evotec

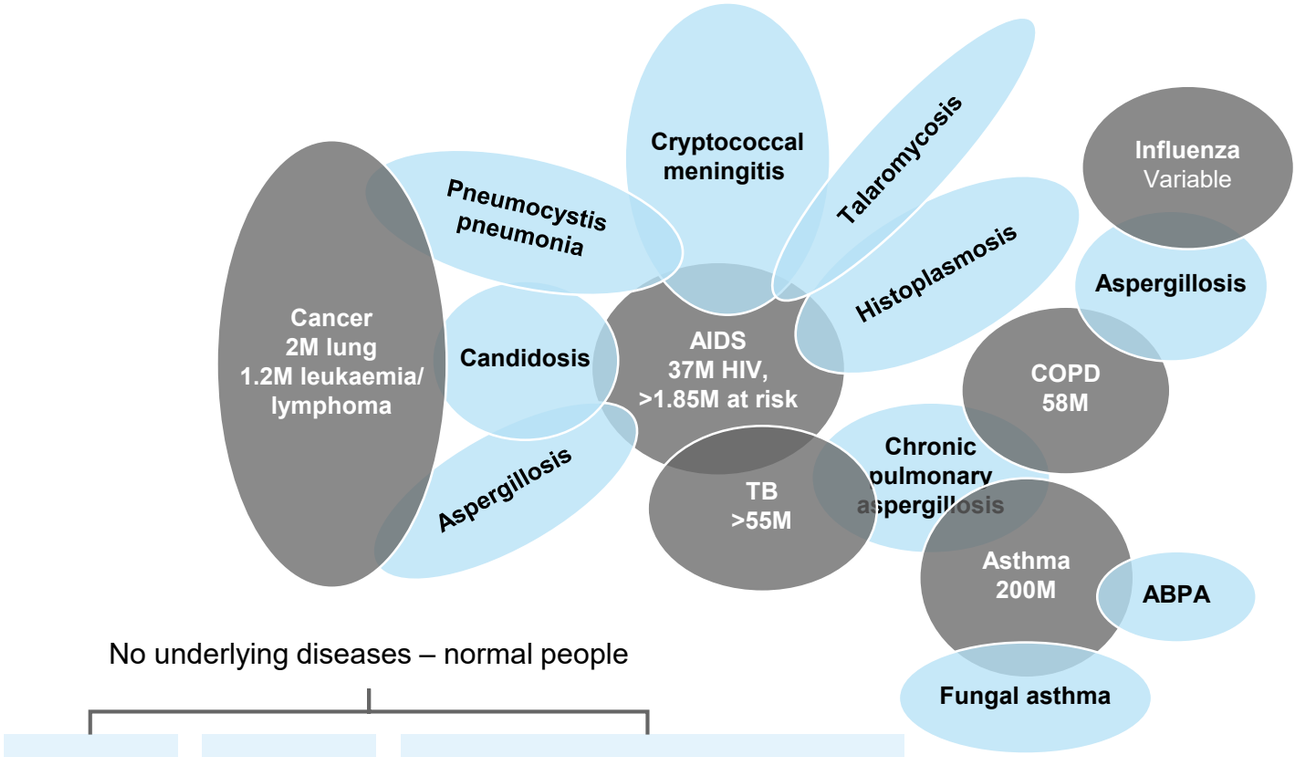
#RESEARCHNEVERSTOPS

Evotec

Integrated Drug Discovery in Antifungal Research

Global burden of fungal disease

- Globally, over 300 million people are afflicted with a serious fungal infection and 25 million are at high risk of dying or losing their sight
- Estimates for the global burden of fungal diseases are based on population and disease demographics (age, gender, HIV infected, asthma etc.)
- Fungal diseases can be
  - Acute and severe (i.e. cryptococcal meningitis and fungal eye infection (keratitis)
  - Recurrent (i.e. *Candida* vaginitis or oral candidiasis in AIDS)
  - Chronic (i.e. chronic pulmonary aspergillosis or fungal hair infection (tinea capitis)



Gaffi - Global Action For Fungal Infections - https://gaffi.org/why/fungal-disease-frequency/
ABPA, allergic bronchopulmonary aspergillosis; AIDS, acquired immunodeficiency syndrome; COPD, chronic obstructive pulmonary disease; TB, tuberculosis)

Relevant, seamless and state of the art

Integrated value chain

PDC

Best-in-class Drug Discovery & Early Development

- Translation from idea to development candidate
- Drug discovery solutions based on multidisciplinary capabilities across biology and chemistry

Preclinical studies & INDiGO

- Translation from *in vitro* to patient
- Seamless transition from development candidate through to pre-clinic and IND

Small molecules high-end CMC

- GMP manufacturing for clinical development programmes and Niche scale commercial API and Drug Product

Biologics Molecule Optimization – J.MD™

- ML based sequence evaluation for lead selection of best molecules for rapid and efficient manu-facturability and formulation stability

Biologics process development – JP3®

- Formulation design from clinical to commercial with powerful analytical, ML and high-throughput tools

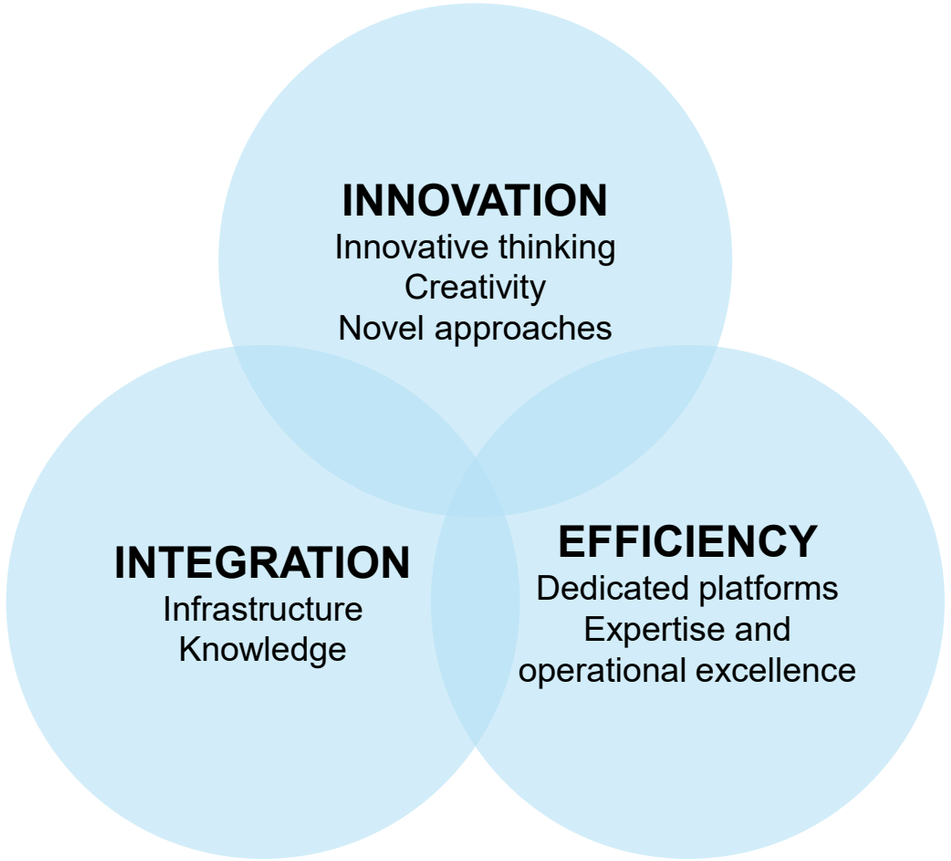
Biologics high-end CMC – J.POD®

Modular and flexible clinical and commercial manufacturing at highest quality and low costs

- Seamless integration

Evotec is confronting the renewed challenge in Infectious Diseases

Innovation and operational excellence



~200 Scientists dedicated to ID across multiple functions

Fully integrated drug discovery and disease biology

Combining world-class expertise and platforms

- **Deep rooted heritage in ID:** High calibre scientists
- **Strong expertise:** Dedicated technologies and scientific platforms
- **Global partner network:** Foundations, BRIDGES, KOLs
- **EvostrAIn™:** Highly valuable collection of ~10,000 strains from clinic and reference collections including ~300 fungal isolates
- ***In vivo* pharmacology and biomarker solutions:** Efficacy, PK/PD, *ex vivo* platform

- **Designing a TPP-driven strategy & screening cascade**
  - From HTS to Lead identification and optimization
  - Clear criteria for progression
  - Link between *in vitro* activity and *in vivo* efficacy
- **Supporting the development of multiple therapeutic modalities**
  - From small molecules to biologics and large molecules
  - Traditional and non-traditional target
  - Full integration with DMPK, Immunology and Toxicology platforms
- **Development of disease models representing pathological conditions**
  - Use of clinical isolates from seriously ill patients
  - Qualify the model on the basis of host infection biomarkers
  - Apply imaging technologies for real time evaluation of progression and infection distribution

The anti-infective autobahn: from discovery biology to the clinic

Seamless program progression from discovery to development

Discovery biology

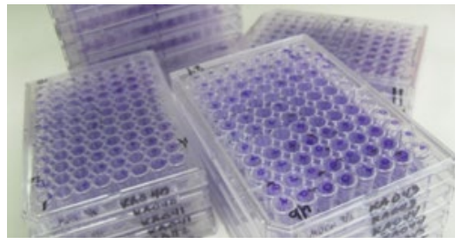
- Standard microbiology on a large strain collection (MIC, MIC_{90}, TKC, FoR, PAE, resistant mutants characterization, etc.)
- Target identification
- MoA/MoR determination and molecular profiling
- Omics and sequencing technologies
- Generation of engineered bacteria
- Target or Whole-cell based assay development

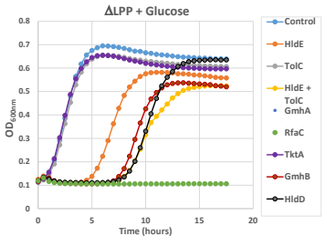
Integrated Drug Discovery

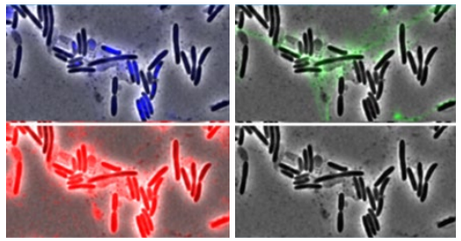
- Label-free bacterial intracellular compound accumulation assay
- Phenotypic screening (Biolog)
- Vivo-mimetic screening
- Target-based screening including fragment approaches
- Medicinal chemistry
- Computational chemistry and structure based drug design
- Highly efficient DMTA cycles

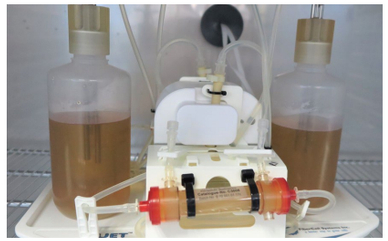
Translational microbiology and PK/PD

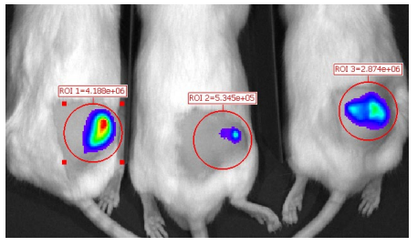
- State of the art *in vivo* DMPK
- *In vivo* microbiology for efficacy profiling
- *In vivo* and *in vitro* PK/PD platforms including Hollow fibre systems
- Mathematical modelling and simulations
- Translation of discovery data to the clinical setting

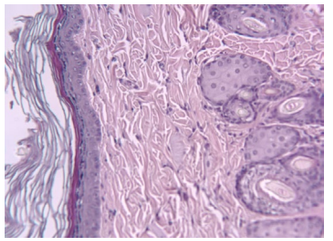








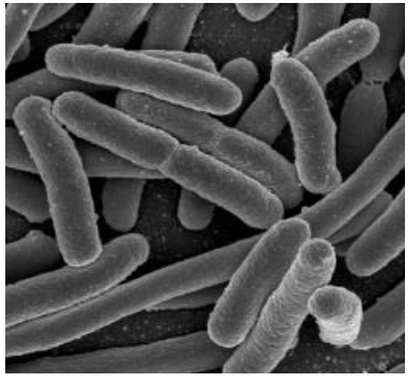


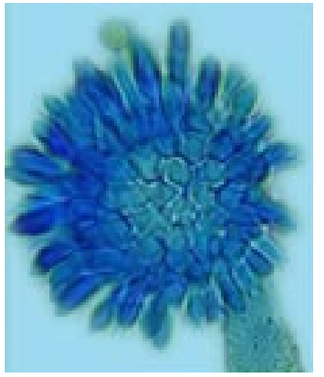


EvostrAIn™: a dedicated resource for AMR programs

Evotec's collection of characterised strains and clinical isolates

- Constantly evolving resource of thousands of primary clinical isolates and reference strains
- Broad collection of bacteria, fungi, viruses & parasites
  - ~10,000 strains from the clinic and culture collections
  - Global, recent sources with continual refreshment
  - High degree of characterisation (susceptibility profiles, mechanisms of resistance and *in vivo* drug response, genome sequences available)
  - Includes isogenic mutant strains and mutant libraries
- Used to establish spectrum of activity and potential clinical use of new antimicrobials
- Rapidly build bespoke panels
  - Guide SAR
  - MoA / MoR (target validation & identification)
  - TPP validation
  - Translational activities





EvostrAIn™

Bacteria, fungi, viruses

Bacteria: Gram positive pathogens

- *Staphylococcus aureus* incl. MRSA, VISA & VRSA strains
- β-Haemolytic *streptococci* groups A, B, C & G
- *Streptococcus pneumoniae* (including penicillin, macrolide, fluoroquinolone, cephalosporin & MDRSP resistant strains)
- Vancomycin Resistant *Enterococci* (VRE)
- *Bacillus* species
- *Listeria* species
- *Corynebacterium* and *Propionibacterium* species
- *Clostridium difficile* (multiple ribotypes incl. 012, 027 & 078)
- Other *Clostridia* (including C. *perfringens*)
- Constituents of gut microbiota
- Mycobacteria (*Mtb* & non-*MTb*)

Bacteria: Gram negative pathogens

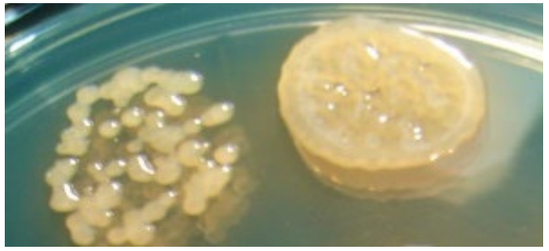
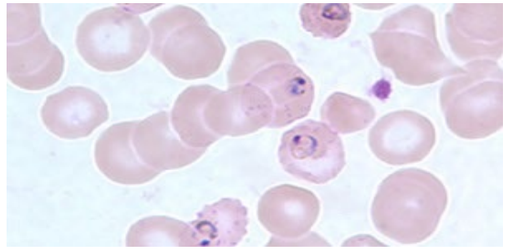
- *E. coli* including Extended Beta lactamase producing strains
- *Klebsiella pneumoniae* Carbapenemase producing strains (KPCs & MDR XDR)
- *Acinetobacter baumannii* incl. MDR XDR
- *Pseudomonas* spp. including MDR XDR
- *Haemophilus influenzae*
- *Bacteroides* spp.
- *Neisseria gonorrhoeae* and *N. meningitidis*
- Intestinal pathogens: *Vibrio* spp, *Campylobacter* spp incl. pylori, *Salmonella* spp, *Shigella* spp, *Yersinia* spp.
- *Legionella* spp. and Chlamydia (ongoing)
- Other Enterobacteriaceae: *Enterobacter, Proteus, Citrobacter, Serratia, Prividencia & Moraganella*
- *Burkholderia* and *Stenotrophomonas*

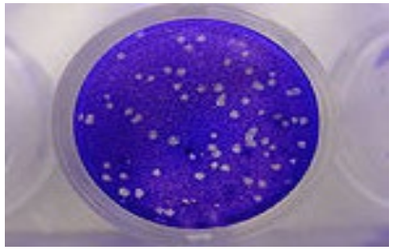
Fungi

- *Aspergillus* spp. (resistant to azoles, polyenes and echinocandins)
- *Candida* spp. (resistant to azoles, polyenes and echinocandins)
- *Mucorales*
- *Cryptococcus*
- Dermatophytes (*Fusarium*)

Viruses

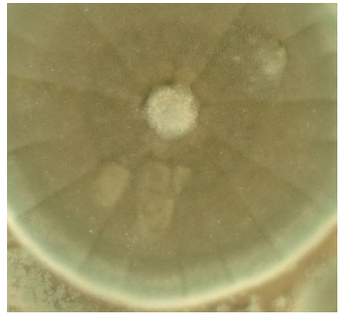
- SARS-CoCV2 (all VOC)
- Influenza virus
- Respiratory syncytial virus
- Human rhinovirus
- Human parainfluenza virus
- Human metapneumovirus
- Hepatitis B virus

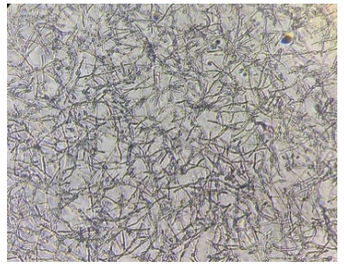




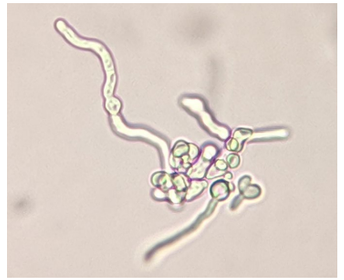


Breadth and depth of microbiology expertise

Characterisation of microbiological activity







- Antimicrobial Susceptibility Testing and hit follow-up
  - Primary screening
  - Panels of fully susceptible organisms and multidrug resistant strains that exhibit a broad range of resistant mechanisms and phenotypes. Clinically relevant and recently isolated bacterial, fungal and viral panels.
  - Large and rapidly evolving collection of highly characterised clinical isolates and strains (EvostrAIn™)
- Potencies of compounds (MIC/MFC/MPC/IC_{50})
  - Standardised methods where available (CLSI, EUCAST), miniaturized methods when required (non CLSI)
  - Detailed screening against bespoke panels of pathogens
  - Additional visual endpoint for fungal MEC (minimum effective concentration), defined as lowest concentration in which abnormal, short, and branched hyphal clusters are observed.
  - Enzyme and cellular assays
  - Time Kill Curves
- Combination studies
  - Checkerboard assays to assess synergy, additive activity, and antagonism
  - Mathematical modelling of data

Testing of a panel of clinical *Candida* isolates

Case study

- **Assessment of the *In Vitro* Antifungal Activity of SCY-078 Against a Panel of Susceptible and Resistant Clinical *Candida* Isolates from Europe**
- Collaboration with University Hospital of South Manchester, Manchester, UK
- Evaluation of the *in vitro* antifungal activity of SCY-078 against a panel of recent clinical *Candida* isolates from the EU
- A panel of 270 clinical *Candida* strains isolated from referring hospitals throughout the was tested for sensitivity against SCY-078, Amphotericin B, Anidulafungin, Micafungin, Caspofungin, Fluconazole, Voriconazole and Posaconazole
- Panel composition: *C. albicans* (n=99), *C. glabrata* (n=72), *C. guilliermondii* (n=17), *C. krusei* (n=14), *C. lusitaniae* (n=10), *C. parapsilosis* (n=41), *C. dubliniensis* (n=6), *C. inconspicua* (n=1), *C. kefyr* (n=2) and *C. tropicalis* (n=9)
- SCY-078 demonstrated potent, broad spectrum activity against a panel of clinical *Candida* isolates. Notably, SCY-078 retained activity against all of the echinocandin- and azole-resistant *C. glabrata* isolates tested.

SCY-078 MICs vs DRUG RESISTANT *C. glabrata*

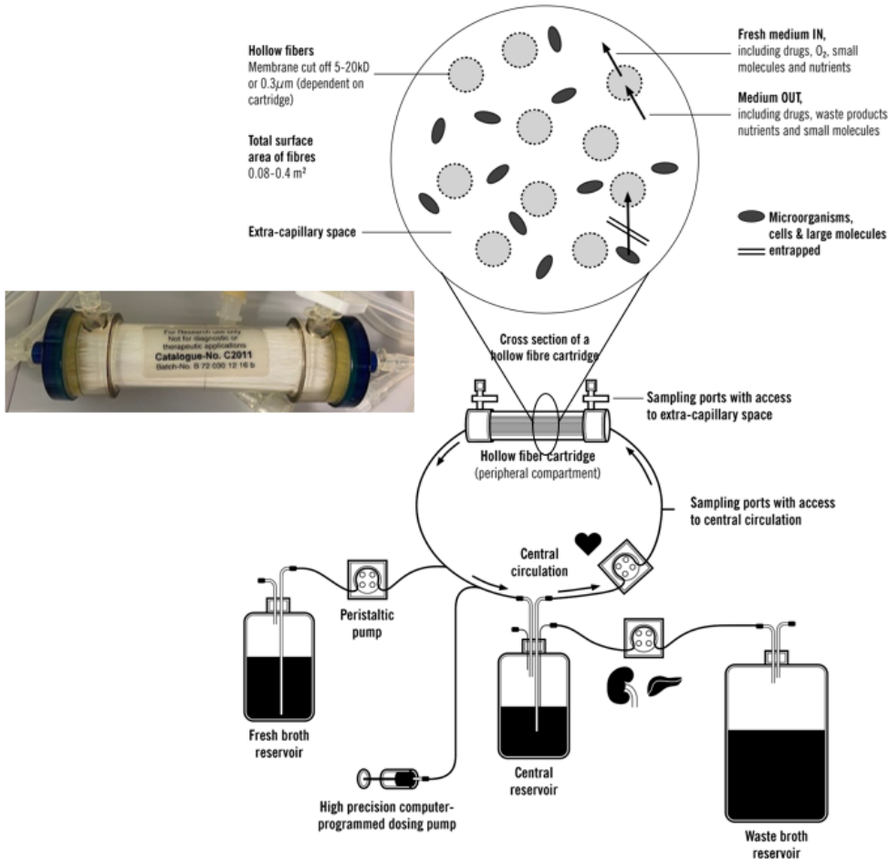
C. glabrata Isolates	Total (N=72)	Anidulafungin Resistant (N=64)	Micafungin Resistant (N=43)	Anidulafungin and Micafungin Resistant (N=41)	Fluconazole Resistant (N=7)
SCY-078 MIC (µg/mL)	MIC_{50} = 1 MIC_{90} = 1 Range 0.125 - 2	MIC_{50} = 1 MIC_{90} = 1 Range 0.25 - 2	MIC_{50} = 1 MIC_{90} = 1 Range 0.25 - 2	MIC_{50} = 1 MIC_{90} = 1 Range 0.25 - 2	Range 0.25 - 1

Presented at ASM Microbe 2017

Hollow Fibre Infection Model (HFIM)

Understanding the PK/PD relationship

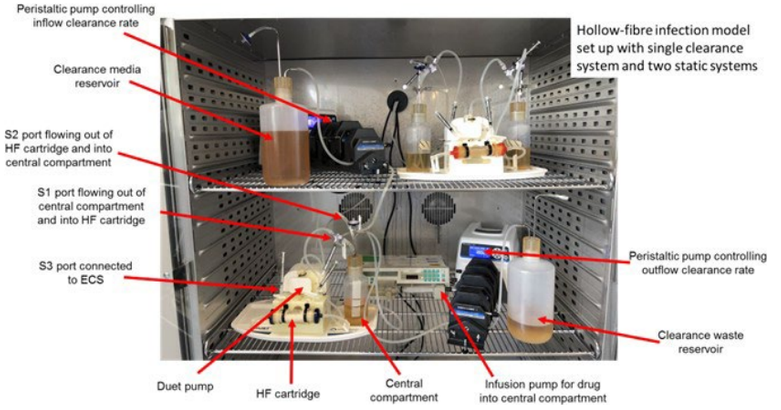
- The Hollow Fibre Infection Model provides a dynamic *in vitro* method of assessing the impact of a time course of drug exposure(s) on a cell or combination of cells
- The most capable *in vitro* model for evaluating PK/PD indices and optimising dosing regimens for bacterial or fungal killing and suppressing the amplification of drug resistant mutant subpopulations
- Two principal compartments:
  - Central reservoir and associated tubing which constitutes a circulating system
  - A hollow fibre cartridge containing thousands of permeable capillaries, sealed at both ends within a tubular polycarbonate shell. The extracapillary space (ECS) outside the fibres but within the cartridge housing contains the target organism.
- Drug-infused growth medium in the central reservoir is continuously pumped to the hollow fibre cartridge, rapidly passes through the capillaries and equilibrates with medium in ECS. Nutrients and oxygen are continuously refreshed, waste products are removed
- Using infusion and clearance pumps any PK profile (animal, human) including drug combinations can be simulated
- System can also be applied to determine resistance development
- **HFIM study designs can be tailored to specific requirements or bespoke client protocols**



Hollow Fibre Infection Model

Capabilities, capacity and experience at Evotec

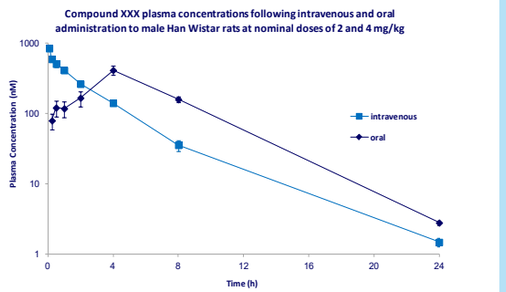
- Experience at Evotec with HFIM for:
  - *Mycobacterium tuberculosis* H37Ra
  - Bacteria: *Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, …*
  - Fungi: *Aspergillus fumigatus*
- Containment level 2 laboratory space dedicated to running HFIM studies
- Up to 34 hollow fibre cartridges[1)] can be operated in parallel for different organisms, using variable drug infusion and clearance rates, with study durations ranging from hours to 6 weeks
- Dedicated team of scientists trained to operate the system
- Full range of microbiology support including alternative endpoints, whole-genome sequencing and bioinformatics services to support resistance characterisation studies, and mechanism of action determination
- Bioanalysis facilities for LC-MS analysis of PK samples
- A dedicated PK/PD modelling team
  - Experimental design for single- and multiple-drug experiments
  - Generation of infusion parameters (duration, rates, frequency) to reproduce specific PK profile
  - Mathematical models for interpretation of pharmacokinetic and pharmacodynamic data



[1)] Depending on model type and duration

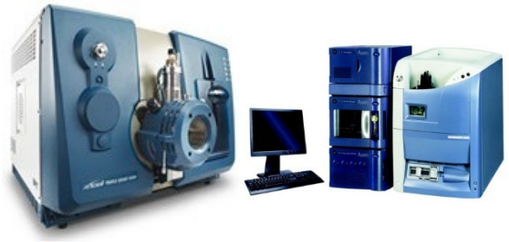
Pharmacokinetic profiling in anti-infective drug discovery

From *in vivo* characterisation to bioanalysis and biomarker assessment



PK Parameter	Intravenous	Oral
Dose (mg/Kg)	1.83 ± 0.04	4.07 ± 0.02
C_0 / C_{max} (ng/mL)	492 ± 25	206 ± 80
T_{max} (h)	-	4
V_{dss} (L/Kg)	6.9 ± 0.3	-
CL (mL/min/Kg)	34.6 ± 0.3	-
Liver Blood Flow (%)	48.1 ± 0.5	-
AUC_{inf} (ng.hr/mL)	880 ± 28	1248 ± 380
Bioavailability (%)	-	64 ± 0.2

- **Standard and specialised PK studies in multiple rodent species:**
  - Administration routes; intravenous (prolonged infusion), per oral, intraperitoneal, subcutaneous, intra cerebrospinal, intramuscular, pulmonary (nebulized, aerosolized), iPrecio & Alzet pumps
- **Sampling types:** jugular vein cannulation, cardiac puncture, tail vein microsampling
- **Matrices:** blood, plasma, CSF, BALF, whole tissues, bile, urine and faeces
- PK in infected animals assessing impact of disease state on drug exposure
- Data directly translated into efficacy studies to optimise outcomes
- PK experiments designed to accompany PK/PD profiling programmes

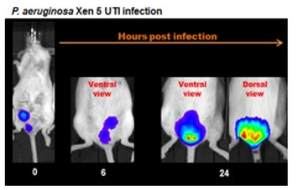


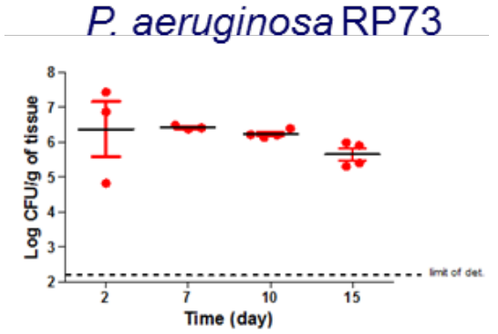
- **State-of-the-art bioanalytics**
  - Highly sensitive biophysical methods including LC-MSMS
  - Bioassay techniques: tracking biological activity
- **Biomarker quantification:** pathogen/infection specific and host response

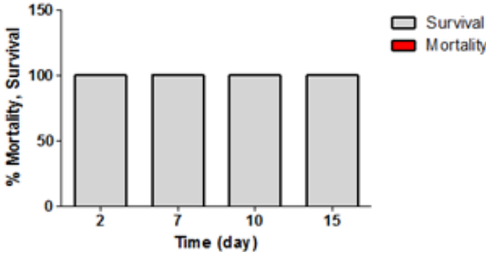
Comprehensive translational microbiology platform

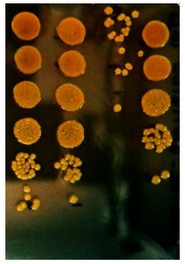
Integrating *in vivo* pharmacology and DMPK

- State-of-the-art animal facilities (**BSL 2 & 3**) to house rodents, immunocompromised animals, and multiple backgrounds (incl. dogs and monkeys AAALAC accredited)
- Target validation, tolerability studies, DMPK studies, PK/PD studies, *in vivo* MoA studies, efficacy screening
- Multiple hosts and fully validated models of infection: Rat, mouse, guinea pig, hamster, cotton rat, rabbit
- Multiple routes of infection include: lung, thigh, blood (sepsis), skin, urinary tract, GI tract, vagina, bone
- Full range of endpoints: pathogen burden (culture, qPCR, biomarkers), host response
- Real time imaging of microbes during infection: IVIS, MRI, CAT, PET
- New model development programme
- Invertebrate screening model for bacteria & fungi (wax moth larva rapid screening models)







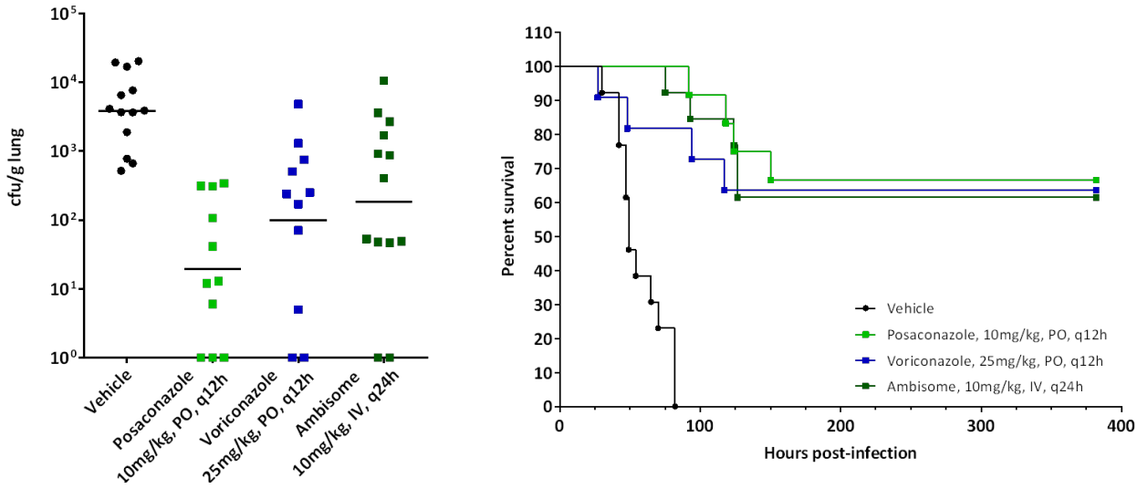


Breadth of *in vivo* models of fungal infection

Summary and *Aspergillus* lung infection as example

Model	Pathogen	Animal species	Immune system
Acute Sepsis	*A. fumigtaus, A. flavus, A. terreus*	Mouse	neutropenic
	C. albicans, C. glabrata, C. tropicalis	Mouse	neutropenic
	C. auris	Mouse	neutropenic
	C. neoformans	Mouse	neutropenic
Chronic Sepsis	*C. albicans*	Mouse	competent
Brain	*C. neoformans*	Mouse	neutropenic
	C. albicans	Mouse	neutropenic
GI tract	*C. albicans*	Mouse	competent
	C. albicans	Rat	competent
Skin	*M. pachydermatis*	Guinea pig	competent
	T. mentagrophytes	Guinea pig	competent
Vaginal	*C. albicans*	Mouse	competent
	C. albicans	Rat	competent
Lung	*A. fumigatus, A. flavus, A. terreus*	Mouse	neutropenic
Lung *aerosolised*	*A. fumigatus*	Mouse	neutropenic
Oropharyngeal	*C. albicans*	Mouse	neutropenic

Neutropenic murine model of *A. fumigatus* lung infection. Survival and lung burden as endpoints:

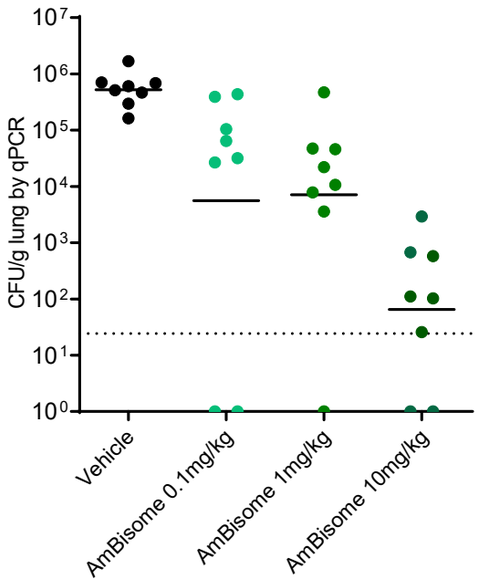


Additional endpoints: cytokines, cellular response, galactomannan, β glucan, histopathology

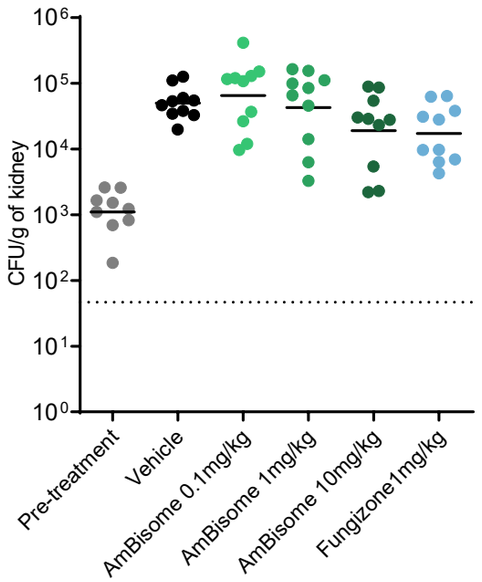
In vivo pharmacology – fungal infection models

Example Data[2, 3]

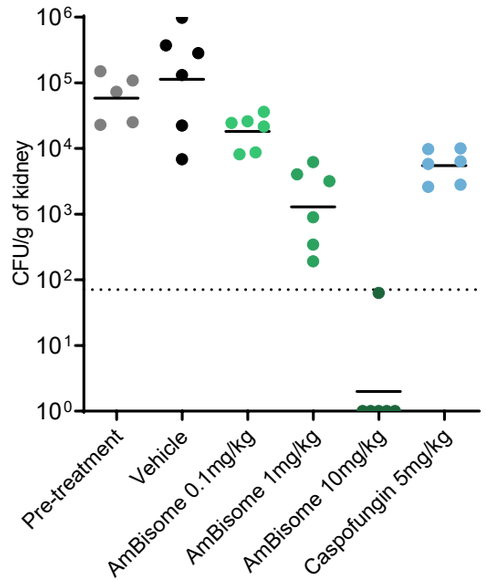
A. fumigatus ATCC204305 IPA[1)] Lung Burden



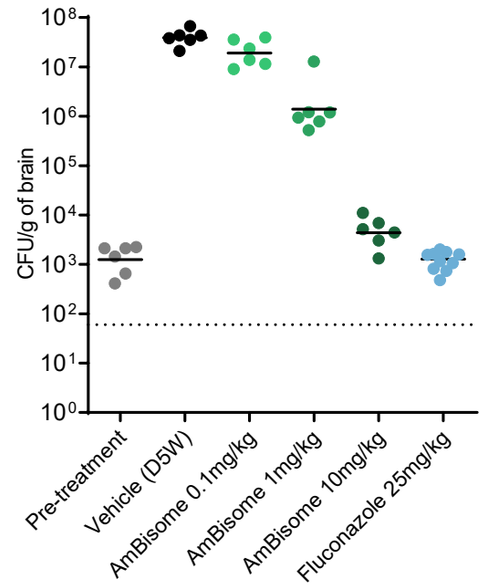
A. terreus AT49 Sepsis Kidney Burden



C. glabrata ATCC2001 Sepsis Kidney Burden



C. neoformans HA99 Sepsis Brain Burden



[1)] IPA= Invasive pulmonary *Aspergillosis*, preferred measure of burden for IPA model is qPCR due to known difficulties recovering *Aspergillus* from lung tissues using standard microbiological culture
[2)] Extended duration models are available to determine survival
[3)] Additional types of models including vaginal and oral candidiasis, gastrointestinal tract infection and dermatophyte skin infection models are also available

Aspergillosis model

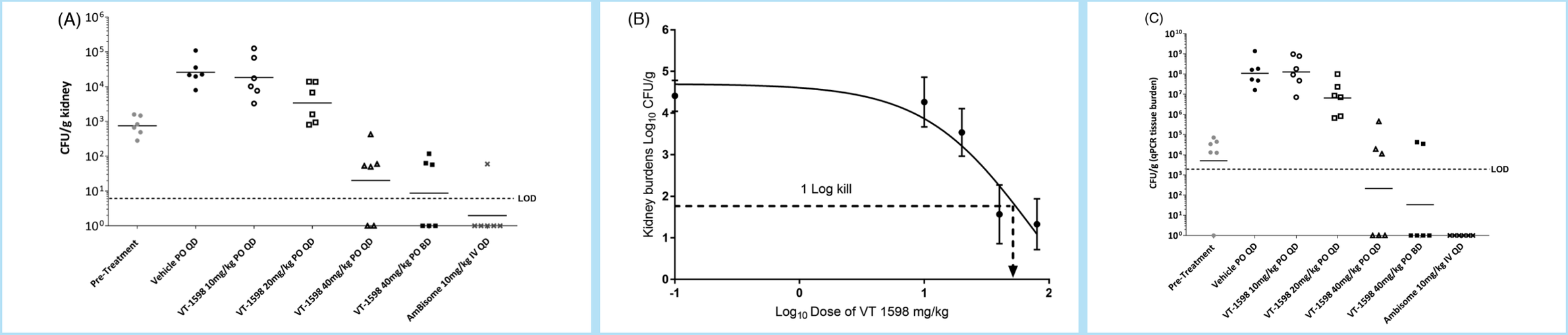
Case study

VT-1598 dose-dependent reductions in fungal burdens in a neutropenic mouse acute model of disseminated invasive aspergillosis using *Aspergillus fumigatus* ATCC 204305 to infect

A. Fungal burdens were determined by quantitative culture of kidney samples collected 1 day after the last treatment

B. Kidney burden/dose response relationship to determine 1-log killing dose (i.e., the dose that would reduce fungal burden prior to treatment by 1-log)

C. Fungal burdens were determined by measuring fungal DNA by qPCR in kidney samples collected 1 day after the last treatment. For each dose group shown in panels A and B, solid bar within each data set represents mean value. Dashed lines represent limit of detection (LOD).

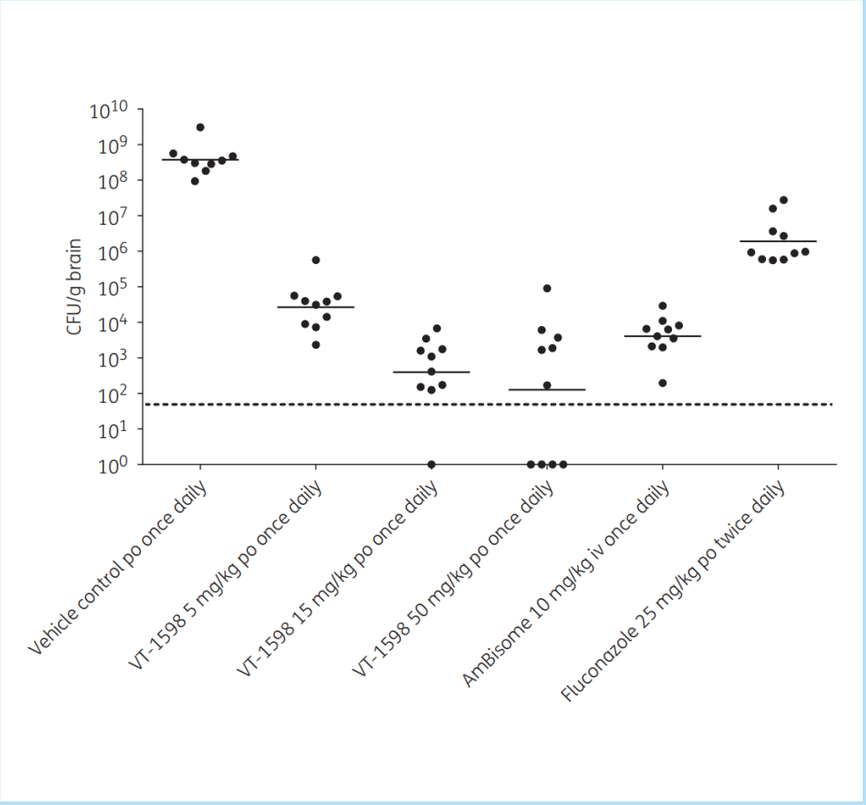


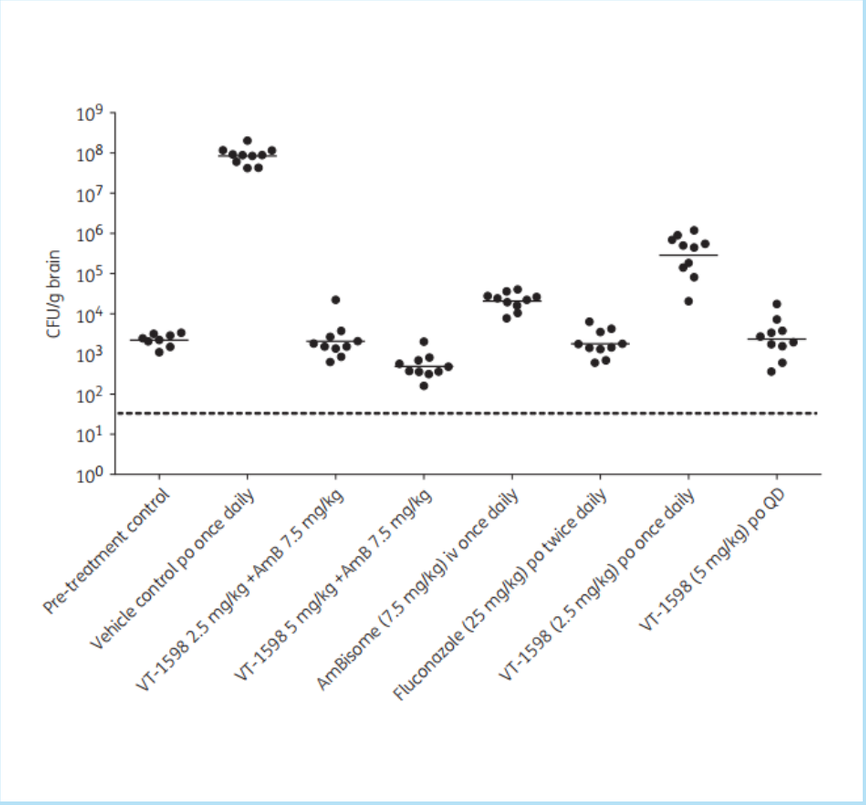
Garvey EP, Sharp AD, Warn PA, Yates CM, Atari M, Thomas S, Schotzinger RJ.Med Mycol. 2020 Jun 1;58(4):505-513. doi: 10.1093/mmy/myz092.

Cryptococcal meningitis model

Case study

- VT-1598 maintains dose-dependent reduction in brain fungal burden in treating murine cryptococcal meningitis after a 6 day drug washout
- VT-1598/AmBisome combination increases antifungal efficacy of both monotherapies in treating murine cryptococcal meningitis
- Treatment began 1 day after inoculation of *C. neoformans* H99 and lasted for 14 days, with brain samples collected 6 days after the last dose

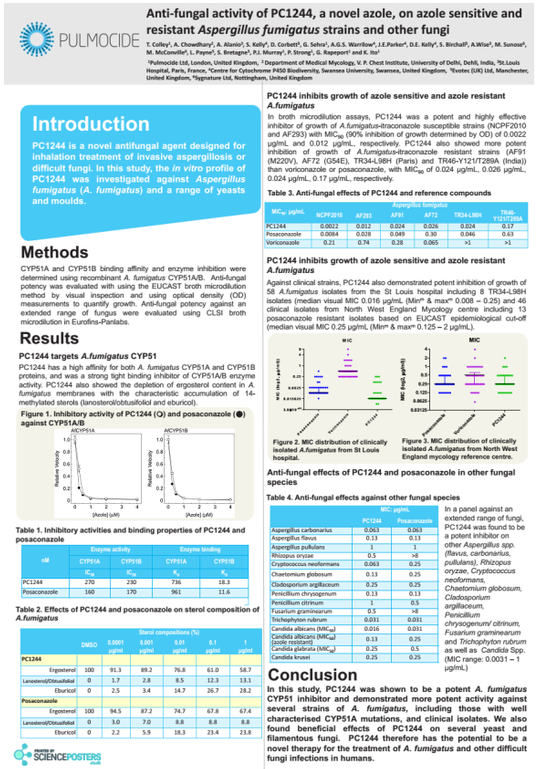




Garvey EP, Sharp AD, Warn PA, Yates CM, Schotzinger RJ.J Antimicrob Chemother. 2018 Oct 1;73(10):2815-2822. doi: 10.1093/jac/dky242.

Fungal disease models at Evotec

Publications



Anti-fungal activity of PC1244, a novel azole, on azole sensitive and resistant *Aspergillus fumigatus* strains and other fungi

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Introduction

PC1244 is a novel antifungal agent designed for inhalation treatment of invasive aspergillosis or difficult fungi. In this study, the *in vitro* profile of PC1244 was investigated against *Aspergillus fumigatus* (*A. fumigatus*) and a range of yeasts and moulds.

Methods

CYP51A and CYP51B binding affinity and enzyme inhibition were determined using recombinant *A. fumigatus* CYP51A/B. Anti-fungal potency was evaluated with using the EUCAST broth microdilution method by visual inspection and using optical density (OD) measurements to quantify growth. Anti-fungal potency against an extended range of fungus were evaluated using CLSI broth microdilution in Eurofins-Panlabs.

Results

PC1244 targets *A.fumigatus* CYP51

PC1244 has a high affinity for both *A. fumigatus* CYP51A and CYP51B proteins, and was a strong tight binding inhibitor of CYP51A/B enzyme activity. PC1244 also showed the depletion of ergosterol content in *A. fumigatus* membranes with the characteristic accumulation of 14-methylated sterols (lanosterol/obtusifolol and eburicol).

Figure 1. Inhibitory activity of PC1244 (○) and posaconazole (●) against CYP51A/B

Table 1. Inhibitory activities and binding properties of PC1244 and posaconazole

nM	Enzyme activity		Enzyme binding	
	CYP51A	CYP51B	CYP51A	CYP51B
	IC_{50}	IC_{50}	K_d	K_d
PC1244	270	230	736	18.3
Posaconazole	160	170	961	11.6

Table 2. Effects of PC1244 and posaconazole on sterol composition of *A.fumigatus*

	DMSO	Sterol compositions (%)				
		0.0001 µg/ml	0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	1 µg/ml
PC1244						
Ergosterol	100	91.3	89.2	76.8	61.0	58.7
Lanosterol/Obtusifolol	0	1.7	2.8	8.5	12.3	13.1
Eburicol	0	2.5	3.4	14.7	26.7	28.2
Posaconazole						
Ergosterol	100	94.5	87.2	74.7	67.8	67.4
Lanosterol/Obtusifolol	0	3.0	7.0	8.8	8.8	8.8
Eburicol	0	2.2	5.9	18.3	23.4	23.8

PC1244 inhibits growth of azole sensitive and azole resistant *A.fumigatus*

In broth microdilution assays, PC1244 was a potent and highly effective inhibitor of growth of *A.fumigatus*-itraconazole susceptible strains (NCPF2010 and AF293) with MIC_{90} (90% inhibition of growth determined by OD) of 0.0022 µg/mL and 0.012 µg/mL, respectively. PC1244 also showed more potent inhibition of growth of *A.fumigatus*-itraconazole resistant strains (AF91 (M220V), AF72 (G54E), TR34-L98H (Paris) and TR46-Y121/T289A (India)) than voriconazole or posaconazole, with MIC_{90} of 0.024 µg/mL, 0.026 µg/mL, 0.024 µg/mL, 0.17 µg/mL, respectively.

Table 3. Anti-fungal effects of PC1244 and reference compounds

MIC_{90} µg/mL	*Aspergillus fumigatus*					
	NCPF2010	AF293	AF91	AF72	TR34-L98H	TR46-Y121/T289A
PC1244	0.0022	0.012	0.024	0.026	0.024	0.17
Posaconazole	0.0094	0.028	0.049	0.30	0.046	0.63
Voriconazole	0.21	0.74	0.28	0.065	>1	>1

PC1244 inhibits growth of azole sensitive and azole resistant *A.fumigatus*

Against clinical strains, PC1244 also demonstrated potent inhibition of growth of 58 *A.fumigatus* isolates from the St Louis hospital including 8 TR34-L98H isolates (median visual MIC 0.016 µg/mL (Min* & max* 0.008 – 0.25) and 46 clinical isolates from North West England Mycology centre including 13 posaconazole resistant isolates based on EUCAST epidemiological cut-off (median visual MIC 0.25 µg/mL (Min* & max* 0.125 – 2 µg/mL).

Figure 2. MIC distribution of clinically isolated *A.fumigatus* from St Louis hospital.

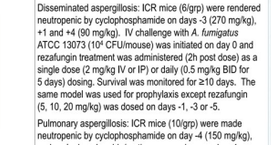

Figure 3. MIC distribution of clinically isolated *A.fumigatus* from North West England mycology reference centre.

Anti-fungal effects of PC1244 and posaconazole in other fungal species

Table 4. Anti-fungal effects against other fungal species

	MIC µg/mL	
	PC1244	Posaconazole
Aspergillus carbonarius	0.063	0.063
Aspergillus flavus	0.13	0.13
Aspergillus pullulans	1	1
Rhizopus oryzae	0.5	>8
Cryptococcus neoformans	0.063	0.25
Chaetomium globosum	0.13	0.25
Cladosporium sirgillaceum	0.25	0.25
Penicillium chrysogenum	0.13	0.13
Penicillium citrinum	1	0.5
Fusarium graminearum	0.5	>8
Trichophyton rubrum	0.031	0.031
Candida albicans (MIC_{50})	0.016	0.031
Candida albicans (MIC_{50}) (azole resistant)	0.13	0.25
Candida glabrata (MIC_{50})	0.25	0.5
Candida krusei	0.25	0.25

In a panel against an extended range of fungi, PC1244 was found to be a potent inhibitor on other *Aspergillus* spp. *(flavus, carbonarius, pullulans), Rhizopus oryzae, Cryptococcus neoformans, Chaetomium globosum, Cladosporium argillaceum, Penicillium chrysogenum/ citrinum, Fusarium graminearum* and *Trichophyton rubrum* as well as *Candida* Spp. (MIC range: 0.0031 – 1 µg/mL)

Conclusion

In this study, PC1244 was shown to be a potent *A. fumigatus* CYP51 inhibitor and demonstrated more potent activity against several strains of *A. fumigatus*, including those with well characterised CYP51A mutations, and clinical isolates. We also found beneficial effects of PC1244 on several yeast and filamentous fungi. PC1244 therefore has the potential to be a novel therapy for the treatment of *A. fumigatus* and other difficult fungi infections in humans.

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CIDARA THERAPEUTICS

CD101 Lung Epithelial Lining Fluid (ELF) Concentrations Substantiate Its Use For Prophylaxis Treatment As Evident In Mouse Disseminated and Pulmonary Aspergillosis Models

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[1]Cidara Therapeutics, San Diego, CA; [2]Evotec, Manchester, UK; [3]NeoSome Life Sciences, Lexington, MA

INTRODUCTION AND PURPOSE

Rezafungin (previously CD101) has demonstrated robust efficacy as prophylaxis and treatment in mouse antifungal models of aspergillosis. The distribution of rezafungin into lung ELF was studied to further substantiate the observed efficacy.

METHODS

Rezafungin (20 mg/kg IP; human equivalent dose) was administered to ICR mice. At 0, 1, 3, 6, 12, 24, 48, and 72 hours post-dose, 3 mice/timepoint were euthanized for plasma and bronchoalveolar lavage fluid (BALF) collection with 2x 0.5 mL flushes of saline. Urea levels for plasma/ BALF normalization for lung ELF volume calculation were quantified using a spectrophotometry-based assay. Rezafungin concentrations in plasma/BALF samples were measured by LC-MS/MS.

Disseminated aspergillosis: ICR mice (6/grp) were rendered neutropenic by cyclophosphamide on days -3 (270 mg/kg), +1 and +4 (90 mg/kg). IV challenge with *A. fumigatus* ATCC 13073 (10^4 CFU/mouse) was initiated on day 0 and rezafungin treatment was administered (2h post dose) as a single dose (2 mg/kg IV or IP) or daily (0.5 mg/kg BID for 5 days) dosing. Survival was monitored for ≥10 days. The same model was used for prophylaxis except rezafungin (5, 10, 20 mg/kg) was dosed on days -1, -3 or -5.

Pulmonary aspergillosis: ICR mice (10/grp) were made neutropenic by cyclophosphamide on day -4 (150 mg/kg), and cyclophosphamide/cortisone was given on day -1 (150/175 mg/kg). Intranasal challenge with *A. fumigatus* AF293 (10^5 CFU/mouse) was initiated on day 0 and prophylaxis with a single dose of rezafungin (IP; 5, 10, and 20 mg/kg) or posaconazole (PO; 2 and 10 mg/kg) was started 1 day prior to infection. Survival was monitored for 10 days.

RESULTS

Rezafungin ELF concentrations reached a maximum by 4h and were comparable between plasma and ELF beyond 24h post-dose in total-drug concentrations but much higher based on free-drug concentrations (99.2% plasma protein binding), the ELF/Plasma AUC ratio was 0.80 for total-drug and 100 based on free-drug exposures, respectively (fig. below).

RESULTS

For prophylaxis of disseminated aspergillosis, a single dose of 10 or 20 mg/kg showed 100% survival regardless of prophylaxis day. Survival in the 5 mg/kg group improved with prophylaxis given closer to the day of challenge (fig. below).

In the more challenging pulmonary aspergillosis model, an increase in survival was also observed from a single prophylaxis rezafungin dose. The human dose (400 mg) equivalent dose of 2 mg/kg was not protective and only showed a significant increase in survival at 10 mg/kg (5x higher than human AUC).

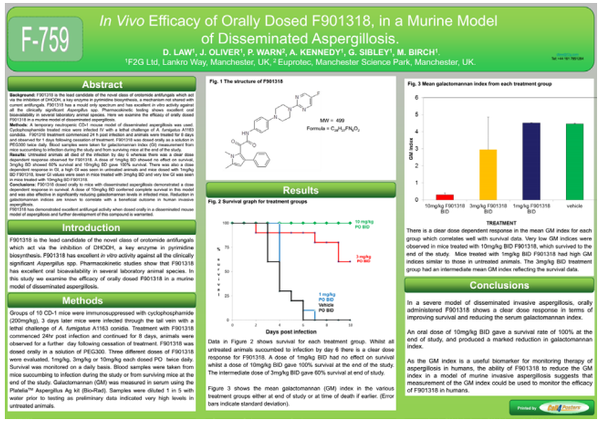
For treatment of disseminated aspergillosis, rezafungin by IV or IP showed a significant increase in survival compared to vehicle. Survival was comparable with either a single 2 mg/kg dose or 0.2 mg/kg BID x 5d (fig. below), confirming that a single-dose, front-loading regimen was equally effective[1].

CONCLUSIONS

With a long plasma half-life in human (133 h) and extensive lung ELF exposures, these findings suggest that rezafungin could be a viable candidate for prophylaxis and treatment of aspergillosis.

REFERENCE

1. Lakota et al. 2017. Pharmacological basis of CD101 efficacy: exposure shape matters. Antimicrob Agents Chemother 61:e00758-17. https://doi.org/10.1128/AAC.00758-17.



F-759

In Vivo Efficacy of Orally Dosed F901318, in a Murine Model of Disseminated Aspergillosis.

D. LAW[1], J. OLIVER[1], P. WARN[2], A. KENNEDY[1], G. SIBLEY[1], M. BIRCH[1].
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Abstract

Background: F901318 is the lead candidate of the novel class of orotomide antifungals which act via the inhibition of DHODH, a key enzyme in pyrimidine biosynthesis. F901318 has excellent *in vitro* activity against all the clinically significant *Aspergillus* spp. Pharmacokinetic studies show that F901318 has excellent oral bioavailability in several laboratory animal species. Here we examine the efficacy of orally dosed F901318 in a murine model of disseminated aspergillosis.

Methods: A temporary neutropenic CD1 mouse model of disseminated aspergillosis was used. Cyclophosphamide treated mice were infected IV with a lethal challenge of *A. fumigatus* A1163 conidia. Treatment with F901318 commenced 24hr post infection and continued for 8 days, animals were observed for a further day following cessation of treatment. F901318 was dosed orally as a solution in PEG300. Three different doses of F901318 were evaluated, 1mg/kg, 3mg/kg or 10mg/kg each dosed PO twice daily. Survival was monitored on a daily basis. Blood samples were taken from mice succumbing to infection during the study or from surviving mice at the end of the study. Galactomannan (GM) was measured in serum using the Platelia™ Aspergillus Ag kit (Bio-Rad). Samples were diluted 1 in 5 with water prior to testing as preliminary data indicated very high levels in untreated animals.

Results: Untreated animals all died of the infection by day 5 whereas there was a clear dose dependent response observed for F901318. A dose of 1mg/kg BID showed no effect on survival, whilst a dose of 3mg/kg BID gave 100% survival at the end of the study. The 3mg/kg BID treatment group had an intermediate mean GM index reflecting the survival data. 3mg/kg BID response to 1% v high GI was very similar to BID dosed at mice treated with 3mg/kg BID and very low GI was seen in mice treated with 10mg/kg BID F901318.

Conclusions: F901318 dosed orally to mice with disseminated aspergillosis demonstrated a clear dose dependent response in the mean GM index for each group which correlates well with survival data. Very low GM indices were observed in mice treated with 10mg/kg BID F901318, which survived to the end of the study. Mice treated with 1mg/kg BID F901318 had high GM indices similar to those in untreated animals. The 3mg/kg BID treatment group had an intermediate mean GM index reflecting the survival data.

An oral dose of 10mg/kg BID gave a survival rate of 100% at the end of study, and produced a marked reduction in galactomannan index.

As the GM index is a useful biomarker for monitoring therapy of aspergillosis in humans, the ability of F901318 to reduce the GM index in a model of murine invasive aspergillosis suggests that measurement of the GM index could be used to monitor the efficacy of F901318 in humans.

Introduction

F901318 is the lead candidate of the novel class of orotomide antifungals which act via the inhibition of DHODH, a key enzyme in pyrimidine biosynthesis. F901318 has excellent *in vitro* activity against all the clinically significant *Aspergillus* spp. Pharmacokinetic studies show that F901318 has excellent oral bioavailability in several laboratory animal species. In this study we examine the efficacy of orally dosed F901318 in a murine model of disseminated aspergillosis.

Methods

Groups of 10 CD-1 mice were immunosuppressed with cyclophosphamide (200mg/kg), 3 days later mice were infected through the tail vein with a lethal challenge of *A. fumigatus* A1163 conidia. Treatment with F901318 commenced 24hr post infection and continued for 8 days, animals were observed for a further day following cessation of treatment. F901318 was dosed orally in a solution of PEG300. Three different doses of F901318 were evaluated, 1mg/kg, 3mg/kg or 10mg/kg each dosed PO twice daily. Survival was monitored on a daily basis. Blood samples were taken from mice succumbing to infection during the study or from surviving mice at the end of the study. Galactomannan (GM) was measured in serum using the Platelia™ Aspergillus Ag kit (Bio-Rad). Samples were diluted 1 in 5 with water prior to testing as preliminary data indicated very high levels in untreated animals.

Fig. 1 The structure of F901318

Results

Fig. 2 Survival graph for treatment groups

Data in Figure 2 shows survival for each treatment group. Whilst all untreated animals succumbed to infection by day 6 there is a clear dose response for F901318. A dose of 1mg/kg BID had no effect on survival whilst a dose of 10mg/kg BID gave 100% survival at the end of the study. The intermediate dose of 3mg/kg BID gave 60% survival at end of study.

Figure 3 shows the mean galactomannan (GM) index in the various treatment groups either at end of study or at time of death if earlier. (Error bars indicate standard deviation).

Fig. 3 Mean galactomannan index from each treatment group

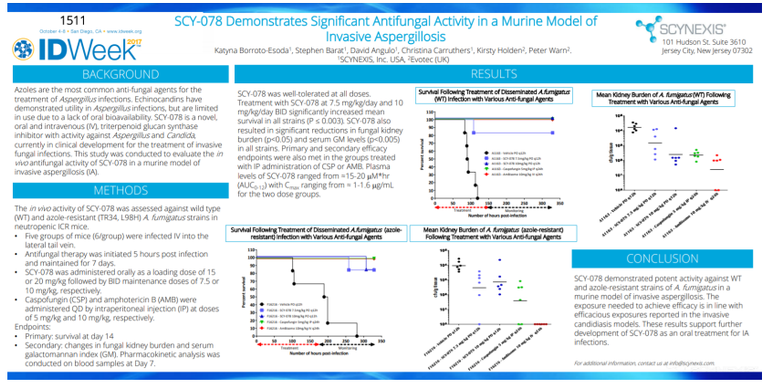
Conclusions

In a severe model of disseminated invasive aspergillosis, orally administered F901318 shows a clear dose response in terms of improving survival and reducing the serum galactomannan index.

An oral dose of 10mg/kg BID gave a survival rate of 100% at the end of study, and produced a marked reduction in galactomannan index.

As the GM index is a useful biomarker for monitoring therapy of aspergillosis in humans, the ability of F901318 to reduce the GM index in a model of murine invasive aspergillosis suggests that measurement of the GM index could be used to monitor the efficacy of F901318 in humans.

Poster by Zoetis Discovery



1511

IDWeek

SCY-078 Demonstrates Significant Antifungal Activity in a Murine Model of Invasive Aspergillosis

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BACKGROUND

Azoles are the most common anti-fungal agents for the treatment of *Aspergillus* infections. Echinocandins have demonstrated utility in *Aspergillus* infections, but are limited in use due to a lack of oral bioavailability. SCY-078 is a novel, oral and intravenous (IV), triterpenoid glucan synthase inhibitor with activity against *Aspergillus* and *Candida*, currently in clinical development for the treatment of invasive fungal infections. This study was conducted to evaluate the *in vivo* antifungal activity of SCY-078 in a murine model of invasive aspergillosis (IA).

METHODS

The *in vivo* activity of SCY-078 was assessed against wild type (WT) and azole-resistant (TR34, L98H) *A. fumigatus* strains in neutropenic ICR mice.

- Five groups of mice (6/group) were infected IV into the lateral tail vein.
- Antifungal therapy was initiated 5 hours post infection and maintained for 7 days.
- SCY-078 was administered orally as a loading dose of 15 or 20 mg/kg followed by BID maintenance doses of 7.5 or 10 mg/kg, respectively.
- Caspofungin (CSP) and amphotericin B (AMB) were administered QD by intraperitoneal injection (IP) at doses of 5 mg/kg and 10 mg/kg, respectively.

Endpoints:

- Primary: survival at day 14
- Secondary: changes in fungal kidney burden and serum galactomannan index (GM). Pharmacokinetic analysis was conducted on blood samples at Day 7.

RESULTS

SCY-078 was well-tolerated at all doses. Treatment with SCY-078 at 7.5 mg/kg/day and 10 mg/kg/day BID significantly increased mean survival in all strains ($P \leq 0.003$). SCY-078 also resulted in significant reductions in fungal kidney burden ($p<0.05$) and serum GM levels ($p<0.005$) in all strains. Primary and secondary efficacy endpoints were also met in the groups treated with IP administration of CSP or AMB. Plasma levels of SCY-078 ranged from ≈15-20 µM*Hr (AUC_{0-12}) with C_{max} ranging from ≈ 1-1.6 µg/mL for the two dose groups.

Survival Following Treatment of Disseminated *A.fumigatus* (WT) Infection with Various Anti-fungal Agents

Mean Kidney Burden of *A. fumigatus* (WT) Following Treatment with Various Anti-fungal Agents

Survival Following Treatment of Disseminated *A.fumigatus* (azole-resistant) Infection with Various Anti-fungal Agents

Mean Kidney Burden of *A. fumigatus* (azole-resistant) Following Treatment with Various Anti-fungal Agents

CONCLUSION

SCY-078 demonstrated potent activity against WT and azole-resistant strains of *A. fumigatus* in a murine model of invasive aspergillosis. The exposure needed to achieve efficacy is in line with efficacious exposures reported in the invasive candidiasis models. These results support further development of SCY-078 as an oral treatment for IA infections.

For additional information, contact us at info@scynexis.com

› J Antimicrob Chemother. 2020 Oct 1;75(10):2919-2924. doi: 10.1093/jac/dkaa243.

Intermittent micafungin for prophylaxis in a rat model of chronic Candida albicans gut colonization

Peter Warn [1], Pia Thommes [1], Andrew Sharp [1], Abdul Sattar [1], Nasrullah Undre [2], Jose Alejandro Palacios-Fabrega [2], Andreas Karas [2]

Antifungal Activity of Novamycin® (NP339) *in vivo* in Respiratory Models of Fungal Infection

NovaBiotics
NATURE'S ANTIMICROBIALS

D. K. Mercer[1], V. M. S. Duncan[1], L. K. Katvars[1], D. W. Smith[1], T. Shaw[1], K. Holden[2], P. Warn[2] and D. A. O'Neil[1]
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www.novabiotics.co.uk

Introduction

Pulmonary infections caused by fungi (including *Aspergillus* spp. and *Exophiala* spp.) continue to pose a significant risk to particular patient groups including immunocompromised individuals and those with cystic fibrosis, and there remains an obvious unmet clinical need for new, improved therapies to combat these infections. The incidence of fungal pathogens demonstrating resistance, or insensitivity to current antifungal therapies is becoming increasingly common, and poses a significant problem to healthcare providers due to the negative outcomes for these patients. Novamycin® (NP339) has previously been demonstrated *in vitro* to be a rapidly acting fungicidal peptide effective against a broad spectrum of pathogenic fungal species, including, but not exclusively *Aspergillus* spp. and *Exophiala* spp. (Poster 7289). Novamycin® (NP339) has been formulated for direct delivery into the lung in aerosolised form, and is intended as a monotherapy or to be applied in combination with existing standard of care antifungals to tackle clinically challenging respiratory fungal infections.

Methods

We previously demonstrated *in vitro* efficacy of Novamycin® (NP339) against *Aspergillus* spp. and *Exophiala* spp. The activity of nebulised Novamycin® (NP339), as a monotherapy (nebulised twice daily (BD) at 1-10 mg/mL for 10 min) and in combination with intravenous and oral azoles and amphotericin B (AmBisome), was assessed in preliminary *in vivo* experiments utilising established murine models of invasive pulmonary aspergillosis. Efficacy was determined by measuring the survival, weight and temperature for up to 6 days post treatment of nebulised Novamycin® (NP339) alone or as an adjunct to systemic standard of care therapies.

Results

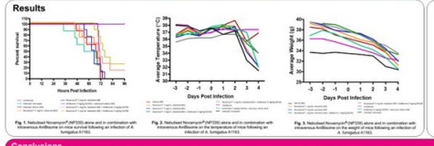

Fig. 1. Nebulised Novamycin® (NP339) alone and in combination with Intravenous AmBisome in mice survival following an infection of *A. fumigatus* A1163.

Fig. 2. Nebulised Novamycin® (NP339) alone and in combination with Intravenous AmBisome on the temperature of mice following an infection of *A. fumigatus* A1163.

Fig. 3. Nebulised Novamycin® (NP339) alone and in combination with Intravenous AmBisome on the weight of mice following an infection of *A. fumigatus* A1163.

Results

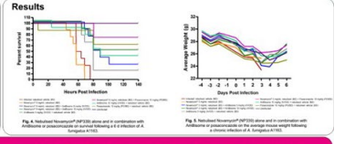

Fig. 4. Nebulised Novamycin® (NP339) alone and in combination with Amphotericin B (AmBisome) on survival following a 4 X infection of *A. fumigatus* A1163.

Fig. 5. Nebulised Novamycin® (NP339) alone and in combination with Amphotericin B (AmBisome) on the average mouse weight following a chronic infection of *A. fumigatus* A1163.

Conclusions

Novamycin® (NP339), in combination with intravenous amphotericin B and oral posaconazole in the 96h and 140h clinically relevant *in vivo* rodent models of *Aspergillus* spp. lung infection demonstrated a greater survival as well as better indicators of health such as weight and temperature compared to the monotherapies. This preliminary data suggest that the combination of Novamycin® (NP339) with systemic antifungals may be a useful strategy in addressing disseminated fungal infection, improving outcomes from treatment with standard of care therapies alone. Our data also suggests that Novamycin® (NP339) can be viably delivered via nebulisation. Novamycin® (NP339) is distinct from current therapies in its rapidly fungicidal membranolytic mechanism of action with proven potent *in vitro* data and with these initial *in vivo* findings warrants further optimisation and investigation as an adjunct inhaled antifungal therapy for use in invasive aspergillosis alongside standard of care systemic therapies.

#RESEARCHNEVERSTOPS

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