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The challenges of developing and identifying a pipeline for drug development for *Aspergillus fumigatus*-induced lung infections

Yuyao Zhao

North London Collegiate School (Singapore), 130 Depot Road, 109708, Singapore

nancyzhao802@gmail.com

Abstract. Aspergillosis, a lung infection brought on by Aspergillus, is particularly dangerous for people with impaired immune systems. Aspergillosis is difficult to prevent since the spores are found in the environment on a regular basis. Healthy people are usually unaffected, but those with compromised immune systems must deal with the challenges of medication resistance and reinfection throughout therapy. Additionally, Aspergillus fumigatus is listed as a fungal priority pathogen by the World Health Organization. To tackle this developing pandemic and improve outcomes for those afflicted, effective management measures and further research are required. This paper analyses the challenges faced in the drug pipeline against Aspergillus fumigatus, so as to address the spread of the disease of Aspergillosis lung infections. Recognizing these challenges could help researchers consider other possible modes of treatment and what to look out for. The paper proposes that a possibility could be a combination of various drugs, along with immunotherapy, in order to tackle the disease effectively. This means that continued research and efforts are still needed in the drug development pipeline for Aspergillus fumigatus, preferably with more funding. The threat it poses is significant, as its increased infection rate suggests it has the potential to become an epidemic in the next 50 years. Thus, a breakthrough would be significant in ensuring higher survival rates among patients.

Keywords: aspergillus fumigatus, drug development, drug discovery pipeline, challenges of developing a drug.

1. Introduction

Aspergillus fumigatus is a mould pathogen responsible for causing Aspergillosis, a lung infection that can be fatal to people who are immunocompromised or take immunosuppressants. These patients are often those who have received transplants in the past, for instance, liver and kidney transplants, or who are currently undergoing chemotherapy and radiotherapy. The more heavily immunosuppressed the patient, the higher the possibility of mortality after a serious infection. Currently, the infection of Aspergillosis is diagnosed. In the drug development pipeline, Osherov and Kontoyiannis raised the question of whether the glass was half-full or half-empty in 2016. This meant that as of 2016, researchers were questioning whether the progress in the drug development pipeline could be considered optimistic or pessimistic.

In order to cure lung infections caused by Aspergillosis, this paper evaluates the difficulties faced throughout the medication development process for *Aspergillus fumigatus*. Researchers will be better

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equipped to tackle these issues if they can recognize and comprehend these difficulties and look into other therapeutic options. Researchers can create more efficient treatments by better understanding illness causes, patterns of medication resistance, and dynamics of reinfection. Because of its rising infection rate, which raises the possibility of a pandemic in the ensuing decades, *Aspergillus fumigatus* poses a serious concern. Because of this, improvements in the creation of new antifungal drugs and treatment plans would significantly improve patient survival rates. This research emphasizes the significance of addressing the difficulties encountered in developing drugs to combat *Aspergillus fumigatus*. More efficient medicines can be developed and the outcomes for people with Aspergillosis lung infections can be improved by taking into account different treatment modalities and stepping up research efforts. In order to combat this new disease and protect public health, it is essential to continue spending money and working together in this field.

2. Historical development of aspergillus treatment since 2000s

As of 2016, the three main antifungal drug classes used to treat aspergillosis are echinocandins that prevent biosynthesis (anidulafungin, micafungin, caspofungin), polyene amphotericin B-deoxycholate (including the lipid formulations) which causes cell lysis through binding fungal membrane ergosterol, and triazoles which prevent ergosterol biosynthesis (isavuconazole, posaconazole, voriconazole, itraconazole) [1]. Based on the research by Osherov and Kontoyiannis, the use of triazoles can have contraindications in the form of drug interactions when used in treatment. These contraindications can result in significant side effects, which can be chronic or acute. Chronic side effects include alopecia and neuropathy, while acute side effects include central nervous system toxicity and hepatic toxicity [2]. The use of chronic voriconazole has also been linked to skin cancer, photoaging, and bone fluorosis. Owing to its saturable and nonlinear pharmacokinetics, voriconazole treatment is also considered costly and technically complex, needing regular monitoring. There is also a threat of such drugs being no longer as effective later on, due to the potential of increasing drug resistance of such fungus that has been reported in different countries, as highlighted by Mayr and Flora in their 2011 review, showing that the resistance has been observed in Manchester (15% of all cases) [3], Nijmegen (10%), France, Spain, Denmark, China, USA, and UK. One finding in the study was that the increase in azoles and the use of the therapy lead to long-term resistance for the aspergillus, though this does not extend to in vivo or in vitro resistance to echinocandins. Yet a limitation remains that the degree of resistance could be underdiagnosed, which would be significant since it could be more widespread than originally thought. Besides, effective drugs include the nephrotoxicity that can occur from the use of amphotericin B formulations, and echinocandins only act in a narrow spectrum [4]. This has resulted in the use of anidulafungin and voriconazole combinations for the infection, and its resulting meningitis as a related condition being treated by flucytosine and amphotericin.

Yet despite the challenges faced, there has been no additional commercialization of drugs to treat aspergillosis since 2001, when echinocandins were launched. This has also been seen as highly challenging since resources tend to be channelled according to researchers Mayr and Lass-Florl to other chronic conditions like diabetes. The large-scale funded studies and drug screening for fungal-based diseases like aspergillus were done in the 1990s [3, 4], but these resulted in only a deeper understanding of the toxicity levels these could have, with no other results in terms of effectiveness [5]. This only served to lessen attention and research due to the potential safety risks of the studies for human participants. As a result, mainly smaller laboratories and biotech companies have been the ones in 2010 onwards working on potential human drug trials in phases 1 to 3. Researchers have also referred to a "valley of death" leading to a bottleneck in the drug development pipeline, in terms of the difficulty of gaining approval from the U.S. Food and Drug Administration [6]. Furthermore, the use of mouse models for testing has also led to questions on how successful this would actually be for humans, leaving researchers with the dilemma of undertaking greater toxicity risk using human participants, or using mice studies but not really moving forward significantly in research in a manner that was done in the past.

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Examining the developments in aspergillus drugs allows a better understanding of the possibility of developing new formulations from current drugs. Furthermore, another aspect would be in terms of how this has been combined with immunotherapy.

As shown in Table 1, the main compounds that have had some human clinical testing are F901318, ASP2397, E1210/APX001, and T-2307. Different compounds act differently. F901318 was made in the UK, and it was originally done through screening using chemicals and other drugs, known as a compound library screen. F901318 was found to be effective by inhibiting DHODH/URA1, also known as dihydroorotate, specifically in the fourth step relating to pyrimidine biosynthesis, which is an enzymatic step. DHODH for humans, compared to the fungi, has an estimated 20% similarity but is inhibited 200 times less using F901318 compared to how much it would be inhibited for the fungi [7]. In laboratory settings, in vitro studies have shown that F901318 can successfully limit the growth of azole-resistant fungi similar to aspergillus, specifically fusarium, fumigatus, scedosporium, and dimorphic fungi. However, F901318 remained ineffective against candida and mucorales. This was followed by in vivo testing through oral administration of a dose of 10 milligrams per kilogram of body weight for patients, yielding a 100% survival rate within two weeks. The levels of safety were also significant in terms of being well tolerated for a maximum test amount of 4 milligrams per kilogram of body weight, for a single dose without any negative effects and with high levels remaining, administered intravenously. Researchers have also considered the success of possible dose escalation [8].

Table 1. 2017 review in Medical Mycology by Nir Osherov and Dimitrios P Kontoyianniss.

Antifungal	Mode of action	In vitro MIC A. fumigatus	In vivo murine IA infection: active dose	Human trials Phase 1
Small molecules				
T-2307	Mitochondrial function	0.01-1 μg/ml	1 mg/kg Subcutaneous	Yes, 2015
E1210/APX001	GPI-anchor inhibitor	0.03-0.13 μg/ml	25 mg/kg, oral	Yes, ongoing
ASP2397	Unknown	0.06-0.5 μg/ml	4 mg/kg	Yes, ongoing
F90138	DHODH inhibitor, pyrimidine biosynthesis	< 0.06 μg/ml	10 mg/kg, oral	Yes, ongoing
Amphotericin B (AMB) formulations and conjugate	Disruption of fungal membrane	0.125-0.75 μg/ml	10 mg/kg	No
AMB-nanoparticles		0.25-1 μg/ml	5 mg/kg	No
AMB–arabinogalactan AMB-PEG		1-8 μg/ml	7 mg/kg	No
Repurposed drugs				
Clofazimine	Antimycobacterial DNA binding	Only effective in combination with caspofungin or Posaconazole	Not determined	No
Trichostatin A	HDAC inhibitor	4 μg/ml, synergy with caspofungin	Not determined	No

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Table 1. (continued).

MGCD290	HDAC inhibitor	8- >32 μg/ml, synergy with azoles	Not determined	No
Geldanamycin	Hsp90 inhibitor	4 μg/ml (MEC), synergy with caspofungin	Not determined	No
Tacrolimus (FK506)	Calcineurin inhibitor	0.01-0.06 μg/ml (MEC), synergy with caspofungin	Not determined	No
Cyclosporin	Calcineurin inhibitor	0.5-1 μg/ml (MEC)	Not determined	No
Natural Products				
Psoriasin	Zinc chelation	1 μM	5 mg/kg	No
Humidimycin	HOG pathways inhibitor	Only active in combination with caspofungin	Not determined	No

3. Broad spectrum formulations and Amphotericin B deoxycholate (d-AMB)

Amphotericin B deoxycholate (d-AMB) has been in use since 1947 and has been one of the most effective and potent drugs for treating fungi-related illnesses. However, the main issue with using Amphotericin B deoxycholate (d-AMB) also lies in its potency, especially nephrotoxicity and acute infusion reactions which result from the potency and toxicity of the drug itself. As such, other preferred formulations are liposomal amphotericin B, and amphotericin B lipid complex, despite their increased cost, being lipid formulations [9,10]. Other new formulations in use for Amphotericin B with low toxicity are Amphotericin B nanoparticle suspensions, which have both high efficacy and low toxicity in vivo, also shown in Table 1. These suggest significant progress in the development of aspergillosis drug treatment, despite the issues with toxicity existing when it comes to particularly potent formulations, or concerns with cost when it comes to allowing for greater accessibility for patients. Another key challenge is the fact that the novel broad-spectrum formulations and Amphotericin B deoxycholate formulations were also not put into human clinical trial phases as of 2017.

4. Possible reasons for a bottleneck in the drug development pipeline

As examined above, the cost and challenges for *Aspergillus fumigatus* drug development and treatment include cost and toxicity, especially nephrotoxicity. This has also resulted in drug development for *Aspergillus fumigatus* being postponed for clinical human trials, although in vitro studies may seem promising.

Another key reason for the potential bottleneck in the development of *Aspergillus fumigatus* is also the increased resistance of the fungus to certain drugs. With the increasing resistance, this means that there is the risk that the drug itself will also develop resistance during the clinical trial phase. The current resistant drugs that researchers are aware and conscious of are fluconazole and ketoconazole, though these have been widely recognised to involve intrinsic resistance against *Aspergillus fumigatus*, being completely ineffective against aspergillus. Possible antifungal agents that could be effective against aspergillus are isavuconazole, voriconazole, posaconazole, and itraconazole, however, there is a possibility of developing resistance to either of the drugs by *Aspergillus fumigatus*, either in vitro or in vivo studies. As of 2016, according to Leonardelli et al, there was no evidently clear reason for the molecular mechanism of the resistance for *Aspergillus fumigatus* [11]. The current hypothetical molecular mechanism which has been suggested by Edlind et al. as of 2001 is an amino acid substitution occurring in Cyp51Ap, which was discovered through in silico comparisons of candida albicans and *Aspergillus fumigatus* [12]. The general finding was comparing the residues for candida albicans and

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Aspergillus fumigatus, the T315 residue was not conserved. Rather, for *Aspergillus fumigatus*, there was a replacement by the I301 nonpolar isoleucine; as opposed to how for candida albicans, T315A nonpolar alanine replaced the T315 polar residue [13].

Furthermore, drug development pipelines are also affected by patents, and the existing efficacy of antifungals in dealing with the disease caused by *Aspergillus fumigatus*. According to a paper by Robbins, Spitzer, Yu, et al. in 2015, the use of drugs that are off-patent could increase efficacy in terms of antifungal drug discovery. This diverges from the past approaches which traditionally focused on genome mining and structural biology. A notable example of one of the successes cited by Robbins, Spitzer, Yu et al. was the use of a compound library together with existing known antifungals for diverse fungi species, using sublethal dose concentrations. It enabled the effective discovery of clofazimine, an antimycobacterial which had an effective antifungal activity that was unreported in the past. This could then be synergized with posaconazole and caspofungin against Aspergillus fumilgatus and candida albicans.

5. Critical evaluation of current perspectives and other challenges

According to a review conducted by Hsu, Tamma, and Fisher in 2022 among aspergillosis patients, one of the key challenges is being immunocompromised. This applies to both adults and children but has been more prominently reviewed in terms of mortality and morbidity rates among immunocompromised children. This is a factor that drug development would need to take into consideration, in comparison to other existing treatments.

Based on the 2022 review, the lack of sufficient analysis on drug treatments for pediatric dosing and children's tolerance level remains a key issue that any new drugs have to be taken into account. Newer agents are also recommended to consider safe dosing guidelines for children and adults, including pediatric treatment of invasive aspergillosis. Current drugs which are in use include combination antifungal therapy, echinocandins, liposomal amphotericin B, isavuconazole, posaconazole, and voriconazole. However, there remain some knowledge gaps for optimum doses for both adults and children, given the high levels of potential nephrotoxicity that can result in terms of side effects.

For future drug development, a key challenge that has been identified is the presence of invasive aspergillosis among patients suffering from acute leukemia, out of which ≥10% have been identified as children. These include allogeneic hematopoietic cell transplant recipients and patients affected by graft versus host disease (GVHD) [14]. High risk for invasive aspergillosis is also among those taking immunosuppressant drugs, and drug manufacturers would have to take this into consideration in terms of possible contraindications that could decrease the efficacy of drugs.

One possible approach is a pre-emptive form of antifungal drug treatment through diagnostics the moment that there have been elevated fungal biomarkers detected, even prior to a full-blown infection occurring. This could be identified through fungal PCR testing and serum galactomannan combined with radiographic findings. Drugs for pre-emptive driven antifungal therapy for aspergillosis could help improve morbidity rates in the long term and decrease potential fatalities. Furthermore, given at a lower dose, the use of azoles as a form of pre-emptive treatment could decrease the levels of nephrotoxicity.

Diagnostic-driven therapy is also an aspect that can be linked to the drug development pipeline. As early as 2011, experts Sartori, Steinmann, Evers, and Jantzer from McKinsey research have placed an emphasis on a value-driven drug development pipeline, enabling collaboration between commercial and research and development teams from Phase 1 or Phase 2 [15], so as to prove that drugs are effective. For aspergillosis drugs, this could help bring down the cost of drug development and allow new classes of drug treatments and a better rate of return, also providing broad market access for manufacturers. Having the risk-sharing agreements in place much earlier would also involve consulting stakeholders, specifically regulators, health technology assessment bodies, and medical practitioners. This would also mean that for drug companies, antifungals as a form of pre-emptive diagnostic-driven treatment could become more widespread. While the main concern raised for this is the possibility of abuse and *Aspergillus fumigatus* resistance developing, a further argument can be made that these can be resolved through the use of strict and tight controls. A key impetus for this would also be a need to ensure that

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stricter regulatory controls and better education for patients are available. In this way, it could be particularly beneficial for patients, lowering the risk and incidences of full-blown invasive aspergillosis infections, while facilitating the development of drugs that could have a significant effect in the event of any potential pandemic or epidemic for invasive aspergillosis.

6. Recommendations

From Table 1 and the analysis above, it suggests that the antifungal drug discovery pipeline remains but the challenge is in getting the other drugs analyzed in Table 1 to also be included in the clinical trials stages. Without this, four compounds remain in clinical trials as of the review examined by Osherov and Kontoyiannis in 2017, in comparison to 15 possible compounds including the natural products that could also be put in the drug development pipeline in terms of the human clinical trial phase. In order to enable this, researchers need to emphasize the need to consider new forms of drug development rather than relying on the existing number of drugs that have been in place for an extended period of time, especially since the 1950s, yet continue to have potentially high levels of nephrotoxicity or increased cost. Any new drug development requires low toxicity and a wide therapeutic index, possible oral and intravenous administration, and a broad spectrum.

Another consideration is manufacturers' profitability, as having a market which is too "niche" and only targeting *Aspergillus fumigatus* is unlikely to be commercially viable and may then not enjoy a sufficient competitive advantage as new drugs compared to the old ones. This could then serve as a disincentive for manufacturers due to the small size of the market and limited profitability. An economic model needs to be developed, where price and sales volume are disassociated from one another or funding needs to be provided by governments. It is also of ultimate importance that resistance does not continually threaten the shelf life of existing drugs, which can only be prevented through the overuse of new compounds and by taking special care to engage in thoughtful integration.

Hence, in order to tackle the possible bottleneck in the drug development timeline, the solutions proposed also include educating people on using existing antifungals responsibly. This can make a significant difference in preventing further drug resistance. In a manner of speaking, the drug development pipeline needs to be 'refilled', and this should ideally be done prior to the development of any kind of further resistance which could occur in the meantime. Based on past research and literature reviews, this seems to be the most viable approach to be undertaken on a short-term foreseeable basis. In order to effectively manage aspergillosis, a number of other individuals and groups need to be consulted: funding agencies, health economists, policymakers, industry, basic scientists, and clinical mycologists. Such funding would include The Binational Science Foundation, which has previously provided a study grant for earlier studies on aspergillosis. Through synergistic efforts, this would then facilitate what has been termed 'rational drug development' in order to manage aspergillosis and prevent possible epidemics.

7. Conclusion

The growing threat to lung health posed by *Aspergillus fumigatus* is highlighted in this research, especially for those who are immunocompromised. Analysis has been done on the difficulties encountered in developing drugs to treat *Aspergillus fumigatus*, including drug resistance and reinfection. According to the study, immunotherapy together with a variety of medications may be a useful strategy for treating aspergillosis lung infections. However, given the disease's potential to spread like wildfire in the future, more financing and research are required to combat it more successfully. There is a need for further investigation into certain medication combinations and immunotherapeutic techniques in the future. A more thorough investigation of the underlying causes of medication resistance and reinfection would also be beneficial to the article. Future studies should concentrate on examining different therapeutic modalities, looking at potential pharmacological targets, and developing diagnostic techniques for early recognition of *Aspergillus fumigatus* infections.

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