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NP339; A Therapeutic Candidate for Respiratory Fungal Infection

Daniel Smith, Laura Simpson, Emma Lovie, Deborah O'Neil

- Chronic and acute fungal infection of the respiratory tract is an increasing health challenge in an expanding group of susceptible patient populations (including cystic fibrosis (CF), non-Tuberculosis mycobacteria, allergic bronchopulmonary aspergillosis, immunocompromised etc.). Acute infections carry a high mortality rate and chronic infections can have significant impact on prognosis and quality of life (e.g., CF patients).

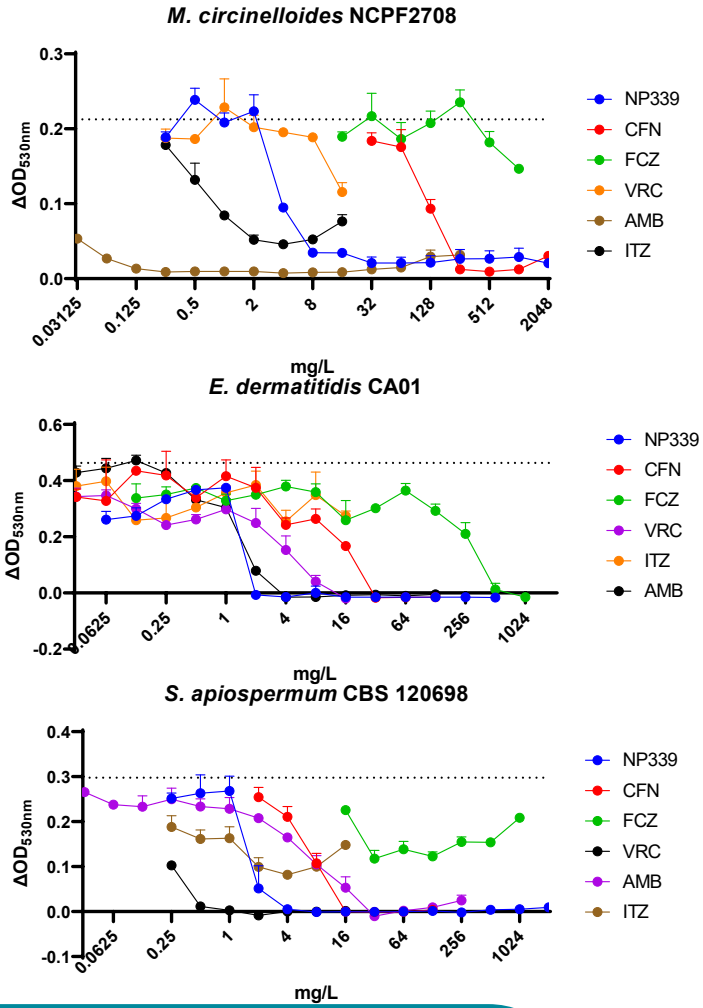
- Current systemic treatment options are limited by well-described, toxicity, drug-drug interaction and efficacy shortcomings.

- NP339 is a novel, cationic, hydrophilic antifungal peptide, ideally suited for contained local delivery via the respiratory route. We present data demonstrating NP339s' broad-spectrum activity against key pathogens associated with respiratory fungal infections, its anti-biofilm activity and *in vivo* efficacy of nebulised NP339 in a murine model of invasive aspergillosis.

NP339 demonstrates broad spectrum activity *vs.* clinically relevant moulds & yeast

Genus	species	Strain	NP339	CFN	FCZ	VRC	ITZ	AMB
Aspergillus	*fumigatus*	ATCC-MYA-3626	4	32	512	0.5	4	2
Rhizopus	*oryzae*	NCPF2504	8	128	512	8	0.5	0.5
Rhizopus	*microsporus*	NCPF2776	8	128	512	8	0.5	0.5
Rhizomucor	*pusillus*	NCPF2265	2	32	512	0.25	0.25	0.25
Mucor	***circinelloides***	**NCPF2708**	8	**128**	**>1024**	**>16**	**>16**	**0.25**
Cunninghamella	*bertholletiae*	NCPF2878	64	>128	>1024	>16	1	2
Absidia	*corymbifera*	NCPF2326	4	128	>1024	>16	0.5	0.125
Exophiala	*jeanselmei*	NCPF2377	64	128	512	<0.5	0.5	1
Exophiala	*dermatitidis*	SP4002	2	NT	NT	NT	NT	NT
Exophiala	*dermatitidis*	SP4493	3	NT	NT	NT	NT	NT
Exophiala	*sp*	SP5884	2	NT	NT	NT	NT	NT
Exophiala	*dermatitidis*	SP5973	2	NT	NT	NT	NT	NT
Exophiala	*sp*	SP6339	20	NT	NT	NT	NT	NT
Exophiala	***dermatitidis***	**CA01**	2	**32**	**512**	**8**	**>16**	**2**
Scedosporium	*apiospermum*	CBS 129968	16	32	32	1	4	4
Scedosporium	*apiospermum*	ATCC-MYA-3634	16	16	>1024	0.5	2	8
Scedosporium	*apiospermum*	ATCC-MYA-3635	64	16	>1024	0.5	1	1
Scedosporium	*apiospermum*	NCPF2869	32	64	>1024	<0.25	0.5	1
Scedosporium	*apiospermum*	CBS 127621	2	8	>1024	0.5	2	0.5
Scedosporium	***apiospermum***	**CBS 120698**	4	**8**	**>1024**	**1**	**4**	**4**

Table 1: Minimum inhibitory concentrations (MIC) of respiratory pathogens against a range of antifungal compounds including NP339. Antimicrobial susceptibility tests carried out following CLSI M38 document. MIC defined as inhibiting 100 % of fungal growth. Representative inhibition curves are presented for select fungi. CFN, caspofungin; FCZ, fluconazole; VRC, voriconazole; ITZ, itraconazole; AMB, amphotericin B



NP339 active *in vitro* despite using standardised testing, not OPTIMISED for membrane acting agents such as antimicrobial peptides

Duncan V, Smith DW, Simpson L, Lovie E, Katvars L, Berge L, Robertson J, Smith S, Munro C, Mercer D and O'Neil D. Preliminary Characterisation of NP339, a Novel Polyarginine Peptide with Broad Antifungal Activity. *AAC*. May 2021.

NP339 is active *vs. E. dermatitidis* biofilm cells

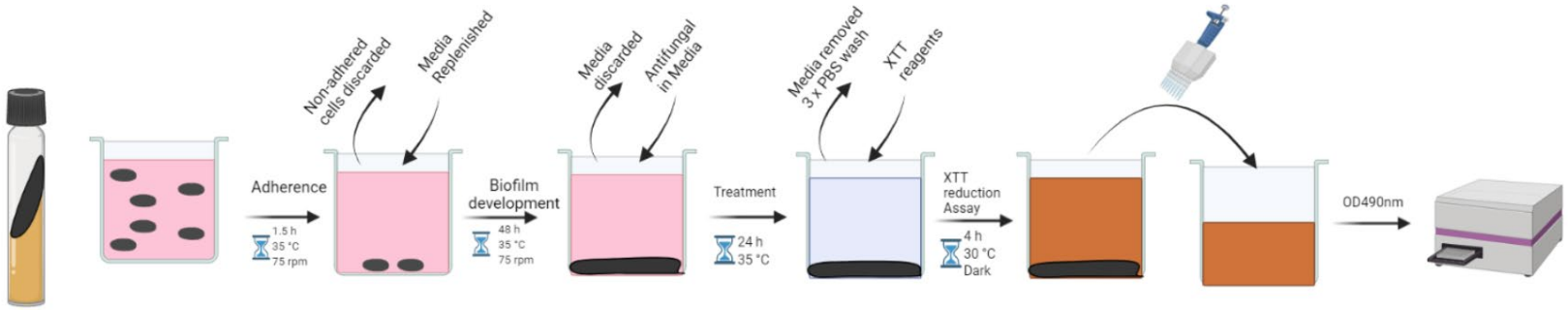


Figure 1: Experimental design for challenge of *E. dermatitidis* biofilms using an XTT reduction assay. Method adapted from Seneviratne *et al*., 2015 Illustration created in BioRender

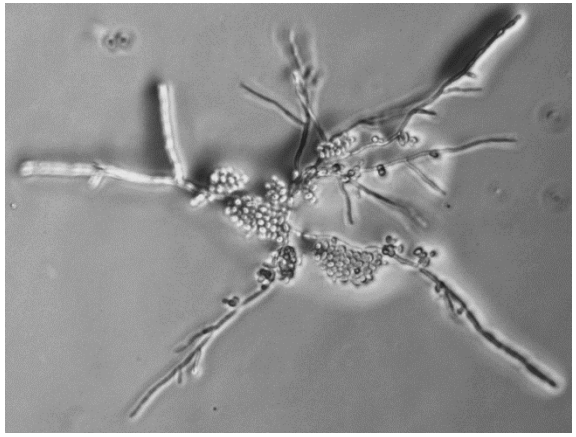


Figure 2: *E. dermatitidis* CA01 colony on Potato dextrose agar, 48 h, 35 °C

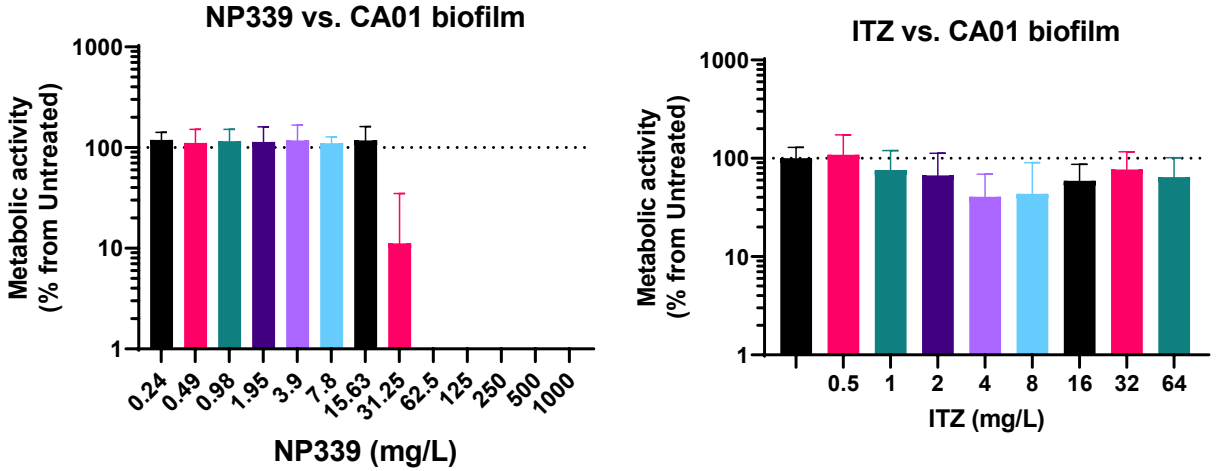


Figure 3: *E. dermatitidis* CA01 Biofilm metabolism following treatment of **A** NP339 or **B** ITZ. Metabolism quantified using XTT reduction assay and normalised to untreated control

NP339 **kills** *E. dermatitidis* biofilm cells, whereas itraconazole (ITZ) does not.

NP339 is active *in vivo* model of invasive pulmonary aspergillosis

- Immunosuppressed, male CD1 mice were infected via intranasal administration of *A. fumigatus*.
- NP339 was nebulised (n=8 mice/treatment group) at 5mg/mL for BD.
- Lung burden of *A. fumigatus* was assessed by plating of homogenates at clinical end point.

Nebulised administration of NP339 resulted in a **reduction in mean burden** per group compared to vehicle of 1.43 log_{10} cfu/g. Sterilisation of lungs was observed in 4 animals suggesting that NP339 is **fungicidal**.

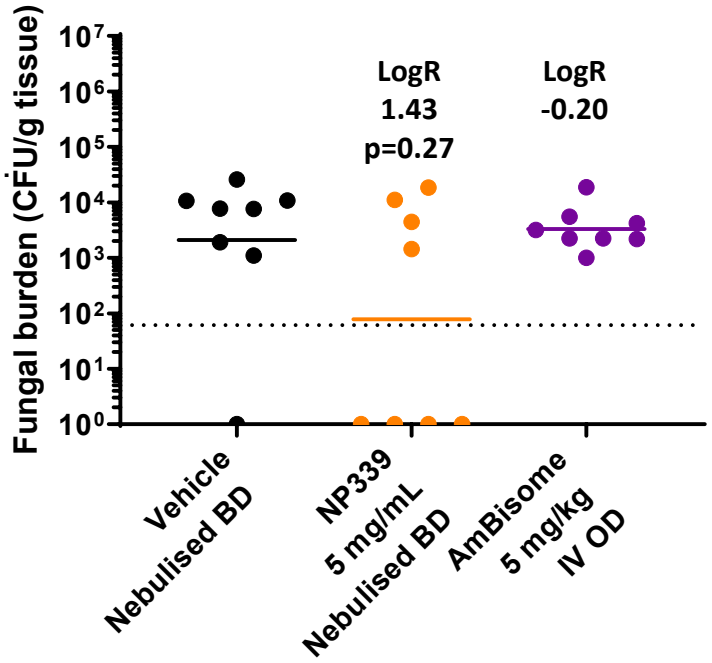


Figure 4: Murine lung burden of *A. fumigatus* at clinical end point following nebulised NP339 or IV AmBisome

Summary

- NP339 is broad spectrum; active against fungal pathogens associated with chronic and acute respiratory disease
- highly differentiated mechanism of action ensures resistance & metabolic state agnosticism
- Nebulised NP339 reduces lung burden in murine model of invasive pulmonary aspergillosis
- Combined with the favourable physicochemical properties for contained local delivery, NP339 is a candidate worthy of further investigation as a much needed therapy for fungal infection of the respiratory tract

Duncan V, Smith DW, Simpson L, Lovie E, Katvars L, Berge L, Robertson J, Smith S, Munro C, Mercer D and O'Neil D. Preliminary Characterisation of NP339, a Novel Polyarginine Peptide with Broad Antifungal Activity. *AAC*. May 2021.