



# Neue Antimykotika

# Neue Wirkstoffe gegen Pilze

WK-Code	Wirkstoff (WK) Name	WK-Gruppe	Wirksam gegen:	Abstract
--	Tamoxifen	Östrogenrezeptor-Antagonist	<i>C. albicans</i> ; <i>S. cerevisiae</i> ; <i>Cryptococcus</i> spp.	<a href="#">M-2130</a>
AN 2718	Benzoxaborole	Proteinsyntheseinhibitor	Dermatophyten	<a href="#">F1-1176</a>
FG 3409	k.A. („small molecule“)	k.A.	Sprosspilze/+ Hyphomyceten	<a href="#">F1-1177</a> ; <a href="#">F1-1178</a> ; <a href="#">F1-1179</a> ; <a href="#">F1-1180</a> ; <a href="#">F1-1181</a> ; <a href="#">F1-1182</a> ; <a href="#">M-1178</a>
FR290581	Sodarín-Derivat	Sodarín	<i>Candida albicans</i> ; <i>Pneumocystis jirovecii</i>	<a href="#">F1-1183</a>
BAL8557	Isavucoazol	Azol	Sprosspilze/+ Hyphomyceten	<a href="#">M-2137</a> ; <a href="#">M-2140</a> ; <a href="#">M-1226</a>
ITF2534	Triazol	Azol	<i>Cryptococcus</i> spp.	<a href="#">M-2127</a>
ITF2537	Azol- Wirkungsverstärker	Histondeacetylase-Inhibitor	<i>Cryptococcus</i> spp.	<a href="#">M-2127</a>
MGCD290	Azol- Wirkungsverstärker	Histondeacetylase-Inhibitor	<i>C. albicans</i>	<a href="#">M-2129</a>
NB-002	Nanoemulsion	Öl/Wasser-Emulsion	Dermatophyten	<a href="#">M-2134</a> ; <a href="#">M-2135</a>
--	Gentianaviolett	Farbstoff	<i>C. albicans</i> Biofilme	<a href="#">M-1566</a>

# F1-1176

## Antifungal Activity and Mechanism of Action of a Benzoxaborole, AN2718, which is in Development for the Treatment of Tinea Pedis

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**Background:** AN2718 is a member of a new class of antifungals, benzoxaboroles, which inhibit fungal growth by blocking protein synthesis. AN2718 is being developed for the topical treatment of tinea pedis including the hard to treat moccasin-type, which at present is only treatable with oral antifungals.

**Methods:** MICs were determined according to CLSI guidelines. The *Candida albicans* and *Aspergillus fumigatus* cytoplasmic leucyl-tRNA synthetases (LeuRS) were over-expressed in *E. coli* as N-terminal six-histidine-tagged proteins and purified by standard techniques using a nickel column. Enzyme inhibition was determined by the inhibition of leucine incorporation into crude baker's yeast tRNA measured by TCA precipitation. To obtain a crystal structure for the *C. albicans* cytoplasmic leucyl-tRNA synthetase we over-expressed the C-terminal six-histidine-tagged editing domain.

**Results:** AN2718 has a broad-spectrum of antifungal activity with MIC<sub>90</sub> of 1 µg/mL, 0.25 µg/mL, 1 µg/mL, 0.5 µg/mL for *C. albicans* (n=100), *C. glabrata* (n=100), *Trichophyton mentagrophytes* (n=100) and *T. rubrum* respectively (n=100). We show that AN2718 inhibits cytoplasmic LeuRS from molds, *A. fumigatus*, and from yeasts, *C. albicans*, with an IC<sub>50</sub> of 2 µM and 4.2 µM, respectively. An analogue of AN2718, AN3018, was shown by co-crystal structure determination to bind to the editing active site as an adduct with AMP, a surrogate for the terminal ribonucleotide of tRNA. The boron in AN3018 was bound to the *cis*-diol on the ribose of AMP in the active site.

**Conclusions:** AN2718 Inhibits LeuRS by the OBORT mechanism of trapping tRNA<sup>LEU</sup> in the editing site of LeuRS. AN2718 has good MIC<sub>90</sub> activity against the dermatophytes, *T. rubrum* and *T. mentagrophytes*, which supports its use as a topical agent to treat tinea pedis.

# F1-1177

## FG3409: A Novel Small Molecule Antifungal Agent with Activity against *Aspergillus* spp.

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**Background:** FG3409 is a first in class small molecule antifungal agent that exhibits a novel mechanism of action and demonstrates activity against a broad range of filamentous fungi. The objective of this study was to compare the *in vitro* activity of FG3409 with that of amphotericin (AMB), and itraconazole (ITZ) against a panel of *Aspergillus* clinical isolates from the UK.

**Methods:** Susceptibility tests were performed using the microdilution plate modification of the CLSI M38-A2 method against clinical isolates of *Aspergillus* comprising 36 *A. fumigatus*, 17 *A. terreus*, 19 *A. flavus*, and 19 *A. niger*. The panel included isolates known to be resistant to ITZ.

**Results:** MIC distributions for FG3409, AMB, and ITZ were as follows. MICs are in mg/L, MIC<sub>90</sub> is the MIC value inhibiting 90% of strains.

	FG3409	AMB	ITZ	FG3409	AMB	ITZ
	Range	Range	Range	MIC <sub>90</sub>	MIC <sub>90</sub>	MIC <sub>90</sub>
<i>A. fumigatus</i>	0.008-0.06	0.5-4	0.5-16	0.03	2	2
<i>A. terreus</i>	0.015-0.03	2-4	0.5-1	0.03	4	1
<i>A. niger</i>	0.015-0.25	0.5-1	0.5-16	0.06	1	4
<i>A. flavus</i>	0.015-0.06	1-4	0.5-4	0.03	2	1
All strains	0.008-0.25	0.5-4	0.5-16	0.06	2	2

FG3409 was the most potent of the agents tested, and exhibited excellent *in vitro* activity against all 4 *Aspergillus* species. *A. terreus* and *A. flavus* were highly susceptible to FG3409 with *A. niger* displaying slightly higher MICs than other species. Seven isolates displayed resistance to ITZ (MICs >4mg/L) and all were highly susceptible to FG3409.

**Conclusions:** FG3409 is a novel antifungal agent that is highly active against the most clinically relevant *Aspergillus* spp. and exhibits greater potency across all species than other antifungal drugs tested. Isolates that demonstrate resistance to azoles were shown to be highly susceptible to FG3409 as were species such as *A. terreus* and *A. flavus* which are often resistant to agents within the polyene class of drugs.

# F1-1178

## ***In Vitro* Activity of FG3409 Compared to Posaconazole and Voriconazole Against a Panel of 163 Clinical Fungi**

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**Background:** Despite advances in antifungal therapy, certain fungi are resistant to all available antifungal agents. As a result, there is an urgent need for new agents with activity against such resistant fungi. FG3409 is the first of a novel class of antifungal agent. Here we compared the spectrum and potency of FG3409 with posaconazole (POSA) and voriconazole (VORI) against a range of clinical fungi, including aspergilli, agents of hyalohyphomycosis, phaeohyphomycosis, dermatomycosis and the endemic mycoses.

**Methods:** Testing methods are outlined in CLSI document M38-A2. All organisms were tested in microdilution format except dimorphic fungi and some species requiring extended incubation which were tested by macrobroth method. MIC ranges were 0.06-32µg/ml (FG3409) and 0.015-8µg/ml (POSA/VORI). POSA and VORI endpoints were determined as the lowest concentration giving 100% growth inhibition, 50 and 100% growth reduction endpoints were determined for FG3409.

**Results:** The FG3409 MIC<sub>50/90</sub> for all fungi was 0.125/>32µg/ml using both endpoints. The MIC<sub>50/90</sub> for POSA and VORI were 0.06/8µg/ml and 0.5/>8µg/ml respectively. FG3409 was inactive against 37 isolates. After eliminating data for these fungi, the MIC<sub>90</sub> fell to 0.5/2 (50/100%) µg/ml showing that FG3409 is highly active against species that do not display outright resistance. When the same criteria were applied to POSA and VORI, the MIC<sub>90</sub> fell to 1 and 2µg/ml respectively, showing that FG3409 compared favorably to both drugs. FG3409 had excellent activity against aspergilli, the endemic mycoses and dermatophytes, and in addition was active against *Scedosporium prolificans*, *Fusarium solani*, *F. oxysporum*, *Scopulariopsis brevicaulis* and *Paecilomyces variotii* which were resistant to VORI and in some cases POSA.

**Conclusions:** FG3409 has potent activity against a broad range of fungi including some azole-resistant species and is an excellent candidate for further development.

# F1-1179

## Pharmacokinetics of the Novel Antifungal Agent, FG3409 in Mouse

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**Background:** FG3409 is a new small molecule antifungal agent belonging to a novel structural class displaying a mechanism of action not seen before in the treatment of human mycoses. The compound demonstrates excellent *in vitro* and *in vivo* activity against *Aspergillus* spp. Detailed PK studies are required to help interpret *in vivo* efficacy data.

**Methods:** PK profiling in the mouse was performed in order to determine total drug concentrations in plasma, and tissue distribution following IV and oral dosing. FG3409 was solubilised in appropriate vehicles for IV injection, and formulated as a suspension or a solution for oral dosing. Blood and tissue samples were taken at appropriate time intervals. Plasma was analysed following protein precipitation with acetonitrile and tissues were homogenised in PBS followed by precipitation with acetonitrile. Concentrations of FG3409 in these matrices were determined using LC/MS/MS techniques.

**Results:** The PK parameters of FG3409 following a 20mg/kg IV dose in CD-1 mice are shown in the table. FG3409 shows excellent distribution into key tissues such as kidney and lung and can also be detected in brain and skin tissue, although levels were lower than those seen in plasma after a single dose. The kinetics of FG3409 dosed orally varied depending on formulation, however, dosing as a suspension with a reduced particle size resulted in an oral bioavailability of ~50%.

**Conclusions:** FG3409, when dosed intravenously in mouse, has a plasma half life of 4.1h and is well distributed into important tissues including lung, kidney and brain. Oral bioavailability of up to 50% can be achieved resulting in potentially therapeutic levels by this route.

		<b>C<sub>max</sub></b> <b>(ng/ml)</b>	<b>AUC</b> <b>(ng-h/ml)</b>	<b>Elimination t1/2</b> <b>(h)</b>	<b>Vd</b> <b>L/kg</b>	<b>Cl</b> <b>L/h/kg</b>
	Plasma	7013	31817	4.1	3.6	0.62
FG3409	Kidney	8811	59292	4.6	2.2	0.33
	Lung	9537	50804	4.8	2.7	0.39

# F1-1180

## FG3409: eADMET Profile of a First in Class Antifungal Agent

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**Background:** FG3409 is a novel small molecule antifungal agent that displays *in vitro* activity against a broad range of filamentous fungi and demonstrates excellent efficacy in murine models of *A. fumigatus* infection. The eADMET properties of this analogue were assessed to determine the potential for liabilities associated with drug-drug interactions (DDI's), cytotoxicity, mutagenicity, and hERG inhibition. Many agents used currently to treat serious fungal disease exhibit drug-drug interactions and toxicity issues.

**Methods:** CYP inhibition (human liver microsomes), CYP induction (human hepatocytes), Ames test, Caco-2 permeability, mammalian cell viability, and hERG inhibition assays were performed using validated, industry recognised methods. In addition to *in vitro* ADMET profiling, short term *in vivo* tolerability was also investigated prior to assessment of FG3409 in *in vivo* efficacy studies.

**Results:** FG3409 did not exhibit cytotoxic activity against several mammalian cell lines at levels  $>20\mu\text{g/ml}$ , and did not inhibit *E. coli* or *S. aureus* in standard bacterial MIC tests. The 5 main CYP isoforms (1A, 2C19, 2C9, 2D6 and 3A4) were not inhibited by FG3409 ( $\text{IC}_{50}$ 's  $>25\mu\text{M}$ ) and there was no significant induction of the two common CYP inducible isoforms (1A and 3A4). The Caco-2 permeability assay suggested that FG3409 was permeable and not a substrate for Pgp. The Ames test failed to show mutagenic activity using 2 *Salmonella* strains with or without S9 fraction activation. The predicted  $\text{IC}_{50}$  for the hERG assay (patch clamp) was  $30.5\mu\text{M}$  indicating a low likelihood for QT prolongation at efficacious doses in animals. The compound was extremely well tolerated in laboratory animals at doses higher than what is considered to be the therapeutic dose required for *in vivo* efficacy.

**Conclusions:** FG3409 demonstrates a clean cytotoxicity profile. The compound was well tolerated in rodents, even at very high doses, and the lack of inhibition or induction of the main CYP isoforms and lack of interaction with Pgp suggest that the compound has a low potential for DDI's.

# F1-1181

## ***In Vivo* Efficacy of a New Antifungal Agent, FG3409 in a Murine Model of Disseminated Aspergillosis**

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**Background:** There is an urgent need for new classes of antifungal agents due to concerns of toxicity and resistance of current agents. FG3409 is a member of a new class of small molecule agents with a novel mode of action and potent *in vitro* activity against *Aspergillus* spp. and other filamentous fungi. In this study the efficacy of FG3409 was compared with caspofungin (CAS) and itraconazole (ITZ) in a survival model of *A. fumigatus* infection.

**Methods:** Male CD1 mice were immunosuppressed with cyclophosphamide generating temporary neutropenia for 5-6 days. 3 days post immunosuppression mice were infected IV with an LD<sub>90</sub> challenge of *A. fumigatus* A1163. 5 h post infection mice were treated with CAS 1 or 5mg/kg IP once daily, oral ITZ 25mg/kg TDS/BD. FG3409 was dosed in DMSO/cremophor IV 20mg/kg TDS or as a nanoparticulate suspension (1, 2, 5, 10, 20 or 50mg/kg) IV once daily. Mice were treated for 9 days and observed for a further 2 days.

**Results:** Vehicle controls exhibited 90-100% mortality (mean survival time (MST) 3.4-5.5 days). CAS (1 and 5 mg/kg) and ITZ resulted in survivals of 90, 100 and 90% respectively. All FG3409 formulations were well tolerated with no local irritation, phlebitis or systemic signs of intolerance. FG3409 20mg/kg TDS formulated in DMSO/cremophor gave 100% survival (superior to vehicle  $p < 0.001$  and equivalent to CAS and ITZ). FG3409 administered as a nanosuspension had 0, 20, 50, 60, 100 and 100% survival for 1, 2, 5, 10, 20 and 50mg/kg (MST 4, 6.5, 9, 9.5, >11 and >11 days respectively); all doses other than 1mg/kg were superior to vehicle at improving survival (e.g. vehicle vs. 20mg/mg  $p < 0.001$ ).

**Conclusions:** FG3409 was well tolerated in mice at doses up to of 60mg/kg/day and demonstrated a dose dependent response at improving survival following a lethal *A. fumigatus* infection. FG3409 was at least as effective as CAS and ITZ in this murine model of disseminated aspergillosis.

# F1-1182

## Efficacy of FG3409 a New Antifungal Agent, in Reducing Tissue Burden in Murine Models of Disseminated Aspergillosis

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**Background:** FG3409 is the first of a new class of antifungals with a novel mode of action. FG3409 is active *in vitro* against *Aspergillus* and other fungi and improves survival in *A. fumigatus* (AF) murine models. Here FG3409 formulated in cremophor/DMSO (DC) or as a nanosuspension (N) and dosed IV and PO was compared with itraconazole (ITZ) and caspofungin (CAS) in murine tissue burden models.

**Methods:** Male CD1 mice were immunosuppressed with cyclophosphamide (CP) (temporary neutropenia 5-6 days) and infected 3 days later IV with *A. fumigatus* A1163. 5h later mice were treated with FG3409DC (20mg/kg TDS) IV, or FG3409N (20mg/kg BD PO), CAS 1 or 5mg/kg IP once daily, or ITZ 25mg/kg TDS/BD PO. Mice were treated for 4 days. 5 days post infection kidney burden was quantified by culture. In other experiments, mice received a further dose of CP 4 days after the 1<sup>st</sup> dose to induce persistent neutropenia (PN) and were treated similarly. A further experiment compared IV FG3409 N (2, 5, 10 & 50mg/kg OD) with CAS 5mg/kg given once daily for 10 days and kidney burden assessed 12 days post infection.

**Results:** In the 5 day study IV FG3409DC reduced the AF kidney burden by 2.4 log<sub>10</sub>cfu/g superior to ITZ (1.8 log<sub>10</sub>cfu/g reduction). Oral FG3409 was superior to vehicle and reduced the AF kidney burden by 0.84log<sub>10</sub>cfu/g. In the PN model FG3409DC showed a 2.6 log<sub>10</sub>cfu/g reduction compared with 0.23 log<sub>10</sub>cfu/g (ITZ) and 2.0 log<sub>10</sub>cfu/g (CAS) In the 11 day study IV FG3409N was highly effective at reducing burden and 50mg/kg/day sterilized kidneys in 50% of mice: in contrast CAS 5mg/kg sterilized no kidneys p<0.02. FG3409N 20mg/kg/day had similar efficacy to CAS 5mg/kg.

**Conclusions:** FG3409 was highly effective at reducing AF kidney burden following disseminated aspergillosis being at least as effective as CAS and superior to ITZ when administered IV. FG3409N was also effective at reducing counts when dosed orally but was less effective than IV dosing.

# F1-1183

## Antifungal Activity of a Novel Orally Active Sordarin Derivative, FR290581

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**Background:** The antifungal natural product sordarin inhibits protein synthesis by a mechanism involving selective targeting of translation elongation factor 2 in fungi. Because of the weak antifungal activity and poor pharmacokinetic profile of sordarin itself, however, in vivo antifungal activity is negligible. We have discovered a novel derivative, FR290581 (FR), by chemical modification of sordarin, the aglycone unit of sordarin. We report herein the superior antifungal activities of FR in vitro and in vivo.

**Methods:** In vitro antifungal activity against pathogenic fungi was evaluated according to CLSI M27-A2, M38-A, and using animal serum. Therapeutic activity against *Candida albicans* (C.a)- infected ICR mouse models and *Pneumocystis jirovecii* (P.j)-infected SCID mouse model was evaluated orally.

**Results:** FR displayed potent in vitro activity against C.a, *C. tropicalis*, and *C. kefyr*, which was comparable to fluconazole (FLCZ), but showed only negligible activity against *C. parapsilosis*, *C. neoformans*, and *A. fumigatus*. In a mouse systemic candidiasis model of C.a, FR had a comparable survival effect to FLCZ and had fungicidal activity against kidney fungal burden whereas FLCZ had only a fungistatic effect. In mouse oropharyngeal candidiasis, FR had a similar mycological effect in tongue as compared with FLCZ. FR also had more potent in vivo activities against P.j as compared with sulfamethoxazole-trimethoprim (ST). (see table)

**Conclusions:** FR had potent in vivo activity against C.a and P.j compared with existing drugs. The antifungal profile of FR suggests it may be useful for AIDS patients.

		Number of P.j cysts and histological changes in mouse lung		
Group		No. of cysts (x 10 <sup>3</sup> )	Pneumonia (positive)	Mononuclear cell infiltration
Control	-	1187.5±628.9	8/8	impossible for analysis
FR	5mg/kg	0.6±1.6*	0/8	2/8
	20mg/kg	0*	0/8	1/8
ST	120mg/kg	1.1±1.9*	0/8	8/8

\*: significantly difference from the control (p<0.01)

# M-1178

## ***In Vitro* Activity of FG3409 Compared to Posaconazole and Voriconazole Against a Panel of 163 Clinical Fungi**

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<sup>1</sup>Univ. Texas Hlth.Sci. Ctr., San Antonio, TX, <sup>2</sup>F2G Ltd, Manchester, United Kingdom.

**Background:** Despite advances in antifungal therapy, certain fungi are resistant to all available antifungal agents. As a result, there is an urgent need for new agents with activity against such resistant fungi. FG3409 is the first of a novel class of antifungal agent. Here we compared the spectrum and potency of FG3409 with posaconazole (POSA) and voriconazole (VORI) against a range of clinical fungi, including aspergilli, agents of hyalohyphomycosis, phaeohyphomycosis, dermatomycosis and the endemic mycoses.

**Methods:** Testing methods are outlined in CLSI document M38-A2. All organisms were tested in microdilution format except dimorphic fungi and some species requiring extended incubation which were tested by macrobroth method. MIC ranges were 0.06-32µg/ml (FG3409) and 0.015-8µg/ml (POSA/VORI). POSA and VORI endpoints were determined as the lowest concentration giving 100% growth inhibition, 50 and 100% growth reduction endpoints were determined for FG3409.

**Results:** The FG3409 MIC<sub>50/90</sub> for all fungi was 0.125/>32µg/ml using both endpoints. The MIC<sub>50/90</sub> for POSA and VORI were 0.06/8µg/ml and 0.5/>8µg/ml respectively. FG3409 was inactive against 37 isolates. After eliminating data for these fungi, the MIC<sub>90</sub> fell to 0.5/2 (50/100%) µg/ml showing that FG3409 is highly active against species that do not display outright resistance. When the same criteria were applied to POSA and VORI, the MIC<sub>90</sub> fell to 1 and 2µg/ml respectively, showing that FG3409 compared favorably to both drugs. FG3409 had excellent activity against aspergilli, the endemic mycoses and dermatophytes, and in addition was active against *Scedosporium prolificans*, *Fusarium solani*, *F. oxysporum*, *Scopulariopsis brevicaulis* and *Paecilomyces variotii* which were resistant to VORI and in some cases POSA.

**Conclusions:** FG3409 has potent activity against a broad range of fungi including some azole-resistant species and is an excellent candidate for further development.

# M-1566

## Gentian Violet Exhibits Potent Antifungal Activity against *Candida albicans* Biofilms Formed on Denture Acrylic In Vitro

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**Background:** Resistance of microbial biofilms explains the persistence/relapse of many infections, including oral candidiasis (OC). Thus, effective agents against biofilms would be of clinical importance. We recently showed that gentian violet (GV) has potent activity against planktonic *Candida* strains isolated from the oral cavity of HIV-infected patients. However, its activity against *Candida albicans* (CA, the predominant cause of OC in resource limited countries) biofilm has not been evaluated. In this study, we compared the activity of GV vs. fluconazole (FLC) against CA biofilms formed on polymethylmethacrylate substrate.

**Methods:** Two CA isolates, one FLC-susceptible (FLCS) and one FLC-resistant (FLCR) were allowed to form mature biofilm on our denture biofilm model in vitro. These biofilms were then exposed to different concentration of GV (0.03 to 256 µg/ml) and FLC (0.5 to 64 µg/ml). Effect of these agents on biofilm formation was quantified using tetrazolium-based XTT and dry weight (DW) assays. Drug concentration that caused 50% reduction in metabolic activity (RMA<sub>50</sub>) and DW of *Candida* biofilm compared with control was determined. Experiments were performed in triplicate on three separate days.

**Results:** GV showed potent antifungal activity against CA biofilms (RMA<sub>50</sub> 4 and 8µg/ml for FLCS and FLCR, respectively). Moreover, GV caused at least 50% reduction in biofilm mass compared to control (4.09 ± 1.77, 2.92 ±0.47 and 0.74 ±0.29 mg, for control, FLCS and FLCR, respectively P<0.05). FLC did not show any activity against biofilms (RMA<sub>50</sub> >64 µg/ml for the tested organisms).

**Conclusions:** Our results showed that GV exhibited potent activity against denture-associated CA biofilms in vitro. Further studies against expanded panel of strains and in vivo testing are warranted.

# M-2127

## Efficacy of ITF2534 and ITF2357 against Systemic Cryptococcosis

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**Background:** ITF2534 (Italfarmaco, Milan) is a novel triazole with antifungal activity. ITF2357 is a histone deacetylase inhibitor shown to inhibit proinflammatory cytokine expression. ITF2534 alone was tested for antifungal efficacy, and ITF2357 was examined, in combination with fluconazole (FCZ), to determine if its anti-inflammatory activity was beneficial to conventional treatment against cryptococcosis.

**Methods:** The in vitro activity of ITF2534 was tested by CLSI methods. A murine model of systemic cryptococcosis was established in 6-week-old female CD-1 mice by i.v. inoculation of  $1.7 \times 10^6$  yeasts of *C. neoformans* 9759. Treatment (PO, 19 days) began 4 days later with mice receiving no treatment, FCZ at 10 or 50 mg/kg QD, ITF2534 at 10 or 50 mg/kg QD or 50 mg/kg/dose given BID, ITF2357 at 1, 5 or 10 mg/kg QD alone or in combination with FCZ at 10 mg/kg QD. Survival was tallied through 49 days and CFU in the organs determined.

**Results:** In vitro, against *C. neoformans*, ITF2534 had MICs  $\leq 0.5$   $\mu\text{g/ml}$  (6/6 strains) and MFCs of 1  $\mu\text{g/ml}$  (5/6 strains). In vivo, 90% of control mice died between days 19 and 24. No regimen had >50% survival. FCZ at 10 or 50 mg/kg and ITF2534 50 mg/kg BID prolonged survival ( $P < 0.05$ ). ITF at 50 mg/kg QD delayed deaths versus controls. ITF2357 alone had no significant efficacy, but 5 mg/kg delayed deaths versus controls. ITF2357 at 5 or 10 mg/kg plus FCZ at 10 mg/kg had a negative interaction; mice given the combination died sooner than those given FCZ alone ( $P = 0.06$  to  $<0.0001$ ). No surviving mice in any group were free of detectable infection in the brain, the key organ of infection.

**Conclusions:** Overall, ITF2534 at 50 mg/kg BID had efficacy and ITF2357 had minimal activity in vivo against cryptococcosis. Higher dosages of ITF2534 may be required to provide greater protection. FCZ appears to be about 5X as potent as ITF2534. These data warrant further study on ITF2534 for the treatment of cryptococcosis.

# M-2129

## Combination Testing of MGCD290, a Fungal Histone Deacetylase Inhibitor, with Azole Antifungals against a Large Collection of Clinical Fungal Isolates

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**Background:** We previously demonstrated that the fungal histone deacetylase (HDAC) inhibitor MGCD290 (MG; MethylGene, Inc.) demonstrated synergy with azoles against *Candida* and *Aspergillus*. We now report the *in vitro* activity of MG-azole combinations against a large, diverse collection of clinical isolates, including azole-resistant yeasts and molds.

**Methods:** Susceptibility testing was performed by CLSI M27-A3 and M38-A2 broth microdilution methods. Combination testing was performed by checkerboard (MG in combination with fluconazole (FLU), posaconazole (POS) and voriconazole (VOR)). Fractional inhibitory concentration was calculated and defined as: synergy,  $<0.5$ ; indifference,  $\geq 0.5$  but  $<4$ ; and antagonism,  $\geq 4$ .

**Results:** 91 isolates were tested: 30 *Candida* spp., 10 *Aspergillus* spp., 15 zygomycetes, 10 *Cryptococcus neoformans*, 8 *Rhodotorula*, 8 *Fusarium*, 5 *Trichosporon*, and 5 *Scedosporium* spp. MG demonstrated synergy with FLU, POS and VOR against 55 (60%), 46 (51%) and 48 (53%), respectively, of the 91 isolates. FLU + MG synergy was demonstrated for 26/30 (87%) *Candida*. All 23 *Candida* that were not FLU susceptible (2 susceptible dose-dependent and 21 resistant) demonstrated a reduction in FLU MIC that crossed a breakpoint when combined with MG at 0.12-4 mcg/mL. FLU + MG also demonstrated synergy against 6/10 *Aspergillus* spp., and for 5 the FLU MIC was reduced to  $\leq 32$  mcg/mL when combined with MG at concentrations of 4-32 mcg/mL. POS + MG demonstrated synergy against 14/15 zygomycetes tested (9 *Rhizopus* and 5 *Mucor*), with a [POS] of  $\leq 0.25$  mcg/mL when combined with MG for all 14 isolates. VOR + MG demonstrated synergy against 6/8 *Fusarium* tested.

**Conclusions:** MGCD290 demonstrated *in vitro* synergy with azoles against most clinical fungal isolates, including species groups inherently resistant to azoles (e.g. *Mucor*, *Fusarium*). Further evaluation and development of MGCD290-azole combinations is warranted.

# M-2130

## Antifungal Activity of Tamoxifen: Scope and Mechanism

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Screening existing drugs for new biological activities is emerging as an expedient approach to the identification of lead compounds for new drug development. As part of a high-throughput screen for small molecules that cause yeast cell lysis, we identified tamoxifen (TAM) and its closely related analog clomiphene as active fungicidal agents. TAM is used clinically as an estrogen receptor antagonist in the treatment of breast cancer but it was known to have activity against both *C. albicans* and *S. cerevisiae* (MIC 6-8 mcg/mL). We found it also has similar levels of activity against *Cryptococcus neoformans*. The molecular mechanism of the antifungal activity of TAM is not known. Since TAM inhibits calmodulin function in human cells, we hypothesized that the antifungal activity of TAM may be due to inhibition of calmodulin. Based on studies in *S. cerevisiae*, we have developed five lines of evidence supporting calmodulin as the yeast target of TAM. First, TAM treated yeast cells phenocopy cell morphologic, nuclear separation and actin cytoskeletal defects displayed by calmodulin mutants. Second, calmodulin mutants are hypersensitive to TAM. Third, overexpression of wild type calmodulin in yeast induces resistance to TAM. Fourth, calmodulin is mislocalized in TAM-treated yeast cells. Fifth, co-immunoprecipitation studies indicate that TAM disrupts the binding of proteins to yeast calmodulin. In *C. albicans*, TAM treatment inhibits germ tube formation, a known effect of calmodulin inhibitors. Since the structure of calmodulin in yeast has diverged significantly from human calmodulin, our results suggest that TAM and, more generally, triarylethylenes may represent an attractive class of lead compounds for the development of calmodulin-targeted antifungal drugs.

# M-2134

## NB-002, a Novel Nanoemulsion with Anti-Dermatophyte Activity

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**Background:** NB-002 is an oil-in-water emulsion made with pharmaceutically approved ingredients. The nanodroplets have an average diameter of 180 nm, and their size and composition allows for selective uptake into hair follicles and pores. With translateral diffusion to the site of infection (nailbed), this topical formulation has been designed for safe treatment of onychomycosis.

**Methods:** The minimum inhibitory concentrations (MIC) of NB-002 and comparator compounds against clinical isolates of dermatophytes were determined using methodology described by Ghannoum, et. al (J. Clin. Microbiol. 2006;44:4353-4356). For minimum fungicidal concentrations (MFC), an inoculum of  $1-3 \times 10^4$  cfu/ml was used, thereby permitting 10-30 colonies per plate even if there was 99.9% reduction in the initial inoculum.

**Results:** NB-002 was found to be uniformly active against dermatophytes, with MIC<sub>90</sub>/MFC<sub>90</sub> values as indicated in the table. The MIC and MFC ranges were narrow for NB-002 with the nanoemulsion demonstrating fungicidal activity against every isolate. Comparators showed marked heterogeneity in their MIC and MFC responses; MIC<sub>90</sub>/MFC<sub>90</sub> results indicated that the comparators were fungistatic.

**Conclusions:** NB-002 was consistently fungicidal against clinical isolates of dermatophytes while comparators were not. The fungicidal activity is consistent with the kill-on-contact mechanism of action of NB-002 seen in other studies. NB-002 is currently in a double-blind, vehicle-controlled phase 2 clinical trial at multiple sites in North America.

Active	<i>Trichophyton rubrum</i> (n=15)		<i>Trichophyton mentagrophytes</i> (n=13)		<i>Epidermophyton floccosum</i> (n=6)	
	Values (µg/ml)					
	MIC <sub>90</sub>	MFC <sub>90</sub>	MIC <sub>90</sub>	MFC <sub>90</sub>	MIC range	MFC range
NB-002	2	2	4	4	2 - 4	4
Ciclopirox	2	16	2	16	0.25-2	0.25->32
Terbinafine	>1	>1	0.25	>1	0.0313-0.25	0.0313-1
Itraconazole	2	>16	2	>16	0.5	0.5->16
Econazole	0.25	4	0.25	>16	0.0625-0.125	0.0625-4
Griseofulvin	4	4	2	>16	1-2	1-16

# M-2135

## Mechanism of Skin Penetration and Distribution of a Novel Nanoemulsion

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**Background:** Nanoemulsions (NE) are oil-in-water emulsions containing high energy nanometer-sized droplets, stabilized by surfactants. These droplets are bioavailable in dermal tissues, but do not disrupt normal epithelial matrix. To treat fungal infections under the nail plate, we examined the ability of NE to diffuse laterally along tissue planes to reach sites of infection up to 2 cm away from site of skin application.

**Methods:** Permeation of NE, NB-002, into human cadaver skin was assessed via tissue levels (assayed by HPLC) of cetylpyridinium chloride (CPC), a cationic surfactant oriented at the oil-water interface of the NE and used as the marker for nanodroplet delivery. Lateral diffusion experiments, were conducted using a modified diffusion apparatus where the outer dosing area was sealed off from the inner (non-dosing) area using glass rings with an inner diameter of 22 mm. NE was applied to the outer dosing area of a human cadaver skin sample and the non-dosed areas of skin were collected at 24 hours. The collected tissue was divided into a middle area (representing ~ 8 mm of diffusion) and an inner area (representing up to 11 mm of diffusion).

**Results:** The epidermal levels of the NB-002 ( $\mu\text{g CPC/ml}$ ) in the middle and inner areas were 693 and 196, respectively. The dermal levels were 121 and 107, respectively. These data indicate that NE droplets tranverse laterally into skin a distance up to 11 mm from the dosing area. The levels in the epidermis were significantly higher than the minimum fungicidal concentrations for NE against the dermatophytes that cause onychomycosis.

**Conclusion:** Following topical application, NB-002 diffuses laterally along tissue planes to accumulate in the epidermal and dermal tissues at sites up to 1 cm away from the site of application. The safe and efficient delivery route of NE makes it an ideal candidate for treatment of onychomycosis, where dermatophytes infect under the nail plate. NB-002 is currently being studied in a Phase 2 trial in over 400 subjects with onychomycosis.

# M-2137

## Pharmacokinetics, Safety and Tolerability Results of a Dose Escalation Study of Isavuconazole in Neutropenic Patients

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**Background:** Isavuconazole (BAL8557) is an IV and oral broad-spectrum azole antifungal that showed favorable tolerability and efficacy in esophageal candidiasis. Objective of this study was the assessment of increasing doses targeted for prophylaxis of invasive aspergillosis.

**Methods:** In an open-label, multi-center, sequential group trial, patients after chemotherapy for acute myeloid leukemia received 400 and 200 mg of IV isavuconazole on day 1, followed by 200mg bid on day 2, then qd as maintenance treatment. In a second cohort, dosages were doubled. Serial blood samples were taken on day 7, trough levels were monitored on days 2, 3, 5, 7, 14, 21 and EOT. Plasma levels were determined using a validated LC-MS/MS method.

**Results:** The first cohort consisted of 11 patients, the second cohort had 12 patients. PK data from of cohorts were in the range predicted from PK in healthy volunteers. Within 2 to 3 days, the loading dose regimen approached steady-state and there was a dose proportional increase of trough levels approaching steady state. None of the pre-defined criteria were met for preventing progression to the second cohort with doubling of each dosing. Treatment was generally well tolerated, no QTC prolongation, no safety signal with regard to AEs or laboratory was noted.

**Conclusion:** Isavuconazole was well tolerated in neutropenic patients at the given dosages including the dose regimen currently used in phase III for primary treatment of invasive fungal disease. After doubling the dose, PK remained dose proportional.

	Day 1 (mg per dose)	Day 2	Day 3-27	Trough level (µg/mL) day 7	
				Range	Mean and SD
First cohort	400 / 200 / 200	200 / 200	200	1.37 to 2.87	2.06 ± 0.735 (n=5)
Second cohort	800 / 400 / 400	400 / 400	400	2.38 to 5.12	4.06 ± 1.02 (n=6)

# M-2140

## Pharmacokinetics (PK) and Pharmacodynamics (PD) of Isavuconazole in a Murine Model of Disseminated Candidiasis (DC)

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**Background:** Isavuconazole, a triazole, is administered p.o. or i.v. as a water-soluble prodrug and has broad antifungal activity. For triazoles the AUC:MIC ratio is the PD variable which best links drug exposure with effect in candidiasis. We investigated the PK-PD of isavuconazole in a murine model of DC.

**Methods:** A murine model of disseminated candidiasis was used. PK were determined using 3, 9, 24 and 50 mg/kg subcutaneously and analyzed using a 2-compartment PK model. Exposure-response relationships were defined using 0-50 mg/kg and described using an inhibitory sigmoid Emax model with the following parameters: Econ (fungal burden in absence of therapy), Emax (maximal decrement in fungal burden), E50 (dose producing half maximal effect) and slope (H). The endpoint was log<sub>10</sub>CFU/g in the kidney. Dose fractionation studies were performed; the total dose was administered 1, 2 and 4x / day. The PK model was used to transform exposure from dose to peak:MIC, T>MIC and AUC:MIC. Exposure response relationships were recalculated with these measures as the independent variable. The contribution of neutrophils to killing was investigated in persistently vs. temporarily neutropenic mice. The AUC:MIC was linked to the probability of survival following 5 days therapy using a Cox proportional hazard model.

**Results:** The PK were linear: Ka 13.31 h<sup>-1</sup>, Vc 0.063 L, Kcp 6.24 h<sup>-1</sup>, Kpc 14.03 h<sup>-1</sup> and SCL 0.035 L/h (r<sup>2</sup> 0.99). Drug induced killing was described by an inhibitory sigmoid Emax model: Econ 6.09, Emax 2.49, ED50 11.99 mg/kg, H 1.96. The r<sup>2</sup> for peak:MIC, T>MIC and AUC:MIC vs. effect was 40.8, 22.0 and 87.8%, respectively. The AUC:MIC associated with 80% survival probability after 5 days was 3.7x higher in persistently vs. temporarily neutropenic mice.

**Conclusions:** AUC:MIC ratio is the PD variable which best links isavuconazole exposure with the observed effect. Persistently neutropenic hosts require more drug to achieve a high probability of a successful outcome.