

# Evaluation of a New Host-Derived Synthetic Antifungal Peptide (PAC-113) in the Treatment of Oral Candidiasis

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## ABSTRACT

**Background:** Oral candidiasis (OC) is a frequently encountered opportunistic fungal infection for which only limited therapies are available. PAC-113 is a novel, 12 amino acid fragment of histatin 5, a natural component of human saliva, and has shown potent *in vitro* antimicrobial activity. In a recently completed Phase I/II study in 106 HIV positive subjects, PAC-113 (0.15% in a mouthrinse formulation) demonstrated clear activity in the treatment of OC comparable to nystatin (complete response rates of 44.4% vs 40%). Since the antimicrobial activity of cationic peptides has been shown to be sensitive to the presence and concentration of certain ions, the aim of this study was to investigate whether the antifungal activity of PAC-113 could be potentiated based on ion molarity changes to the clinical formulation.

**Methods:** PAC-113 was formulated in either 50 mM (prior clinical formulation) or 12.5 mM of sodium acetate at concentrations of 0.0375, 0.075 or 0.15% (w/v). The *in vitro* fungistatic / fungicidal activities of the PAC-113 solutions against *C. albicans* were compared to control samples without PAC-113 (n = 6).

**Results:** All PAC-113 formulations displayed an excellent dose-dependent growth inhibitory effect against *C. albicans* with a very low IC<sub>50</sub> value of 0.00015%. Cell killing was far more pronounced with the low ion molarity (12.5 mM) PAC-113 solutions resulting in a 2.43±0.48 to 2.74±0.48 log reduction in viable counts, whereas the high ion molarity (50 mM) solutions caused a 0.28±0.22 to 0.50±0.25 log reduction.

**Conclusion:** The *in vitro C. albicans* cell killing data suggest that the antifungal activity of PAC-113 can be increased dramatically when the drug is formulated with lower ion molarity. The current follow-up, Phase II, clinical dose-ranging study with the improved formulation based on the obtained *in vitro* data may translate into increased clinical efficacy in the treatment of OC.

## INTRODUCTION

Histatins are naturally occurring antifungal proteins in human saliva. The major histatins being histatin 1, 3 and 5.

Major Histatins	Sequence
Histatin 1	D S H E K P R H H G A P P R K K E H K H H S H R E F F Y G D Y G S N V L Y D N
Histatin 3	D S H A K R R H H G Y K R K F H K H H S H R . . . . . G . Y R S N V L Y D N
Histatin 5	D S H A K R R H H G Y K R K F H K H H S H R . . . . . G . Y . . . . .

## PAC-113

- PAC-113 is the small peptide domain spanning 12 amino acids present in histatins 3 and 5 that comprises the fungicidal activity.
- In a Phase I/II clinical study in 106 HIV positive subjects, PAC-113 (0.15%, formulation molarity 50mM) demonstrated potent activity in the treatment of OC comparable to nystatin with complete response rates of 44.4% vs. 40%, respectively.
- Histatin and PAC-113 antifungal activity are negatively impacted by the presence of salts.

## AIM

To investigate whether the antifungal activity of PAC-113 could be potentiated based on ion molarity changes to the clinical formulation.

## METHODS

### Evaluation of Antifungal Activity

#### 1. Trypan Blue Exclusion Assay

*C. albicans* (ATCC 10231) cells were cultured to log phase in low ionic strength fungal growth medium, harvested, exposed to trypan blue, followed by microscopic evaluation of dye exclusion as a measure for cell integrity.

#### 2. Growth Inhibition Assay

*C. albicans* (ATCC 10231) cells were cultured as described above. In a microtiter plate serial dilutions of PAC-113 solutions were prepared in the same growth medium. Cells were added (10<sup>6</sup> cells/ml) and incubated for 24 hr at 30°C after which the OD at 620 nm was measured to assess fungal growth.

#### 3. Killing Assay

*C. albicans* (ATCC 10231) cells were cultured as above, harvested and resuspended in a small volume of water. From this suspension, a 20 µl aliquot was added to 1 ml of PAC-113 formulation (approx 10<sup>6</sup> cells/ml). Suspensions were incubated for 1.5 hr at 37°C, diluted in PBS, plated and counted after 48 hr of incubation to determine the % killing and the log reduction in viable counts.

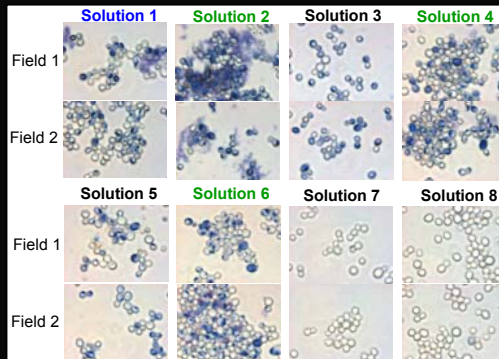
Table 1. PAC-113 solutions used in this study (blinded until completion)

Solution #	PAC-113	Formulation Molarity
1*	0.15% (=1500 µg/ml)	50 mM
2 <sup>^</sup>	0.15% (=1500 µg/ml)	12.5 mM
3	0.075% (=750 µg/ml)	50 mM
4 <sup>^</sup>	0.075% (=750 µg/ml)	12.5 mM
5	0.0375% (=375 µg/ml)	50 mM
6 <sup>^</sup>	0.0375% (=375 µg/ml)	12.5 mM
7	0	50 mM
8	0	12.5 mM

\* prior clinical formulation; <sup>^</sup> current clinical formulation; # patent pending

## Results

### Trypan Blue Exclusion Assay:



## RESULTS

### Growth Inhibition Assay:

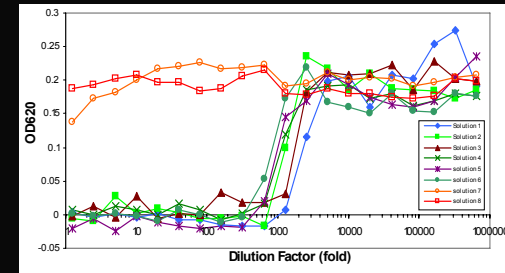


Table 2: IC<sub>50</sub> values

Solution #	IC <sub>50</sub> (dilution)
1	1/2100
2	1/1300
3	1/1800
4	1/1000
5	1/900
6	1/800
7	No activity
8	No activity

### Killing Assay:

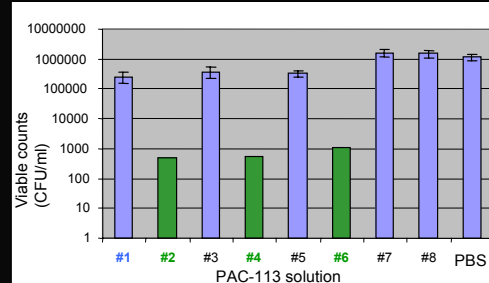


Table 3: Cell killing (percent and log reduction)

Solution #	Cell Killing	
	%	Δ log
1	74 ± 10	0.5 ± 0.3
2	99.7 ± 0.3	2.7 ± 0.5
3	64 ± 10	0.4 ± 0.2
4	99.9 ± 0.1	2.8 ± 0.4
5	52 ± 10	0.3 ± 0.2
6	99.7 ± 0.3	2.5 ± 0.5
7	0	0
8	0	0

## CONCLUSIONS

- All PAC-113-containing formulations are effective in inhibiting the growth of *C. albicans*, showing dose-dependent inhibition curves.
- Reduction of the clinical formulation ion molarity to ¼ of its original concentration clearly potentiates the fungal cell killing efficacy of PAC-113.
- The current follow-up, Phase II, clinical dose-ranging study with the improved formulation based on the obtained *in vitro* data holds great promise for increased clinical efficacy in the treatment of oral candidiasis.