing numbers of immunosuppressive treatments, invasive medical interventions, and limitations of conventional antifungal chemotherapy together with increasing resistance of fungal strains; treatment of IFIs poses a real challenge [4, 5, 7]. Thus, immunotherapy, with and without simultaneous administration of antifungal drugs, represents an interesting approach for the treatment of IFIs. In recent years, progress has been made in the use of existing antifungal immunotherapies, and data generated in clinical research has exploited novel opportunities for improved antifungal therapy. Approaches to tackle IFIs by boosting the host's immunity include application of effector and regulatory cells as well as the administration of recombinant cytokines and growth factors, granulocyte- and granulocyte-macrophage CSF and abs. Novel strategies to treat IFIs in the immunocompromised host target intracellular signaling pathways using small molecular inhibitors such as checkpoint inhibitors. Checkpoint inhibitors have shown significant efficacy in cancer therapy and in first experiments; these molecules demonstrated their antifungal potential in early *Aspergillus* infection. Therefore, checkpoint inhibitors represent a promising novel immunotherapeutic option to treat IFIs, but additional studies followed by clinical trial have to be performed to assess their real antifungal activity.

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Compliance with Ethical Standards

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