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## Background

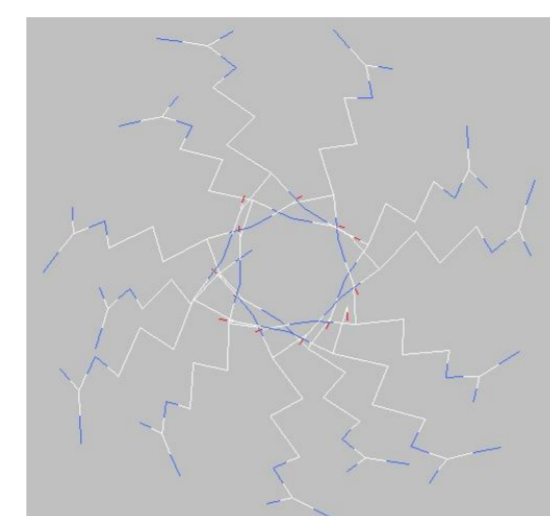
There is a clear clinical & economic need for improved therapeutic options (safety, efficacy, resistance) for the increasing problem posed by systemic *Candida* spp. infections. To address this, NovaBiotics has developed NP339, a fast-acting candidacidal cationic peptide active against *Candida* species, including examples known to be insensitive/resistant to azoles.

## Methods

Antifungal susceptibility testing on >200 *Candida* spp. clinical isolates and other clinically relevant yeasts and fungi was performed using CLSI Approved Standards M27-A3 & M38-A2. The impact of physiological salt concentrations and serum was determined, as was the *in vitro* haemolytic & cytotoxic potential of NP339. Tolerability and activity of NP339 *in vivo* was determined in a murine model of acute candidiasis in which peptide in native & PEGylated forms were administered intravenously 3 h post-*Candida* spp. challenge and yeast burden determined 24 h post challenge.

## Results

In all cases, *Candida* spp. isolates, including isolates known to be insensitive/resistant to azoles were rapidly killed ( $\leq 30$  min), MIC<sub>100</sub> range 1-512  $\mu\text{g/ml}$ , median 2  $\mu\text{g/ml}$ ) following exposure to NP339. NP339 was also active against other clinically relevant yeasts & mould including *Aspergillus* spp., *Fusarium* spp. & *Cryptococcus* spp. The primary mode of action of NP339 is membranolysis. No resistance (acquired or spontaneous) was observed (>60 passages). Activity of NP339 was not significantly inhibited by physiological salt concentrations or serum *in vitro*. Parenterally administered peptide was well tolerated *in vivo*. PEGylated-NP339 successfully reduced fungal burden in a murine model of acute candidiasis (5 mg/kg).

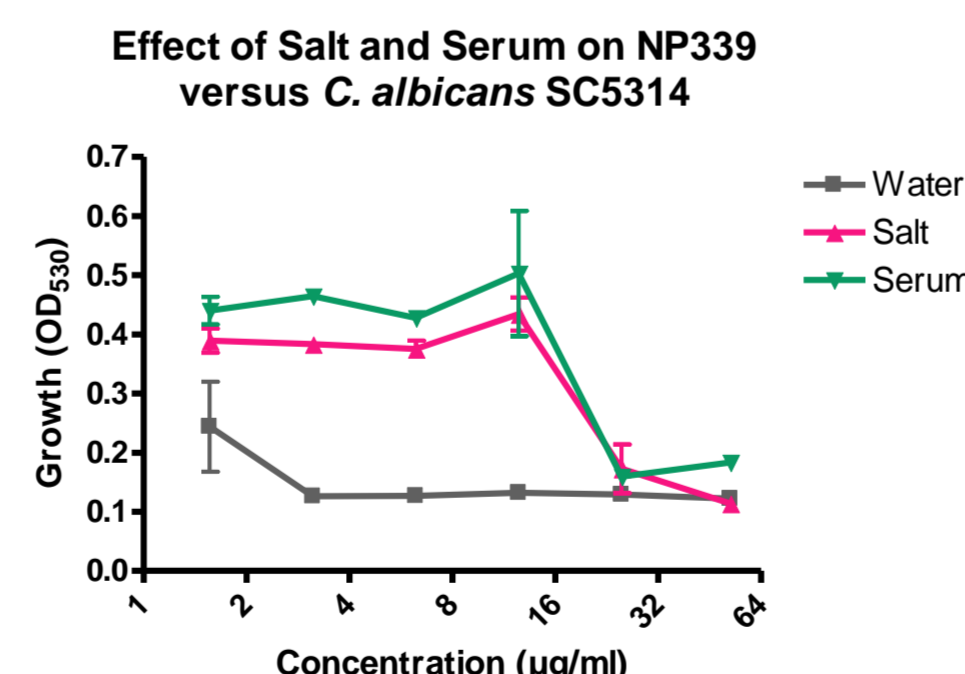


NP339 is an arginine-rich linear peptide (2,048 Da).

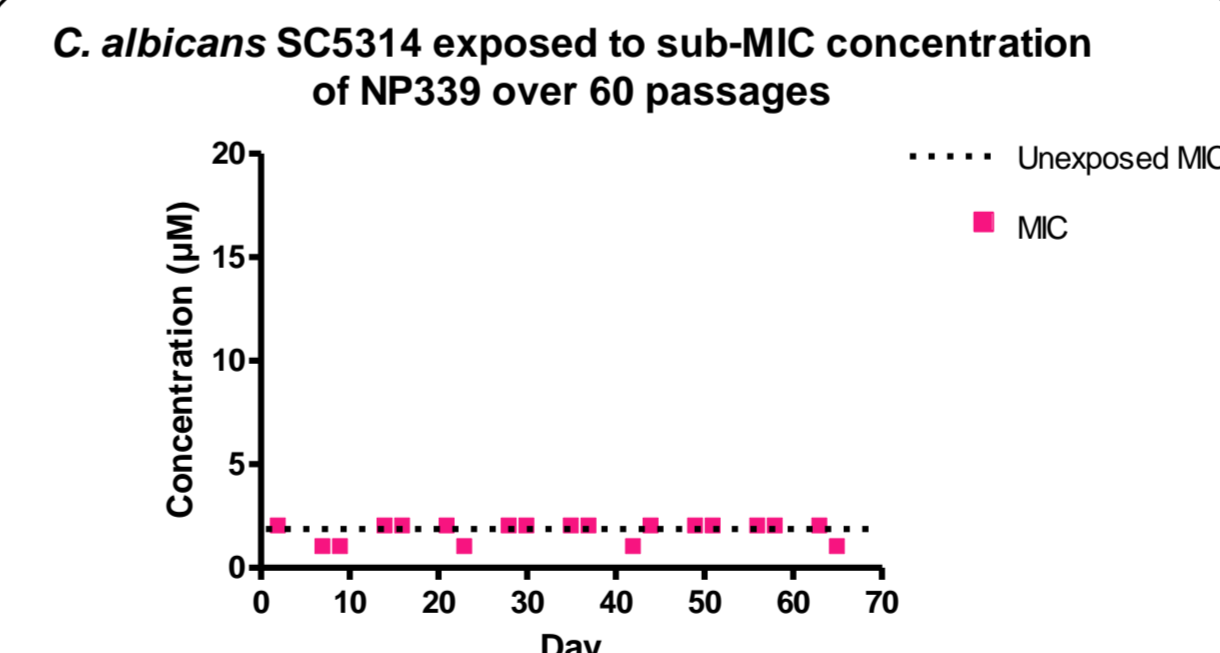
The antifungal susceptibility profile of >80 clinical isolates of *Candida* spp. was determined using CLSI Approved Standard M27-A3 in polystyrene plates.

SPECIES	NUMBER OF STRAINS	MIC <sub>100</sub>
<i>Candida albicans</i>	24	1 - 8
<i>C. parapsilosis</i>	21	1 - >512
<i>C. glabrata</i>	17	1 - 2
<i>C. tropicalis</i>	14	1 - 2
<i>C. krusei</i>	12	1 - 2

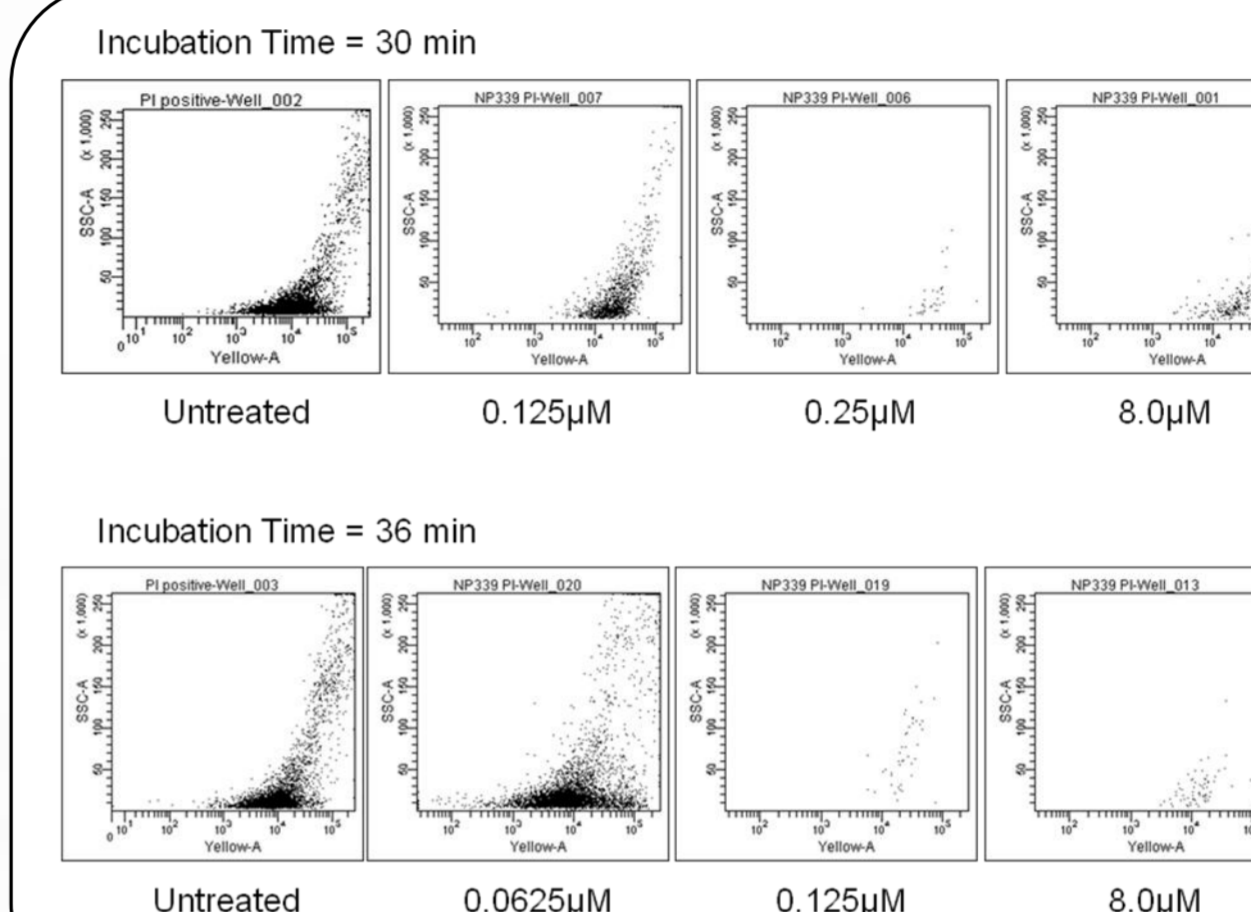
MICs (100% kill) ranged from 1 - >512  $\mu\text{M}$ .



Minimum inhibitory concentration of NP339 were tested against *C. albicans* SC5314 in the presence of physiological salt or serum concentrations.



*C. albicans* SC5314 was exposed to sub-MIC concentrations of NP339 over 65 passages. No increase in MIC was seen over this time demonstrating no increased resistance to NP339.

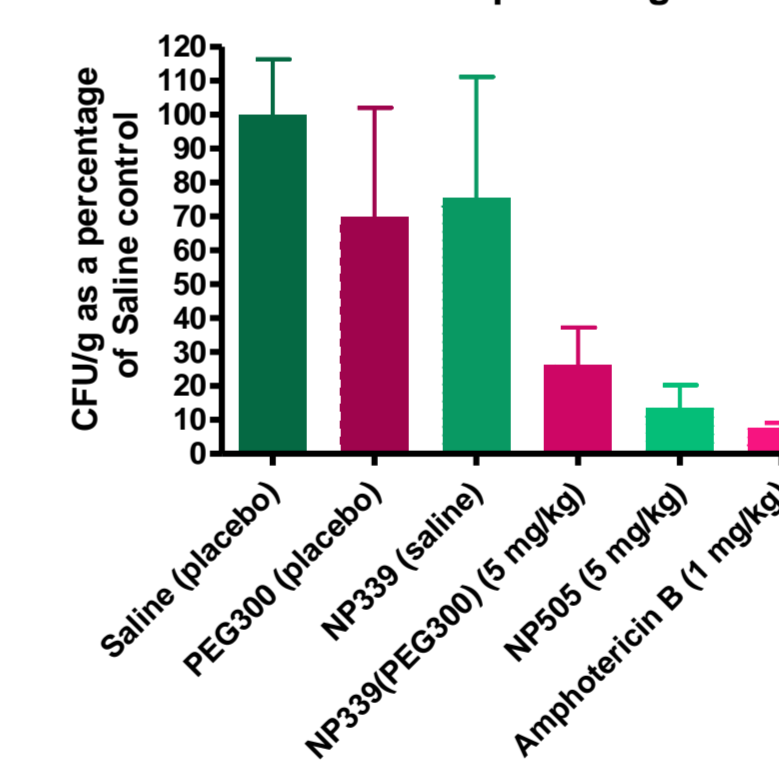


The fungicidal mode of action and rapid time of kill of NP339 was demonstrated by flow cytometric analysis of *C. albicans* SC5314.

Cells were exposed to 0, 0.0625, 0.125, 0.25 and 8.0  $\mu\text{M}$  NP339 for 30 min or 36 min and the scatter plots shown here demonstrate that almost all *C. albicans* SC5314 cells are killed (no detectable events because of lysis of the *C. albicans* SC5314 cells) after 30 - 36 min exposure to  $\geq 0.125$  mM NP339.

The MIC of NP339 is 0.125  $\mu\text{M}$  after 30 min exposure and 0.0625  $\mu\text{M}$  after 36 min exposure. This correlates with the MIC<sub>100</sub> values obtained when the antifungal activity of NP339 was determined by broth microdilution.

Fungal burden (CFU/g as percentage of saline control) *C. albicans* SC5314 24 h-post fungal challenge



Tolerability and activity of NP339 *in vivo* was determined in a murine model of acute candidiasis.

BALB/c mice were infected IV with *C. albicans* SC5314 at a challenge dose of  $2 \times 10^4$  viable yeasts/g mouse body weight. Mice were given a single injection of placebo, NP339(PEG) (IV) or amphotericin B (IP) at 3 h post-infection. Twenty-four hours after infection (21 h post-treatment) kidney burden of *C. albicans* was determined by viable counting.

A single dose of NP339 in the presence of PEG300 successfully reduced fungal burden in a murine model of acute candidiasis. Mice tolerated a dose of NP339 of 10 mg/kg.

## Conclusions

NP339 is an effective, novel fungicidal cationic antimicrobial peptide & a promising candidate for the treatment of *Candida* spp. & other serious yeast and mould infections. NP339 will be formulated for both intravenous & oral delivery. Further research shall assess the suitability of NP339 for co-administration with other antifungals.