A-015

Preclinical Evaluation Shows CD101, a Novel Echinocandin, is Highly Stable with No Hepatotoxicity in Rats

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ABSTRACT

Background: CD101 shows robust efficacy in mouse antifungal models. In preclinical evaluation, studies were conducted to compare the toxicity of CD101 to anidulafungin (ANID), with focus on assessing hepatotoxicity at comparable exposures.

Methods: Metabolic stability and CYP activity were determined with human liver microsomes. Protein binding of CD101 in plasma and dose range toxicity/PK were determined. SD rats (10/sex/group) were administered CD101 (0, 2, 6, and 20 mg/kg) or ANID (40 mg/kg) by IV infusion via the tail vein. Clinical signs, chemistries, hematology, and liver histopathology were studied. Reactive species screening was performed by incubation of CD101 or ANID in PBS at 37°C for 2 h in the presence of 5 M excess of glutathione (GSH). Samples were analyzed using high resolution LC/MS with concurrent low and high energy scans to obtain full scan parent and product fragment ions.

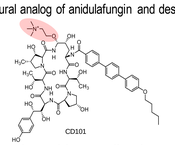
Results: CD101 was stable to biotransformation in liver microsomes and hepatocytes and during in vivo studies. In vitro CYP inhibition studies suggest minimal interaction with recombinant CYP enzymes with IC_{50}s >10 µM. Similar to ANID, CD101 bound avidly (>98%) to human, mouse, rat, and primate plasma proteins. In a 2-wk rat hepatotoxicity screen, animals treated with CD101 did not exhibit effects on BW, hematology, coagulation or urinalysis. No microscopic liver findings were observed for CD101 at all doses. ANID at 40 mg/kg gave similar plasma levels as 20 mg/kg of CD101. At this dose, ANID treatment resulted in reduced BW, decreases in RBC, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, platelet and reticulocyte counts, increases in neutrophil and eosinophil counts, polychromasia and decreased aPTT. Elevated ALT, AST, total bilirubin, cholesterol and globulin, dark and enlarged spleens and single cell hepatocyte necrosis were also observed for ANID. We hypothesized that hepatotoxicity may be due to the inherent chemical lability of ANID generating potentially reactive intermediates. A GSH trapping experiment confirmed the presence of a reactive species for ANID whereas CD101 did not exhibit instability or any reactive intermediate.

Conclusions: Preclinical studies demonstrated CD101 was metabolically stable and was devoid of hepatotoxicity at doses up to 20 mg/kg when dosed in rats for 2 weeks.

INTRODUCTION

CD101 is a novel echinocandin antifungal under development as an IV formulation to treat serious fungal infections. It was designed to be highly stable[1] and exhibits a longer half-life and lower clearance compared with other echinocandins in multiple species.[2,3]

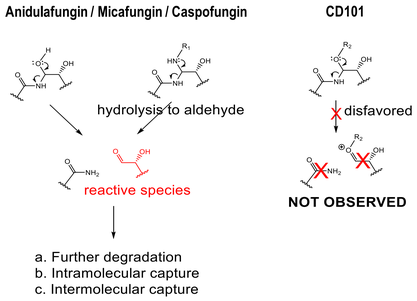
Figure 1. CD101 is a structural analog of anidulafungin and designed to be stable.



Since their introduction, the echinocandins have become increasingly important as antifungals. However, nonclinical toxicology studies for the previous echinocandins have reported hepatotoxicity. A major hypothesis for echinocandin-induced hepatotoxicity is chemical and/or metabolic instability which can generate potentially reactive intermediates. CD101, unlike previous echinocandins, is stable both chemically and metabolically and, therefore, does not generate any reactive intermediates.

INTRODUCTION (cont'd)

Figure 2. Proposed reactive intermediates from degradation of previous echinocandins.



METHODS

- **Metabolic stability:** CD101 at 1 µM was incubated with liver microsomes/hepatocytes from different species for up to 2 hours.
- **Protein binding:** Ultracentrifugation was used to compare human plasma protein binding of CD101 and ANID; cross-species protein binding at 1 µM were also determined by equilibrium dialysis.
- **CYP450 inhibition:** Initial screening using recombinant enzyme assay at 10 µM; further IC_{50} characterization for 2C8 and 3A4 using human liver microsomes with definitive substrates.
- **Pharmacokinetics:** CD101 plasma concentration-time profiles were studied in mice, rats, dogs, cynomolgus monkeys, and chimpanzees following IV administration with analysis of plasma samples by LC-MS/MS.
- **Reactive metabolite screening:** Studied by LC-HRMS detection following incubation with glutathione (GSH) in PBS.
- **Rat hepatotoxicity screening:** Toxicology and hepatic histopathology of CD101 and ANID were compared when administered as 20-minute IV infusions in SD rats for 2 weeks.
- **IND-enabling studies:** Full battery of safety pharmacology and toxicology studies up to 4-weeks as well as genetic toxicology studies were conducted.

RESULTS

Figure 3. CD101 was stable when incubated across different species' hepatocytes.

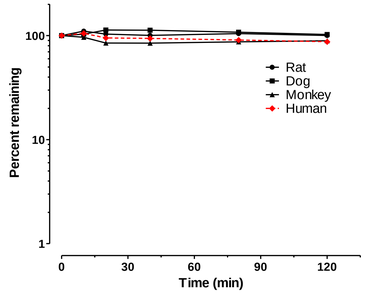


Table 1. Human plasma protein binding comparable to anidulafungin. CD101 also shows same protein binding values across different animal species.

	Concentration (µg/mL)	% Protein Bound (SEM)
CD101	1	98.0 (0.8)
	5	98.3 (0.5)
ANID	1	97.8 (0.8)
	5	98.6 (0.5)

Table 2. CYP450 inhibition was initially screened at 10 µM in incubations of fluorogenic substrates with recombinant CYPs (1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) and 2C8 as well as 3A4 isoforms were further characterized using human liver microsomes to determine definitive IC_{50} values below.

Cytochrome P450 Isoform	IC_{50} (µM)
2C8 (amodiaquine substrate)	25.7
3A4 (midazolam substrate)	29.0
3A4 (testosterone substrate)	>30

Table 3. PK of CD101 and ANID across different animal species; lower clearance of CD101 compared with ANID determined from the same study.

	Rat	Dog	Monkey	Chimp
Anidulafungin				
Cl (mL/h/kg)	64	47	302	25.2
Vz (L/kg)	1.7	0.83	0.8	1.1
$t_{½}$ (h)	22	12	8	30
CD101				
Cl (mL/h/kg)	44	19	18	3.4
Vz (L/kg)	1.7	1.3	0.9	0.4
$t_{½}$ (h)	30	53	40	90

RESULTS (cont'd)

Figure 4. Comparative liver histopathology for CD101 and ANID from 2-week rat hepatotoxicity screening study.

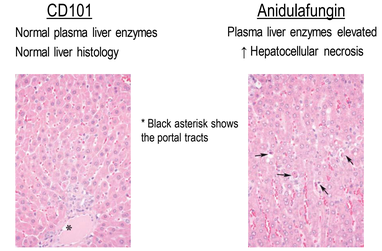


Figure 5. Evidence of reactive intermediate formation from ANID degradation using GSH trapping following 24-hr incubation in PBS.

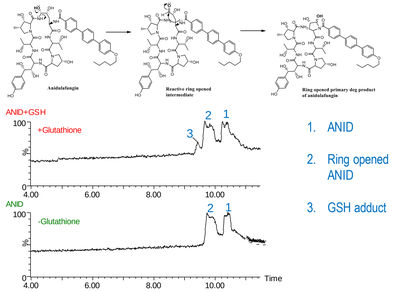
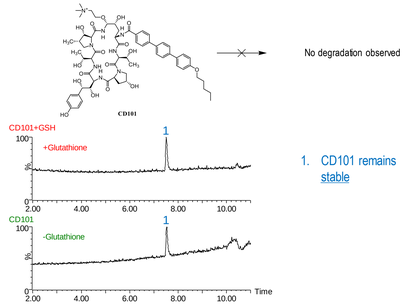


Figure 6. CD101 showing excellent stability following 24-hr incubation in PBS.



RESULTS (cont'd)

Table 4. CD101 was evaluated in a full battery of IND-enabling safety pharmacology/toxicology studies up to 4 weeks in duration in rats and cynomolgus monkeys and in bacterial and mammalian cell in vitro genetic toxicology studies; no CD101-induced systemic adverse microscopic tissue injury in either rats or monkeys, particularly for target organs previously shown to be important for human risk assessment with echinocandins (e.g., liver).

Study Type / Duration	CD101 Summary Findings
GLP mutagenicity	No evidence of mutagenicity
GLP chromosomal aberrations	No evidence of clastogenicity
GLP safety pharmacology	No CNS, respiratory/cardiovascular concern
GLP 4-week study in rats	NOAEL after repeat-dose was 34x animal efficacious plasma exposure
GLP 4-week study in cynomolgus monkeys	NOAEL after repeat-dose was 47x animal efficacious plasma exposure

CONCLUSIONS

- CD101 is highly stable chemically and metabolically.
- No interaction with CYP enzymes – i.e., no biotransformation.
- No evidence of reactive intermediate formation.
- Low clearance in vivo – i.e., plasma half-life.
- No evidence of hepatotoxicity after repeated administration.

REFERENCES

1. ICAAC 2014, Poster F-1592.
2. ICAAC 2014, Poster A-693.
3. ICAAC 2014, Poster A-694.

ACKNOWLEDGEMENTS

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