

Supplementary Table S1. Preclinical and clinical studies of olorofim's pharmacokinetics and pharmacodynamics

Reference (Study type)	Population	ORL Measurement time	OLR dose Route	PK/PD	Safety Tolerability
Hope <i>et al.</i> <i>mBio</i> 2017 (Preclinical)	neutropenic CD-1 male mice infected by:	Before administration	24 mg/kg/q24h IV	Linear PK between 4-15 mg/kg/q8h Protein binding $\approx$ 99% <u>Dose-fractionation study vs. placebo:</u> <i>PD parameter: serum GM at 78 hours</i> 24 mg/kg/q24h: no significant difference 12 mg/kg/q12h: serum GM reduction ($P = 0,028$) 8 mg/kg/q8h: serum GM total suppression ($P = 0,024$)	No adverse events*
	<i>Aspergillus fumigatus</i> TR ₃₄ /L98H (OLR MIC = 0.03 mg/L)	After administration: H2 H4 H8	8 mg/kg/q8h IV	independent of azole susceptibility → time-dependent antifungal effect <u>PK indexes study:</u> <i>PD parameters: serum GM at 78 hours, AUC-GM 0-78 hours</i> C_{max}/MIC : no correlation AUC/MIC : no correlation C_{min}/MIC : $r^2 = 0.983$ (serum GM) and 0.998 (AUC-GM) $T > MIC$ 1 mg/L: $r^2 = 0.996$ (serum GM) and 1.00 (AUC-GM)	
	<i>Aspergillus fumigatus</i> wild-type (OLR MIC = 0.03 mg/L)	3 mice per dose and per measurement time	15 mg/kg/q8h IV	independent of azole susceptibility → time-dependent antifungal effect <u>C_{min}/MIC, serum GM and AUC-GM:</u> <i>Target: 27% reduction of the AUC-GM</i> $C_{min}/MIC = 9.1$ $C_{min} = 0.27$ mg/L <u>Invasive sinusitis cellular model:</u> <i>PD parameter: GM</i> GM total suppression: $C_{min} = 0.3$ mg/L $C_{min}/MIC \approx 10$	
Negri <i>et al.</i> <i>J Infect Dis</i> 2018 (Preclinical)	neutropenic CD-1 male mice infected by 4 <i>Aspergillus flavus</i> strains (OLR MIC = 0.03 mg/L)	Before administration	24 mg/kg/q24h IV	<u>Sinopulmonary aspergillosis murine model:</u> <i>PD parameters: serum GM at 78 hours, AUC-GM</i>	NM
		After administration: between 6-78 hours	8 mg/kg/q8h IV	Near-total suppression of serum GM for 15 mg/kg/q8h Reduction in serum GM comparable to a posaconazole AUC of 47 mg·h/L: $C_{min}/MIC = [9-19]$, mean = 13.38	
		3 mice per dose and per measurement time	15 mg/kg/q8h IV		
Lackner <i>et al.</i> <i>J Antimicrob Chemother</i> 2018 (Preclinical)	neutropenic CD-1 male mice infected by an <i>A.</i> <i>terreus</i> strain (OLR MIC = 0.008 mg/L)	NM	10 mg/kg/q12h orally or IV	$C_{min} VO = 0.8$ mg/L $C_{min} IV = 0.3$ mg/L $AUC_{0-24h} \approx 22.5$ mg·h/L	No adverse events
Seyedmousavi <i>et al.</i> <i>Antimicrob Agents Chemother</i> 2019	neutropenic CD-1 female mice infected by a wild- type <i>A. fumigatus</i> strain (OLR MIC = 0.008 mg/L)	Before administration	2.5 mg/kg single dose IP	Linear PK between 2.5-20 mg/kg $R = 0.96$ (AUC)	No adverse events
		After administration: H0.5 H1 H2 H4 H8	5 mg/kg single dose IP	$C_{max} = 1.345$ mg/L $C_{min} = 0.185$ mg/L $T_{max} = 0.5$ h $AUC_{0-8h} = 4.399$ mg·h/L	
			10 mg/kg single dose IP		
			15 mg/kg single dose IP	$C_{max} = 2.860$ mg/L $C_{min} = 0.122$ mg/L $T_{max} = 0.5$ h $AUC_{0-8h} = 8.478$ mg·h/L	
		2 mice per dose and per measurement time	20 mg/kg single dose IP		

			$C_{\max} = 5.220 \text{ mg/L}$ $C_{\min} = 0.986 \text{ mg/L}$ $T_{\max} = 0.5 \text{ h}$ $AUC_{0-8h} = 16.840 \text{ mg}\cdot\text{h/L}$		
			$C_{\max} = 5.280 \text{ mg/L}$ $C_{\min} = 1.560 \text{ mg/L}$ $T_{\max} = 0.5 \text{ h}$ $AUC_{0-8h} = 27.244 \text{ mg}\cdot\text{h/L}$		
			$C_{\max} = 4.000 \text{ mg/L}$ $C_{\min} = 2.355 \text{ mg/L}$ $T_{\max} = 2 \text{ h}$ $AUC_{0-8h} = 28.475 \text{ mg}\cdot\text{h/L}$		
Dr. Salvatore Febbraro 2015 (Phase I double- blind, randomized, placebo- controlled clinical trial)	40 healthy male volunteers aged between 18 and 45 years divided into 5 cohorts of 8	Before administration	0.25 mg/kg single dose IV		Serious AE: 0% Other AE: epistaxis 16.67% vs. 50% placebo
		During infusion: H1 - H2 - H3 - H4	0.75 mg/kg single dose IV		Serious AE: 0% Other AE: paresthesia 16.67% vs. 0% placebo
		After infusion: H4.5 - H5 - H5.5 - H6 - H7 - H8 - H10 - H12 - H24 - H36 - H48 - H72 - H96 - H120	1.5 mg/kg single dose IV	NM	Serious AE: 0% Other AE: headaches 16.67% vs. 0% placebo
			3 mg/kg single dose IV		Serious AE: 0% Other AE: eczema 16.67% vs. 0% placebo
			4 mg/kg single dose IV		Serious AE: 0% Other AE: epistaxis 16.67% vs. 0% placebo

*Preliminary studies were conducted to determine the maximal tolerated dose.

AE: adverse events. AUC: area under the concentration-time curve. AUC-GM: area under the serum galactomannan-time curve. $C_{\max}$: maximal plasmatic concentration. $C_{\min}$: minimal plasmatic concentration. GM: galactomannan. IP: intraperitoneal. IV: intravenous. NM: not mentioned. OLR: olorofim. PD: pharmacodynamics. PK: pharmacokinetics. PSC: posaconazole. $T_{\max}$: time needed to reach the $C_{\max}$ after administration.

Supplementary Table S2. Preclinical studies evaluating the efficacy of olorofim in animal models of invasive fungal infection.

Reference	Infection model Fungus (OLR MIC) Population	Control	OLR route OLR dose TT duration	Measurement (method)	Efficacy (P)
Hope et al. mBio 2017	Invasive pulmonary infection through nasal inoculation in a neutropenic murine model	Placebo	IV	Survival on day 10 (log-rank test)	Survival on day 10 vs. placebo <u>OLR 24 mg/kg/q24h, all strains</u> (>0.05) Median survival time = 3.5 days Total survival rate = 17.5% <u>OLR 8 mg/kg/q8h, all strains</u> (<0.001) Median survival time ≈ 8 days Total survival rate = 67.5% <u>OLR 15 mg/kg/q8h, all strains</u> (0.001) Median survival time ≈ 8 days Total survival rate = 60% <u>PSC, azole-susceptible strains</u> (0.001) Median survival time > 10 days Total survival rate = 85% <u>PSC, azole-resistant strains</u> (0.001) Median survival time = 3 days Total survival rate = 20%
	<i>Aspergillus fumigatus</i> 2 WT strains (0.03 mg/L) 2 azole-resistant strains (0.03 mg/L) 10 male neutropenic CD-1 mice per group, per strain	IV excipient 10 mg/kg/day oral posaconazole	24 mg/kg/q24h 8 mg/kg/q8h 15 mg/kg/q8h 3 days	Pulmonary histology on day 3 (GMS)	Pulmonary histology on day 3 vs. placebo <u>OLR 15 mg/kg/q8h</u> Reduction in fungal burden Contained hyphae Minimal vascular lesions <u>PSC</u> Reduction in fungal burden Contained hyphae <u>Placebo</u> Severe inflammation, necrosis Hemorrhage, edema Necrotizing vasculitis, vascular invasion
Negri et al. J Infect Dis 2018	Invasive sinopulmonary infection through nasal inoculation in a neutropenic murine model	Placebo	IV	Survival on day 10 (log-rank test)	Survival on day 10 vs. placebo <u>OLR 24 mg/kg/q24h, all strains</u> (NM) Median survival time = 4.5 days Total survival rate ≈ 21% <u>OLR 8 mg/kg/q8h, all strains</u> (NM) Median survival time ≈ 8 days Total survival rate = 52.5% <u>OLR 15 mg/kg/q8h, all strains</u> (NM) Median survival time ≈ 9 days Total survival rate ≈ 69% <u>PSC, all strains</u> (NM) Median survival time: NM Total survival rate: NM <u>Placebo, all strains</u> Median survival time = 2 days Total survival rate = 0%
	<i>Aspergillus flavus</i> 4 strains (0.03 mg/L) 10 male neutropenic CD-1 mice per group, per strain	IV excipient 20 mg/kg/day oral posaconazole	24 mg/kg/q24h 8 mg/kg/q8h 15 mg/kg/q8h 3 days	Pulmonary histology on day 3 (GMS)	Pulmonary histology on day 3 vs. placebo <u>OLR 15 mg/kg/q8h</u> Few or no fungal elements <u>Placebo</u> Severe inflammation, necrosis Hemorrhage, edema

Necrotizing vasculitis, vascular invasion, thrombosis

Lackner <i>et al.</i> <i>J Antimicrob Chemother</i> 2018	Invasive systemic infection through IV inoculation in a neutropenic murine model	Placebo IV excipient	Oral or IV 10 mg/kg/q12h	Survival on day 10 (log-rank test)	Renal fungal burden on death or on day 10 (CFU)	Survival on day 10 vs. placebo <u>OLR IV</u> = 100% (≤ 0.0001) <u>OLR oral</u> = 90% (≤ 0.0001) <u>AMB</u> = 0% (≤ 0.0001) <u>Placebo</u> = 10% Renal fungal burden on day 10 <u>OLR IV</u> 1.99 log₁₀ reduction vs. placebo (≤ 0.0001) <u>OLR oral</u> 1.07 log₁₀ reduction vs. placebo (≤ 0.0001) Survival on day 10 vs. placebo (NM) <u>OLR</u> <i>A. fumigatus</i> = 81% <i>A. nidulans</i> = 88% <i>A. tanneri</i> = 80% <u>Placebo</u> = 10%
	<i>Aspergillus terreus</i> (0.008 mg/L) 10 male neutropenic CD-1 mice per group	1 mg/kg/day IV Amphotericin B	9 days			
Seyedmousavi <i>et al.</i> <i>Antimicrob Agents Chemother</i> 2019	Invasive systemic infection through IV inoculation in a neutropenic murine model		IP	Survival on day 10 (log-rank test)	Serum GM On day 3 and day 10 (EIA)	Serum GM on day 3 vs. placebo (NM) <u>OLR</u> <i>A. fumigatus</i>: Index = 1 on day 3, index $\approx$ 0.9 on day 10 <i>A. nidulans</i>: Index = 1.5 on day 3, index = 0.5 on day 10 <i>A. tanneri</i>: Index = 3.5 on day 3, index = 1 on day 10 <u>Placebo</u> <i>A. fumigatus</i> control: index = 3.5 on day 3 <i>A. nidulans</i> control: index = 3.5 on day 3 <i>A. tanneri</i> control: index = 3.5 on day 3
	<i>Aspergillus fumigatus</i> (0.008 mg/L) <i>Aspergillus nidulans</i> (0.008 mg/L) <i>Aspergillus tanneri</i> (0.06 mg/L) 17 female neutropenic CD-1 mice per group: 10 for survival study 3 for GM measurement and histology 4 for fungal burden measurement	Placebo IP PBS	15 mg/kg/q8h 9 days		Renal fungal burden on day 3 (qPCR)	Renal fungal burden on day 3 vs. placebo <u>OLR</u> <i>A. fumigatus</i>: Mean = 5 pg/μg total DNA (≤ 0.0001) <i>A. nidulans</i>: Mean = 20 pg/μg total DNA (≤ 0.05) <i>A. tanneri</i>: Mean = 50 pg/μg total DNA (≤ 0.05) <u>Placebo</u> <i>A. fumigatus</i> control: Mean = 32 pg/μg total DNA <i>A. nidulans</i> control: Mean = 60 pg/μg total DNA <i>A. tanneri</i> control: Mean = 300 pg/μg total DNA

Renal histology on day 3 vs. placeboOLR

Few or no fungal elements

Placebo

Abundant hyphae

Severe inflammatory infiltrations

Necrosis

Survival on day 10 vs. placebo (NM)OLR*A. fumigatus* = 88%*A. nidulans* = 75%*A. tanneri* = 63%Placebo*A. fumigatus* <40%*A. nidulans* = 0%*A. tanneri* = 0%**Serum GM on day 3 vs. placebo (NM)**OLR*A. fumigatus*:

Index = 1.1 on day 3, index ≈ 1 on day 10

A. nidulans:

Index = 2 on day 3, index = 1.7 on day 10

A. tanneri:

Index = 3.2 on day 3, index = 2.8 on day 10

Placebo*A. fumigatus* control: index = 3.5 on day 3*A. nidulans* control: index = 3.5 on day 3*A. tanneri* control: index = 4 on day 3**Pulmonary fungal burden on day 3 vs. placebo**OLR*A. fumigatus*:

Mean = 0 pg/μg total DNA (≤0.01)

A. nidulans:

Mean = 50 pg/μg total DNA (≤0.001)

A. tanneri:

Mean = 500 pg/μg total DNA (≤0.001)

Placebo*A. fumigatus* control:

Mean = 150 pg/μg total DNA

A. nidulans control:

Mean = 400 pg/μg total DNA

A. tanneri control:

Mean = 11,000 pg/μg total DNA

Pulmonary histology on day 3 vs. placeboOLR

Few or no fungal elements

Placebo

Abundant hyphae

Extensive granulomas with necrosis

Invasive systemic
infection through
inhalation in a CGD
murine model

Seyedmousavi
et al.
Antimicrob
Agents
Chemother 2019

Aspergillus fumigatus
(0.008 mg/L)

Aspergillus nidulans
(0.008 mg/L)

Aspergillus tanneri
(0.06 mg/L)

17 male *gp91^{-/-} phox* CD-
1 mice per group:
10 for survival study
3 for GM measurement
and histology
4 for fungal burden
measurement

Placebo
IP PBS

IP

15 mg/kg/q8h

9 days

Survival on
day 10
(log-rank test)

Serum GM
On day 3 and
day 10
(EIA)

Pulmonary
fungal burden
on day 3
(qPCR)

Pulmonary
histology on
day 3
(GMS)

Survival on day 10 vs. placebo (NM)OLR*S. apiospermum* = 80%*S. boydii* = 100%*L. prolificans* = 100%Placebo*S. apiospermum* = 0%*S. boydii* = 20%*L. prolificans* = 20%**Serum BD on day 3 vs. placebo**OLR*S. apiospermum*: mean = 300 pg/mL (≤ 0.001)*S. boydii*: mean = 100 pg/mL (≤ 0.001)*L. prolificans*: mean $\approx$ 200 pg/mL (≤ 0.0001)Placebo*S. apiospermum* control: mean = 1,200 pg/mL*S. boydii* control: mean = 1,000 pg/mL*L. prolificans* control: mean = 700 pg/mL**Renal fungal burden on day 3 vs. placebo**OLR*S. apiospermum*:
Mean = 100 pg/ μ g total DNA (≤ 0.0001)*S. boydii*:
Mean = 100 pg/ μ g total DNA (≤ 0.0001)*L. prolificans*:
Mean = 100 pg/ μ g total DNA (≤ 0.01)Placebo*S. apiospermum* control:Mean = 600 pg/ μ g total DNA*S. boydii* control:Mean $\approx$ 475 pg/ μ g total DNA*L. prolificans* control:Mean $\approx$ 425pg/ μ g total DNA**Renal histology on day 3 vs. placebo**OLR

Few or no fungal elements

Placebo

Abundant hyphae

Extensive granulomas with necrosis

Scrapings on day 7 of TT vs. placeboOLR

No fungal elements

Reduction in skin lesions

Capillary regrowth

CTZ

No fungal elements

Placebo

Alopecia

Persistent skin lesions

Seyedmousavi <i>et al.</i> <i>Antimicrob</i> <i>Agents</i> <i>Chemother</i> 2021	Invasive systemic infection through IV inoculation in a neutropenic murine model				Survival on day 10 (log-rank test)	
	<i>Scedosporium apiospermum</i> (0.016 mg/L)				Serum BD on day 3 (colorimetric assay)	
Mirbzadeh <i>et al.</i> <i>Antimicrob</i> <i>Agents</i> <i>Chemother</i> 2021	<i>Scedosporium boydii</i> (0.016 mg/L)	Placebo IP PBS	IP	15 mg/kg/q8h	Renal fungal burden on day 3 (qPCR)	
	<i>Lomentospora prolificans</i> (0.03 mg/L)			9 days	Renal histology on day 3 (GMS)	
	17 female neutropenic CD-1 mice per group: 10 for survival study 3 for BD measurement and histology 4 for fungal burden measurement					
	Dermatophytosis in an immunosuppressed guinea pig model	Placebo topical PEG300	Topical	100 μ L of 0.1 mg/mL of orlofin in PEG300	Surface scrapings from inoculation site (optical microscopy)	
	<i>Microsporium gypseum</i> (0.03 mg/L)	1% topical clotrimazole		7 days		
	9 albino female guinea pigs immunosuppressed by corticosteroids, divided into 3 groups					

					Survival on day 30 vs. placebo
Wiederhold <i>et al.</i> <i>Antimicrob Agents</i> <i>Chemother</i> 2018	CNS infection in a murine model	Placebo oral excipient	Oral		<u>OLR 20 mg/kg in 2 administrations</u> Median survival time = 22 days (≤ 0.0001) Total survival rate = 10% (>0.05) <u>OLR 40 mg/kg in 2 administrations</u> Median survival time = 26 days (≤ 0.0001) Total survival rate = 30% (>0.05) <u>FLC</u> Median survival time = 30 days (≤ 0.0001) Total survival rate = 50% (≤ 0.0001) <u>Placebo</u> Median survival time = 9 days Total survival rate = 0%
	<i>Coccidioides immitis</i> (0.016 mg/L)		20 mg/kg/day in 2 or 3 administrations	Survival on day 30 (log-rank test)	<u>OLR 20 mg/kg in 3 administrations</u> Median survival time = 23.5 days (≤ 0.0001) Total survival rate = 30% (>0.05) <u>OLR 40 mg/kg in 3 administrations</u> Median survival time > 30 days (≤ 0.0001) Total survival rate = 80% (0.0007) <u>FLC</u> Median survival time = 28 days (≤ 0.0001) Total survival rate $\approx$ 25% (≤ 0.0001) <u>Placebo</u> Median survival time = 9 days Total survival rate = 0%
	10 male ICR mice per group		40 mg/kg/day in 2 or 3 administrations		
			14 days		
					Brain fungal burden on day 9 vs. placebo
Wiederhold <i>et al.</i> <i>Antimicrob Agents</i> <i>Chemother</i> 2018 (cont.)	CNS infection in a murine model	Placebo oral excipient	Oral		<u>OLR 20 mg/kg in 2 administrations</u> Median = 4.36 log ₁₀ CFU/g (≤ 0.01) <u>OLR 40 mg/kg in 2 administrations</u> Median = 3.41 log ₁₀ CFU/g (≤ 0.01) <u>FLC</u> Median = 1.33 log ₁₀ CFU/g (≤ 0.0001) <u>Placebo</u> Median = 5.53 log ₁₀ CFU/g
	<i>Coccidioides immitis</i> (0.016 mg/L)		20 mg/kg/day in 2 or 3 administrations	Brain fungal burden on day 9 (CFU)	<u>OLR 20 mg/kg in 3 administrations</u> Median = 3.28 log ₁₀ CFU/g (≤ 0.0001) <u>OLR 40 mg/kg in 3 administrations</u> Median = 1.95 log ₁₀ CFU/g (≤ 0.0001) <u>FLC</u> Median = 1.33 log ₁₀ CFU/g (≤ 0.0001) <u>Placebo</u> Median = 5.97 log ₁₀ CFU/g
	10 male ICR mice per group		40 mg/kg/day in 2 or 3 administrations	Brain fungal burden at death or on day 30 for survivors (CFU)	Brain fungal burden on day 30 vs. placebo <u>OLR 20 mg/kg in 2 administrations</u> Median $\approx$ 6 log ₁₀ CFU/g (>0.05) <u>OLR 40 mg/kg in 2 administrations</u> Median $\approx$ 5.5 log ₁₀ CFU/g (>0.05) <u>FLC</u> Median $\approx$ 6 log ₁₀ CFU/g (>0.05) <u>Placebo</u> Median = 5.67 log ₁₀ CFU/g
			14		<u>OLR 20 mg/kg in 3 administrations</u> Median $\approx$ 5.5 log ₁₀ CFU/g (>0.05) <u>OLR 40 mg/kg in 3 administrations</u> Median = 1.13 log ₁₀ CFU/g (≤ 0.0001) <u>FLC</u>

Median $\approx 6 \log_{10}$ CFU/g (>0.05)

Placebo

Median = $5.67 \log_{10}$ CFU/g

AMB: amphotericin B. BD: 1,3- β -D-glucan. CFU: Colony-Forming Unit. CGD: chronic granulomatous disease. CNS: central nervous system. CTZ: clotrimazole. EIA: enzyme immunoassay. FLC: fluconazole. GMS: Grocott-Gömöri Methenamine Silver stain. HE: Hematoxyline-Eosine stain. IP: intraperitoneal. IV: intravenous. MIC: Minimal Inhibitory Concentration. NM: not mentioned. OLR: olorofim. PBS: Phosphate-Buffered Saline. qPCR: quantitative real-time polymerase chain reaction. PSC: posaconazole. TT: treatment. VOR: voriconazole. WT: wild-type.