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ASP2397: A Novel Natural Product with Potent Fungicidal Activity against *Aspergillus* spp. (2) - *In Vivo* Activity against *A. fumigatus*

I. Nakamura, T. Nakai, S. Matsumoto, S. Takeda, S. Akamatsu, S. Uchida, Y. Koide, H. Mitori, T. Noto, and K. Maki
Astellas Pharma. Inc., Tsukuba, Japan

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Astellas Pharma Inc.
Phone: +81-29-829-6082
ikuko.nakamura@astellas.com

Abstract

Background: Therapeutic efficacy of existing agents against invasive pulmonary aspergillosis (IPA) is still insufficient. ASP2397 (ASP) is an injectable agent with a novel mechanism that is actively incorporated into *A. fumigatus* through a membrane transporter. ASP has potent and rapid fungicidal activities against major *Aspergillus* species, *A. fumigatus*, *A. terreus*, *A. flavus*, and *A. nidulans* in vitro. Here, in vivo antifungal activities of ASP were examined in an azole refractory IPA mice model.

Methods: Therapeutic activities against IPA caused by *A. fumigatus* were studied in a neutropenic mouse model generated by treatment with cyclophosphamide. Therapies were either initiated at 6 hr. post infection (p.i.) in the azole sensitive model or on day 1 p.i. to make the model refractory to azole[1]. Posaconazole (PSCZ) was employed as a reference compound because voriconazole (VRCZ) is metabolized rapidly in rodents[2] .

Results: In the azole sensitive mice IPA model of *A. fumigatus*, both 4 mg/kg of ASP (BID) and 10mg/kg of PSCZ (BID) attained 100% survival on day 10 p.i., whereas in the azole refractory IPA model, PSCZ had a modest survival effect (40%) and did not display fungicidal activity or pathological improvement against lung fungal burden of *A. fumigatus*. ASP had superior efficacy with 100% survival and potent fungicidal effect with over 1 $\log_{10}$ cfu/g·lung reduction in the azole refractory IPA model. Histopathological investigation showed that ASP remarkably suppressed disease progression in lung tissue (inflammation/necrosis, hemorrhage and diffusely elongated hyphae). ASP was also effective in an IPA model of azole-resistant *A. fumigatus*, and in combination therapy to compensate for the relatively narrow antifungal spectrum of ASP, ASP augmented the moderate efficacy of PSCZ in the azole refractory IPA model of *A. fumigatus* comparable to ASP mono therapy and PSCZ did not antagonize the antifungal activity of ASP.

Conclusion: ASP has therapeutic potential to improve clinical efficacy for IPA due to excellent in vivo anti-*Aspergillus* activities and reduction in histopathological damage in an animal model, and this may translate to improved efficacy versus standard of care in a clinical setting.

Introduction

- IPA is a major cause of mortality in severely compromised hosts and *Aspergillus fumigatus* causes most case of IPA.
- Newer existing antifungals for IPA are classified into appropriate indications: voriconazole (VRCZ): first choice, posaconazole (PSCZ): prophylaxis or salvage therapy, liposomal amphotericin B (L-AMB): empiric therapy, caspofungin (CAS): second line therapy. However, the efficacy of these drugs for IPA including azole-resistant *Aspergillus* is still insufficient.
- Early therapy is the key factor for successful outcome for IPA. In an advanced rat model, delayed therapy of VRCZ and amphotericin B (AMPH-B) revealed low survival efficacy[1].
- ASP2397 has potent and rapid fungicidal activities in vitro against *Aspergillus* spp. with a novel mechanism (F1590).
- In vivo efficacy of ASP2397 for advanced IPA in an azole refractory model was examined to reveal potential values in clinical setting.
- PSCZ was employed in place of VRCZ, which is metabolized rapidly in rodents[2].

Methods

IPA model: ICR mice treated with 200mg/kg ip cyclophosphamide on days -4 &+1 were intratracheally challenged with *A. fumigatus* conidia on day 0.
Azole sensitive model: Treatments were initiated at 6hr. p.i. and treated for 4 days.
Azole refractory model: Treatments were initiated on day 1 p.i. to establish the advanced IPA and treated for 2 to 3 days.
Treatment: ASP2397 and PSCZ were treated BID subcutaneously and orally, respectively. AMPH-B, L-AMB, and CAS were treated with intravenously QD.
Early fungicidal effects: On day 3 p.i. after two days treatments of the agents, lung fungal burden were determined.
Histopathology: The mice were treated for 3 days. On day 3 p.i. (control and PSCZ) and day 4 p.i. (ASP), lung tissues were stained with hematoxylin and eosin.
Statistical significance: Using the log-rank test under a closed testing procedure in survival and Dunnett's multiple comparison test in fungal burden.

Results

Fig.1. *In vivo* treatment model simulating refractory IPA

Hypothesis: After germination of *A. fumigatus* conidia in patients, antifungal treatment may be less effective
Concept: ***Delayed treatment*** allowing germination of conidia before treatment initiation results in ***azole-refractory***

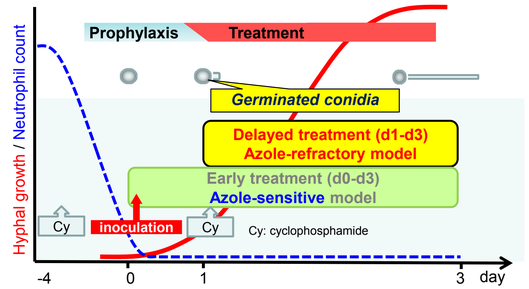
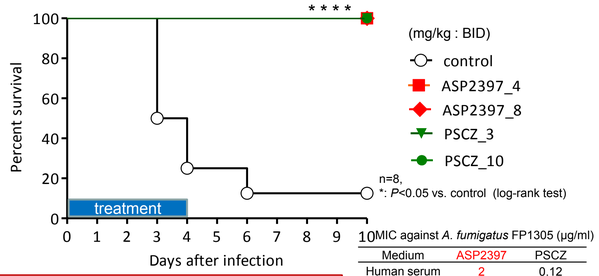


Fig.2. Survival efficacy against azole-sensitive IPA mice model of *A. fumigatus*

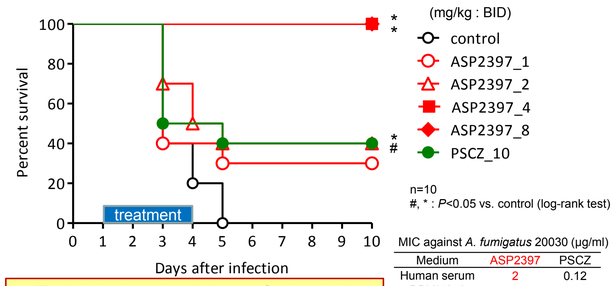


MIC against *A. fumigatus* FP1305 (μg/ml)

Medium	ASP2397	PSCZ
Human serum	2	0.12
RPMI1640	0.12	0.12

- ASP2397 and PSCZ attained 100% survival in the azole sensitive model.

Fig.3. Survival efficacy against azole-refractory IPA mice model of *A. fumigatus*

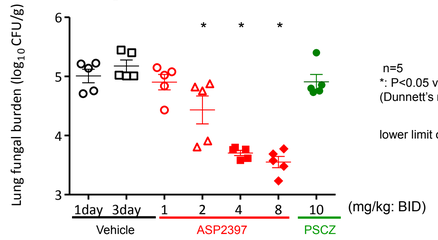


MIC against *A. fumigatus* 20030 (μg/ml)

Medium	ASP2397	PSCZ
Human serum	2	0.12
RPMI1640	0.5	0.25

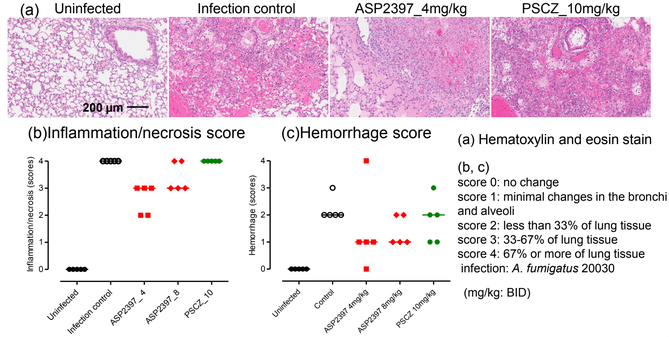
- The late treatment model was refractory to the azole drug.
- ASP2397 (4 and 8 mg/kg BID) demonstrated 100% survival rate despite of reduction of survival effect of PSCZ .

Fig.4. Fungicidal effect against lung fungal burden in azole-refractory IPA mice model of *A. fumigatus* 20030



- ASP2397 (2, 4 and 8 mg/kg, BID) also exhibited significant fungicidal effects against lung fungal burden in the advanced IPA.
- PSCZ (10 mg/kg, BID) did not reduce the lung fungal burden and showed less survival effect as compared with the early treatment model as indicated in Fig. 3.

Fig.5 Lung histopathology in the azole-refractory IPA mice model



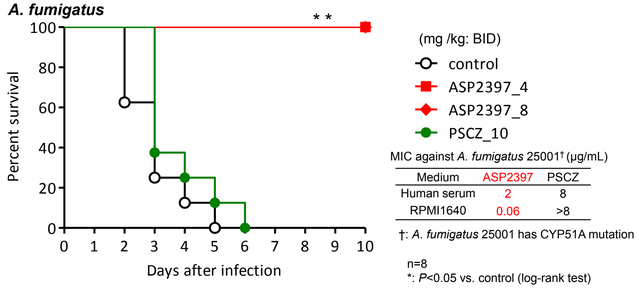
(a) Hematoxylin and eosin stain

(b, c)
score 0: no change
score 1: minimal changes in the bronchi and alveoli
score 2: less than 33% of lung tissue
score 3: 33-67% of lung tissue
score 4: 67% or more of lung tissue
infection: *A. fumigatus* 20030

(mg/kg: BID)

- ASP2397 relieved progress in inflammation/necrosis and hemorrhage in lung caused by *A. fumigatus*.
- PSCZ had little effect on injury in the lung in this study.

Fig.6 Survival efficacy against azole-refractory IPA mice model of azole-resistant *A. fumigatus*



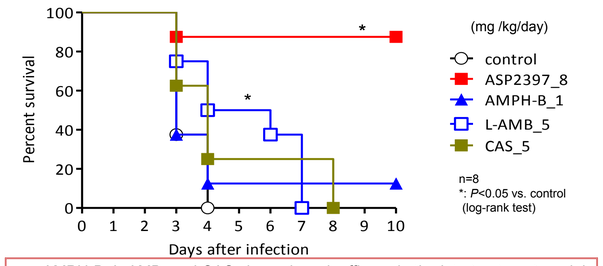
MIC against *A. fumigatus* 25001† (μg/mL)

Medium	ASP2397	PSCZ
Human serum	2	8
RPMI1640	0.06	>8

†: *A. fumigatus* 25001 has CYP51A mutation

- ASP2397 was effective against the azole-resistant *A. fumigatus* even in the late treatment model.

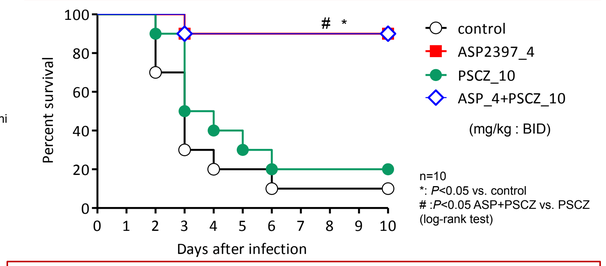
Fig.7 Comparison of ASP2397 with polyenes and echinocandin for late treatment IPA mice model of *A. fumigatus* 20030



- AMPH-B, L-AMB, and CAS showed weak efficacy in the late treatment model
- ASP2397 had a potent survival effect.

Fig.8 Combined effect of ASP2397 and PSCZ in the azole-refractory IPA mice model of *A. fumigatus* 20030

The purpose of combination therapy is:
1. To compensate the narrow spectrum ASP2397
2. Not to reduce dosage but to integrate each antifungal effect at maximum dosages



- ASP2397 had excellent survival efficacy either alone or in combination therapy with PSCZ.
- PSCZ did not antagonize in vivo antifungal effect of ASP2397 in the combination therapy.

Conclusions

ASP2397 has

- Superior survival and early fungicidal effects compared with the existing drugs in azole refractory IPA mice model including azole resistant *A. fumigatus* strain.
- Suppression effects of disease progression in lung tissue of the refractory IPA mice model.
- No antagonism in vivo in combination with existing azole, PSCZ. This can be of great value in the setting of empiric or preemptive therapy where a causative pathogen has not been identified.
- Therapeutic potential to improve mortality of IPA by mono or combination therapies in a clinical setting.

References

1. van de Sandle et. al., Antimicrob Agents Chemother. 2009;53(5):2005-13
2. Roffey et al., Drug Metab Dispos. 2003; 31(6):731-31