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Efficacy of SPR720 in murine models of non-tuberculous mycobacterial pulmonary infection

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Background: Non-tuberculous mycobacterial pulmonary disease (NTM-PD) is increasing worldwide, with *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* as the predominant pathogens. Current treatments are poorly tolerated and modestly effective, highlighting the need for new treatments. SPR719, the active moiety of the benzimidazole prodrug SPR720, inhibits the ATPase subunits of DNA gyrase B, a target not exploited by current antibiotics, and therefore, no cross-resistance is expected with standard-of-care (SOC) agents.

Objectives: To evaluate the *in vitro* activity of SPR719 against MAC and *M. abscessus* clinical isolates, including those resistant to SOC agents, and *in vivo* efficacy of SPR720 in murine non-tuberculous mycobacteria (NTM) pulmonary infection models.

Methods: NTM isolates were tested for susceptibility to SPR719. Chronic C3HeB/FeJ and severe combined immunodeficient murine models of pulmonary infection were used to assess efficacy of SPR720 against MAC and *M. abscessus*, respectively.

Results: SPR719 was active against MAC (MIC_{90}, 2 mg/L) and *M. abscessus* (MIC_{90}, 4 mg/L) clinical isolates. Efficacy of SPR720 was demonstrated against MAC pulmonary infection, both as a monotherapy and in combination with SOC agents. SPR720 monotherapy exhibited dose-dependent reduction in bacterial burden, with the largest reduction observed when combined with clarithromycin and ethambutol. Efficacy of SPR720 was also demonstrated against *M. abscessus* pulmonary infection where monotherapy exhibited a dose-dependent reduction in bacterial burden with further reductions detected when combined with SOC agents.

Conclusions: *In vitro* activity of SPR720 against common NTM pathogens and efficacy in murine infections warrant the continued clinical evaluation of SPR720 as a new oral option for the treatment of NTM-PD.

Introduction

Non-tuberculous mycobacterial pulmonary disease (NTM-PD) is a chronic progressive condition that is acquired via inhalation of non-tuberculous mycobacteria (NTM) from environmental sources. The most common species causing pulmonary disease are members of the slowly growing *Mycobacterium avium* complex (MAC) that have been implicated in approximately 80% of NTM-PD cases, including *M. avium* and *Mycobacterium intracellulare*[1–3] and rapidly growing subspecies of *Mycobacterium abscessus* complex, namely *M. abscessus* subspecies *abscessus*, *M. abscessus* subspecies *bolletii* and *M. abscessus* subspecies *massiliense*. Patients may experience chronic cough, sputum production, shortness of breath, fatigue and systemic manifestations such as fever, night sweats, loss of appetite, and weight loss, and, as the disease progresses, a decline in pulmonary function and impaired quality of life.[4]

Treatment of NTM-PD due to MAC typically involves a combination of different oral antibacterial agents taken over an extended period of 12 months after sputum conversion to culture negative (i.e. a total of approximately 15 to 18 months). Achieving culture conversion has been shown to be associated with lower all-cause mortality[5] but only 60%–70% of patients will convert their sputum cultures to negative within the first 6 months of therapy,[5,6] of whom approximately half will experience recurrence (either relapse or reinfection) within 3 years.[7] First-line agents include a macrolide (azithromycin is preferable to clarithromycin), ethambutol and a rifamycin. These exhibit variable

efficacy, and many patients experience tolerability issues that often result in regimen modifications, thus highlighting the need for new classes of oral agents that may be included in new standard-of-care (SOC) combination regimens.[1] Treatment of pulmonary disease caused by *M. abscessus* complex involves a combination of three or more oral and parenteral antibiotics, and is complicated by multidrug resistance. Although macrolides, amikacin and a β-lactam or imipenem are current drugs of choice, new treatments are needed due to increasing intrinsic resistance to macrolides.[8,9]

SPR719, the active moiety of the phosphate prodrug SPR720, belongs to a novel chemical class (aminobenzimidazoles) targeting the ATPase site located on the gyrase B subunit of the heterotetrameric bacterial gyrase protein, a mechanism that is distinct from that of fluoroquinolones. SPR719 has demonstrated antibacterial activity against NTM pathogens including MAC and *M. abscessus* clinical isolates,[10,11] including those that are resistant to clarithromycin or amikacin,[12] and has been shown to penetrate THP-1 monocytes, which is critical for targeting these intracellular pathogens,[13] and was well tolerated at daily doses up to 1000 mg for 14 days in a Phase 1 randomized, double-blind, placebo-controlled, single ascending dose/multiple ascending dose trial (NCT03796910).[14] SPR720 is currently being evaluated in a Phase 2 dose-ranging clinical study in patients with NTM-PD due to MAC (NCT05496374).

The goals of the current study were to evaluate the *in vitro* antibacterial activity of SPR719 against clinical isolates of MAC and *M. abscessus* and to assess the *in vivo* efficacy of SPR720 alone and in combination with SOC treatment regimens against *M. avium* and *M. abscessus* in murine infection models.[15,16]

Materials and methods

In vitro susceptibility testing

Clinical isolates of MAC and *M. abscessus* complex were tested for susceptibility to SPR719 in accordance with CLSI guidelines.[17] *M. abscessus* was tested in CAMHB, while MAC was tested in CAMHB supplemented with 5% OADC. A total of 105 clinical MAC isolates were tested for susceptibility to SPR719 and comparator antibiotics, including *M. avium* ($n=45$), *M. intracellulare* ($n=28$), MAC ($n=26$) and *M. intracellulare* subspecies *chimaera* ($n=6$). These isolates were collected from diverse specimen sources from patients in different geographic regions in the USA and Japan between 2015 and 2018, and included both isolates that were susceptible to SOC agents and isolates with resistant phenotypes as defined by CLSI criteria.[18] Fifty-three clinical isolates of *M. abscessus* complex, including *M. abscessus* ($n=7$), *M. abscessus* subspecies *abscessus* ($n=33$), *M. abscessus* subspecies *massiliense* ($n=10$) and *M. abscessus/massiliense* hybrid ($n=3$), similarly collected from specimen sources from patients in different geographic regions in the USA between 2016 and 2018, were also tested for susceptibility to SPR719, including isolates that were susceptible or resistant to SOC agents.

Mycobacterial isolates evaluated in murine efficacy models

M. avium (Chester) ATCC 700898 is a standard strain widely used for MAC susceptibility testing and selected for evaluation in the chronic C3HeB/FeJ mouse infection model. The isolate was tested for susceptibility to SPR719 and SOC comparator agents including clarithromycin, ethambutol and rifabutin, which were also evaluated for efficacy in the mouse infection model. *M. abscessus* 1513 was evaluated in the chronic severe combined immunodeficient (SCID) murine model of pulmonary and systemic infection and tested for susceptibility to SPR719 and SOC comparator agents. All procedures involving animals were approved by the Colorado State University Animal Care and Use Committee.

Chronic C3HeB/FeJ mouse infection model

A 60 day chronic C3HeB/FeJ mouse infection model was developed to be representative of NTM-PD since these mice form foci of necrosis in granulomas, as previously described.[15] Mice were infected by aerosol delivery of $1\times10^{8.5}$ cfu/mL of *M. avium* ATCC 700898, which was susceptible to SPR719 with an MIC of 2 mg/L. Untreated groups of mice were evaluated on Days 2, 27 and 61 post-infection (3–6 mice per group) to assess bacterial growth in the lung, spleen and liver, which were extracted, homogenized in 4.5 mL of PBS, plated on 7H11 agar plates, and incubated for ∼30 days to determine growth. On Day 28 post-infection, the oral prodrug SPR720 was administered by oral gavage at 10, 30 and 100 mg/kg/day every 24 h (q24h) (6 mice per group). In a separate preliminary pharmacokinetic study in BALB/c mice, a single dose of SPR720 at 100 mg/kg provided a mean total plasma drug AUC exposure (AUC_{0-inf}) of 72.5 μg·h/mL (CV of 16.7%–88.4%) (data on file), similar to that achieved with a 1000 mg oral dose in healthy volunteers in the Phase 1 clinical trial.[14] Positive control clarithromycin was administered by oral gavage at 250 mg/kg/day. Ethambutol and rifabutin were also delivered by oral gavage at 100 mg/kg/day in combination treatments. The animals were dosed consecutively from Day 28 to Day 60. At the end of the dosing phase (Day 61), lung, spleen and liver were removed, processed and plated on Middlebrook 7H11 media for ∼30 days to enumerate cfu.

Chronic SCID mouse infection model

A chronic SCID mouse infection model previously described by Obregon-Henao *et al.*,[16] was used to evaluate the efficacy of SPR720 against *M. abscessus*. SCID mice were infected with 1×10^{6} cfu/mouse via the tail vein with *M. abscessus* 1513, which was susceptible to SPR719 with an MIC of 2 mg/L. Untreated groups of mice were evaluated on Days 1, 27 and 61 post-infection (3–6 mice per group) to assess bacterial growth in the lung, spleen and liver. On Day 28 post-infection, SPR720 was administered as monotherapy by oral gavage at 25, 50 and 100 mg/kg q24h. SPR720 dosed in mice at 100 mg/kg q24h provided a mean plasma AUC_{0-inf} similar to that achieved with a 1000 mg oral dose in healthy volunteers in a Phase I clinical trial. First-line agents evaluated as monotherapy by oral gavage included clarithromycin (250 mg/kg q24h) and clofazimine (20 mg/kg q24h), while amikacin was dosed subcutaneously (150 mg/g q24h) (6 mice per treatment group). Treatment was initiated 28 days post-infection and continued until Day 60. Bacterial burden in the lung, spleen and livers of infected mice were assessed on Days 2, 27 and 61 after infection by plating onto Middlebrook 7H11 agar and enumerating cfu after 7 days incubation at 37°C.

Statistical analysis

Data are presented using the mean values from 3–6 mice per group performed in a single experiment. Statistical analysis was evaluated by a one-way ANOVA followed by a multiple comparison analysis of variance using a one-way Tukey test (SigmaStat software program). Data are presented using the mean values plus or minus the SEM. Significance was considered with a P value of <0.05.

Results

In vitro susceptibility results

In vitro studies were carried out using the active moiety, SPR719, following CLSI methodology with identification of resistant phenotypes as defined by CLSI criteria.[17,18] Table 1 shows

Table 1. Anti-mycobacterial activity of SPR719 against clinical isolates of MAC and *M. abscessus* collected from diverse specimen sources from patients in the USA and Japan between 2015 and 2018

Organism	Phenotype[a]	*n*	SPR719 MIC (mg/L)		
			Range	MIC_{50}	MIC_{90}
MAC	All	105	0.002–4	1	2
	Amikacin resistant[b]	6	0.25–2	NA	NA
	Clarithromycin resistant	10	0.5–2	1	2
	Linezolid resistant	51	0.12–2	1	2
	Moxifloxacin resistant	47	0.12–4	1	2
	Resistant to ≥2 antibiotics	36	0.12–2	1	2
M. abscessus	All	53	0.12–8	2	4
	Amikacin resistant	2	0.5–2	NA	NA
	Clarithromycin resistant	31	0.25–8	2	4
	Doxycycline resistant	41	0.12–8	2	4
	Cefoxitin resistant	2	2–4	NA	NA
	Imipenem resistant	9	0.5–2	NA	NA
	Linezolid resistant	6	1–4	NA	NA
	Moxifloxacin resistant	51	0.25–8	2	4
	Trimethoprim/sulfamethoxazole resistant	52	0.25–8	2	4

NA, not applicable.
[a]Resistant phenotypes were defined according to CLSI interpretive criteria (CLSI M62, first edition).
[b]Amikacin resistance in MAC was determined using the breakpoints for the liposomal inhaled formulation.

susceptibility results for SPR719 and comparator agents against 105 clinical MAC isolates collected in the USA and Japan from 2015 to 2018. All MAC isolates were inhibited by SPR719 at concentrations of ≤4 mg/L and the MIC_{90} value was 2 mg/L. Among the clarithromycin-resistant isolates, the MICs for SPR719 ranged from 0.5 to 2 mg/L and the MIC_{90} value was 2 mg/L. The MICs for SPR719 against the 47 moxifloxacin-resistant MAC isolates ranged from 0.12 to 4 mg/L and the MIC_{90} value was 2 mg/L, confirming that resistance to fluoroquinolone agents does not impact susceptibility to SPR719, which was expected based on the different binding sites. Thirty-six isolates were resistant to ≥2 antibiotics with SPR719 MICs that ranged from 0.12 to 2 mg/L. *M. avium* ATCC 700898, the organism evaluated for efficacy in the chronic C3HeB/FeJ murine infection model, was susceptible to SPR719 with an MIC of 2 mg/L (Table 2). The MICs of clarithromycin, rifabutin and ethambutol were 1, 0.25 and 4 mg/L, respectively.

Susceptibility results for SPR719 against 53 clinical isolates of *M. abscessus* complex are also shown in Table 1. All isolates were inhibited by SPR719 at concentrations of ≤8 mg/L with an MIC_{90} of 4 mg/L, including isolates that were resistant to SOC agents. Thirty-one isolates were resistant to clarithromycin with SPR719 MICs ranging from 0.25 to 8 mg/L, and 41 isolates were resistant to doxycycline with SPR719 MICs ranging from 0.12 to 8 mg/L. As with MAC, resistance to moxifloxacin did not impact susceptibility to SPR719. *M. abscessus* 1513 was the organism selected for evaluation of efficacy in the chronic SCID mouse model and was susceptible to SPR719 with an MIC of 2 mg/L (Table 2). This organism was resistant to clarithromycin and other SOC agents, including ciprofloxacin, doxycycline, linezolid, sulfamethoxazole and trimethoprim/sulfamethoxazole (Table 2).

Table 2. Activity of SPR719 and SOC and commonly used comparator agents against *M. avium* ATCC 700898 and *M. abscessus* 1513 strains evaluated in murine efficacy models

In vivo test organism	Agent	MIC (mg/L)	S/I/R interpretation[a]
M. avium ATCC 700898	SPR719	2	NA
	Clarithromycin	1	S
	Rifabutin	0.25	NA
	Ethambutol	4	NA
M. abscessus 1513	SPR719	2	NA
	Clarithromycin	>8	R
	Amikacin	2	S
	Cefoxitin	128	R
	Ciprofloxacin	>4	R
	Doxycycline	>16	R
	Linezolid	>16	R
	Sulfamethoxazole	>128	NA
	Trimethoprim/sulfamethoxazole	>4/76	R

I, intermediate; NA, not applicable; R, resistant; S, susceptible.
[a]Susceptibility was assessed according to CLSI interpretive criteria (CLSI M62 first edition).

Efficacy against M. avium in the chronic C3HeB/FeJ mouse infection model

Table 3 shows the changes in bacterial burden of *M. avium* ATCC 700898 in the lung, spleen and liver of C3HeB/FeJ mice following

Table 3. *M. avium* ATCC 700898 bacterial burden (log_{10} cfu/organ) in the lung, spleen, and liver of C3HeB/FeJ mice after treatment with oral SPR720 alone and in combination with clarithromycin (CLR) ± rifabutin (RFB) and/or ethambutol (EMB) or both

Treatment group regimens	No. of mice	Log_{10} cfu ± SEM		
		Lung	Spleen	Liver
Day 1 pretreatment control	3	5.52 ± 0.05	0 ± 0	0 ± 0
Day 27 pretreatment control	3	5.72 ± 0.14	3.16 ± 0.24	3.33 ± 0.05
Day 61 control	3	6.30 ± 0.04	4.20 ± 0.04	5.10 ± 0.08
SPR720 (10 mg/kg q24h)	6	5.86 ± 0.19	3.80 ± 0.07	3.88 ± 0.10
SPR720 (30 mg/kg q24h)	6	5.21 ± 0.44	3.31 ± 0.17	3.40 ± 0.18
SPR720 (100 mg/kg q24h)	6	4.26 ± 0.05	3.21 ± 0.45	3.50 ± 0.52
CLR (250 mg/kg q24h)	6	4.38 ± 0.17	2.41 ± 0.56	2.69 ± 0.11
CLR (250 mg/kg q24h) and RFB (100 mg/kg q24h)	6	4.23 ± 0.06	2.80 ± 0.15	3.76 ± 0.06
CLR (250 mg/kg q24h) and EMB (100 mg/kg q24h)	6	2.78 ± 0.09	2.20 ± 0.54	3.60 ± 0.52
CLR (250 mg/kg q24h), RFB (100 mg/kg q24h) and EMB (100 mg/kg q24h)	6	2.85 ± 0.04	2.81 ± 0.18	3.73 ± 0.03
CLR (250 mg/kg q24h) and SPR720 (30 mg/kg q24h)	6	2.34 ± 0.27	2.24 ± 0.52	2.68 ± 0.73
CLR (250 mg/kg q24h), RFB (100 mg/kg q24h) and SPR720 (30 mg/kg q24h)	6	2.50 ± 0.27	2.40 ± 0.64	2.73 ± 0.57
CLR (250 mg/kg q24h), EMB (100 mg/kg q24h) and SPR720 (30 mg/kg q24h)	6	1.93 ± 0.57	1.70 ± 0.40	2.13 ± 0.67
CLR (250 mg/kg q24h), RFB (100 mg/kg q24h), EMB (100 mg/kg q24h) and SPR720 (30 mg/kg q24h)	6	3.61 ± 0.12	2.85 ± 0.16	3.60 ± 0.52

Dosing regimens: CLR, PO, q24h at 250 mg/kg; EMB, PO, q24h at 100 mg/kg; RFB, PO, q24h at 100 mg/kg; SPR720, PO, q24h at 10, 30 and 100 mg/kg.

treatment with different regimens of SPR720 alone and in combination with clarithromycin ± rifabutin, ethambutol or both. The bacterial burdens (± SEM) in the Day 61 untreated controls in the lung, spleen and liver were 6.30 ± 0.04, 4.20 ± 0.04 and 5.10 ± 0.08 log_{10} cfu/organ, respectively. SPR720 alone showed dose-dependent reductions in bacterial burden in all tissues when compared with the Day 61 untreated control. While SPR720 in combination with other SOC agents showed varying degrees of reduction in bacterial burden, SPR720 in combination with clarithromycin and ethambutol showed the largest reduction in bacterial burden in the lung, spleen and liver (Table 3). Figure 1 shows the changes in pulmonary bacterial burden (log_{10} cfu/lung) after treatment with SPR720 alone and in combination with other agents. SPR720 monotherapy was evaluated at 10, 30 and 100 mg/kg dosed q24h, resulting in a dose-dependent reduction of *M. avium* ATCC 700898 at all doses tested when compared with the Day 61 control group.

The reduction in bacterial burden achieved with all the combinations that included clarithromycin was statistically significant when compared with the Day 61 control ($P < 0.0001$). SPR720 at 30 mg/kg/day improved the efficacy of clarithromycin, resulting in a 3.96 log_{10} cfu reduction in bacterial burden in the lung; the level of reduction of bacterial burden was similar to clarithromycin plus ethambutol, which resulted in a 3.52 log_{10} cfu reduction in bacterial burden in the lung when compared with the Day 61 control (Table 3). A similar log reduction of 3.45 log_{10} cfu was observed with clarithromycin combined with both ethambutol and rifabutin (100 mg/kg). The greatest reduction in bacterial burden was observed with the combination of clarithromycin (250 mg/kg), ethambutol (100 mg/kg) and SPR720 (30 mg/kg), with an average 4.37 log_{10} cfu reduction bacterial burden in the lung; bacterial burden in the lung was reduced by only 2.70 log_{10} cfu relative to the Day 61 untreated control when rifabutin (100 mg/kg) was added to this regimen. It is notable that the regimen consisting of clarithromycin, rifabutin, ethambutol and SPR720 resulted in a lower log reduction in pulmonary bacterial burden than the triple combination of clarithromycin, rifabutin and ethambutol, a finding which requires further evaluation. While the study was not designed to assess statistical differences between treatment groups, *P* values were calculated for groups treated with SPR720-containing combinations compared with those treated with the same combination agents but without SPR720 (Figure 1). Future efficacy studies with additional MAC strains and refinements of the doses of SOC agents may provide better insights as to how SPR720 will fit either as an addition to current regimens, or as a substitute for SOC components.

Efficacy against M. abscessus in the chronic SCID mouse infection model

Table 4 shows the changes in bacterial burden of *M. abscessus* 1513 in the lung, spleen and liver of mice following different treatment regimens of SPR720 alone and in combination with SOC agents. Untreated mice were evaluated on Days 1, 27 and 61 post-infection to assess bacterial growth in the lung, spleen and liver. The bacterial burden in the Day 61 untreated controls in the lung, spleen and liver was 6.55 ± 0.08, 6.74 ± 0.05 and 7.22 ± 0.02 log_{10} cfu/organ, respectively. SPR720 monotherapy showed a dose-dependent reduction in bacterial burden after 28 days of treatment in all tissues compared with the Day 61 untreated control. SPR720 in combination with other SOC regimens also showed varying degrees of reduction. The greatest reduction in bacterial burden in all tissues occurred in mice treated with SPR720 (50 mg/kg) in combination with clarithromycin (250 mg/kg), amikacin (150 mg/kg) and clofazimine (20 mg/kg) q24h. The changes in bacterial burden in the lung for SPR720 alone and in combination are shown as a histogram in Figure 2, which highlights the dose-dependent reduction in log_{10} cfu in

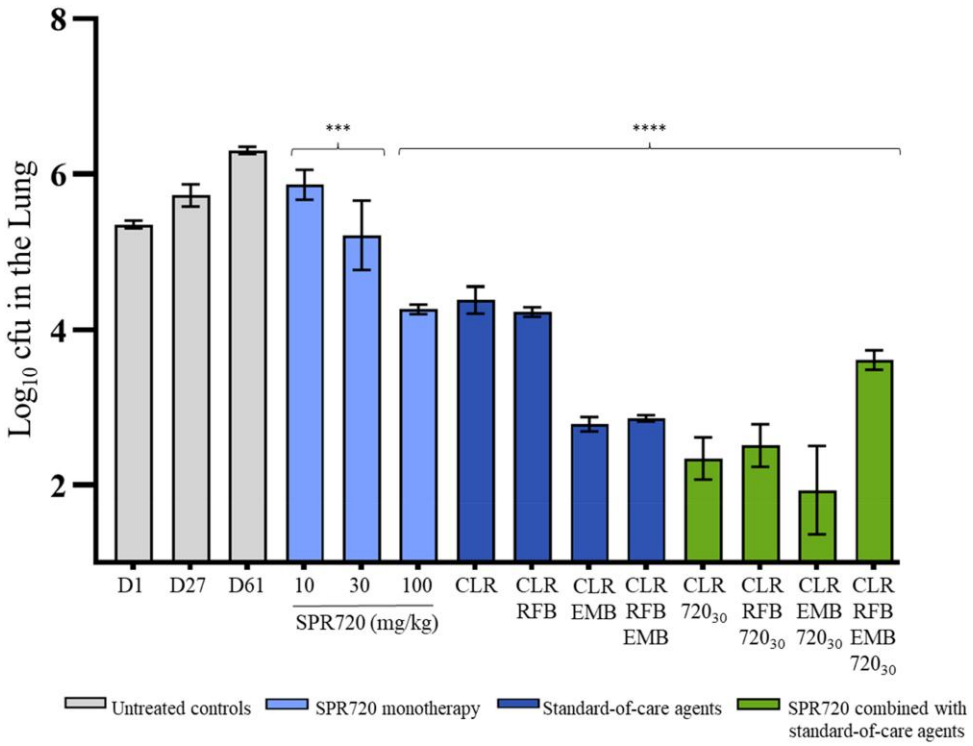

Figure 1. Pulmonary bacterial burden (log_{10} cfu/lung) of *M. avium* ATCC 700898 in C3HeB/FeJ mice after treatment with oral SPR720 alone and in combination with clarithromycin (CLR) ± rifabutin (RFB), ethambutol (EMB) or both in a chronic murine infection model. ***$P < 0.001$; ****$P < 0.0001$ relative to the Day (D) 61 untreated control. The addition of SPR720 to the clarithromycin (CLR) and CLR + RFB treatment regimens further reduced bacterial burden in the lung (P values <0.0001) and SPR720 also improved the outcome when added to the CLR + EMB regimen ($P = 0.0049$), while the addition of SPR720 to the SOC regimen CLR + RFB + EMB resulted in a higher pulmonary bacterial burden relative to the SOC regimen alone ($P < 0.0001$). Dosing regimens: CLR, PO, q24h at 250 mg/kg; EMB, PO, q24h at 100 mg/kg; RFB, PO, q24h at 100 mg/kg; SPR720, PO, q24h at 10, 30 and 100 mg/kg; $SPR720_{30}$ indicates SPR720 was dosed at 30 mg/kg, PO, q24h in combination with SOC agents.

the lung with SPR720 monotherapy at 25, 50 and 100 mg/kg q24h. The *M. abscessus* efficacy study also was not designed to assess statistical differences between treatment groups, and *P* values were calculated for SPR720-treated groups compared with groups treated with the same combination agents but without SPR720 (Figure 2).

Discussion

Current therapeutics for the treatment of NTM-PD remain challenging because of the long treatment duration and poor tolerability. Many patients are left with residual lung dysfunction[19,20] or fail to respond to treatment, relapse or develop macrolide resistance.[21] New oral treatment options are desperately needed that can either be added to or substituted for current SOC agents.

The results of this study highlight the *in vitro* activity of SPR719, a novel aminobenzimidazole, against both MAC and *M. abscessus* complex clinical isolates including those that are resistant to current SOC agents. The primary goal of the study was to assess whether the *in vitro* activity of SPR719 would translate to *in vivo* efficacy of the prodrug, SPR720, in murine efficacy models. The C3HeB/FeJ mouse model was used to evaluate efficacy for SPR720 alone and in combination with other agents against *M. avium* ATCC 700898, which produces a progressive infection resulting in small necrotic foci during granuloma formation.[15] Although the translation of preclinical models of MAC pulmonary infection to patient outcomes is not generally well established, this model may more closely reflect potentially consequential features of NTM-PD in patients, such as the high density of bacteria located in necrotic granulomas and the limited antibiotic penetration into these granulomas, therefore preclinical studies conducted with this model may uniquely inform the clinical potential of new antibiotics. Although SPR720 demonstrated a dose-dependent reduction in bacterial burden in the lung, one limitation of the study was that doses >100 mg/kg were not explored and a full sigmoid E_{max} dose–response relationship could not be described. SPR720 at 30 mg/kg was selected as the mid-point regimen for evaluation in combination with SOC agents. However, the results of this study support the inclusion of SPR720 at 100 mg/kg, which provides a mean plasma AUC_{0-inf} exposure similar to that achieved with a 1000 mg oral dose in healthy volunteers in a Phase 1 clinical trial, in future combination regimens due to the greater reduction in bacterial burden that was observed. As this was a proof-of-concept efficacy study, no pharmacokinetic data were collected and it was not possible to evaluate AUC:MIC exposures versus changes in bacterial burden. Nevertheless, the results from the current study will be important in guiding future pharmacokinetic-pharmacodynamic (PK-PD)

Table 4. *M. abscessus* 1513 bacterial burden (log_{10} cfu/organ) in the lung, spleen and liver of SCID mice after treatment with oral SPR720 alone and in combination with clarithromycin and amikacin (AMK) ± clofazimine (CLF)

		Log_{10}cfu ± SEM		
Treatment group regimens	No. of mice	Lung	Spleen	Liver
Day 1 pretreatment control	3	5.83 ± 0.04	5.58 ± 0.04	6.12 ± 0.04
Day 27 pretreatment control	6	6.39 ± 0.03	5.77 ± 0.11	7.03 ± 0.11
Day 61 untreated control	6	6.55 ± 0.08	6.74 ± 0.04	7.22 ± 0.02
SPR720 (25 mg/kg q24h)	6	4.45 ± 0.07	4.25 ± 0.20	5.07 ± 0.16
SPR720 (50 mg/kg q24h)	6	3.50 ± 0.22	3.89 ± 0.18	4.91 ± 0.29
SPR720 (100 mg/kg q24h)	6	2.71 ± 0.40	3.30 ± 0.16	4.73 ± 0.41
CLF (20 mg/kg q24h)	6	5.08 ± 0.05	4.63 ± 0.13	5.83 ± 0.04
AMK (150 mg/kg q24h)	6	4.53 ± 0.08	4.09 ± 0.04	5.23 ± 0.07
CLR (250 mg/kg q24h)	6	4.46 ± 0.17	5.06 ± 0.05	5.45 ± 0.05
CLR (250 mg/kg q24h) and AMK (150 mg/kg q24h)	6	3.99 ± 0.14	5.07 ± 0.05	4.86 ± 0.12
CLR (250 mg/kg), AMK (150 mg/kg) and CLF (20 mg/kg q24h)	6	3.70 ± 0.25	4.83 ± 0.11	4.33 ± 0.07
CLR (250 mg/kg q24h), AMK (150 mg/kg q24h) and SPR720 (50 mg/kg q24h)	6	2.20 ± 0.22	3.93 ± 0.11	4.92 ± 0.19
CLR (250 mg/kg q24h), AMK (150 mg/kg q24h), CLF (20 mg/kg q24h) and SPR720 (50 mg/kg q24h)	6	1.86 ± 0.16	3.36 ± 0.21	3.96 ± 0.13

Dosing regimens: AMK, subcutaneously, q24h at 150 mg/kg; CLR, PO, q24h at 250 mg/kg; CLF, PO, q24h at 20 mg/kg; SPR720, PO, q24h at 25, 50 and 100 mg/kg; $SPR720_{50}$ indicates SPR720 was dosed at 50 mg/kg, PO, q24h in combination with first-line agents.

studies, confirming the PK-PD index and the magnitude required for bacterial killing against additional strains of MAC.

In the assessment of efficacy of SPR720 in combination with SOC agents, changes in bacterial burden of *M. avium* in the lung as the primary target organ were compared. Changes in bacterial burden in the spleen and liver were assessed to determine the effect on delayed systemic dissemination of the organism. The reduction in log_{10} cfu counts in the lung relative to the Day 61 untreated control was used as the primary measure to compare efficacy of the different regimens. The study was not designed to formally assess any synergy or antagonism nor was it powered to statistically evaluate differences in outcome between the various treatment regimens. While all regimens studied showed reductions in bacterial burden compared with the Day 61 untreated control (P values <0.0001), some difference in cfu reductions were observed for the different regimens. Strikingly, the addition of SPR720 at 30 mg/kg to clarithromycin, rifabutin and ethambutol showed a higher cfu burden when compared with the same regimen excluding rifabutin, which resulted in the lowest cfu burden of all regimens evaluated ($P < 0.0001$), while the greatest reduction in bacterial burden was observed with the combination of clarithromycin, ethambutol and SPR720. The addition of SPR720 to clarithromycin either alone or in combination with ethambutol or rifabutin further reduced bacterial burden relative to the same regimens without SPR720 (P values of 0.0049 to <0.0001). Additionally, the combination of rifabutin with clarithromycin resulted in higher cfu counts in the liver when compared with clarithromycin alone. The rifamycins are well-known inducers of cytochrome P450 enzymes and transporters, which result in reduced exposure to macrolide antibiotics, therefore lower exposure to clarithromycin may be a causative factor in these findings. These results suggest that SPR720 could be a potential substitute for rifabutin in the current SOC regimens, a possibility which will be explored in future efficacy and PK-PD studies with additional strains of MAC and alternate murine models of MAC pulmonary infection.

In this study, the combination of ethambutol with clarithromycin resulted in a higher cfu burden in the liver when compared with clarithromycin alone, in contrast with outcomes in the lung where a further 1.60 log_{10} cfu reduction was observed when ethambutol was added to clarithromycin. The significance of this 1 log_{10} cfu difference in the liver is not clear, although it is possible that the 100 mg/kg dose of ethambutol evaluated in the murine model was high when compared with the exposure achieved with the 25 mg/kg daily dose typically recommended for the treatment of MAC pulmonary disease.[4]

While the improved efficacy of SPR720 in combination with clarithromycin and ethambutol observed in the MAC murine efficacy model warrants further study with additional strains of MAC, *in vitro* time–kill studies with SPR719 and ethambutol have shown increased bacterial killing and suppressed outgrowth of *M. avium* relative to either agent alone[11]. Although the precise mode of action of ethambutol has not been established, it is thought to inhibit cell wall (arabinogalactan) biosynthesis in mycobacteria[22] and may act synergistically to enhance access of SPR719 to intracellular targets in MAC.

The SCID model was the first murine model to explore the efficacy of SPR720 alone and in combination with SOC agents against *M. abscessus* 1513. Mice were infected IV, so all organs showed a similar infection burden on Day 1. SPR720 demonstrated a dose-dependent reduction in bacterial burden in lung, spleen and liver with the mid-range dose of 50 mg/kg being used in combination with other agents. Overall, changes in bacterial burden trended similarly when lung was compared with spleen and liver, although the greatest log reductions were observed in the lung. As this was a preliminary model to explore

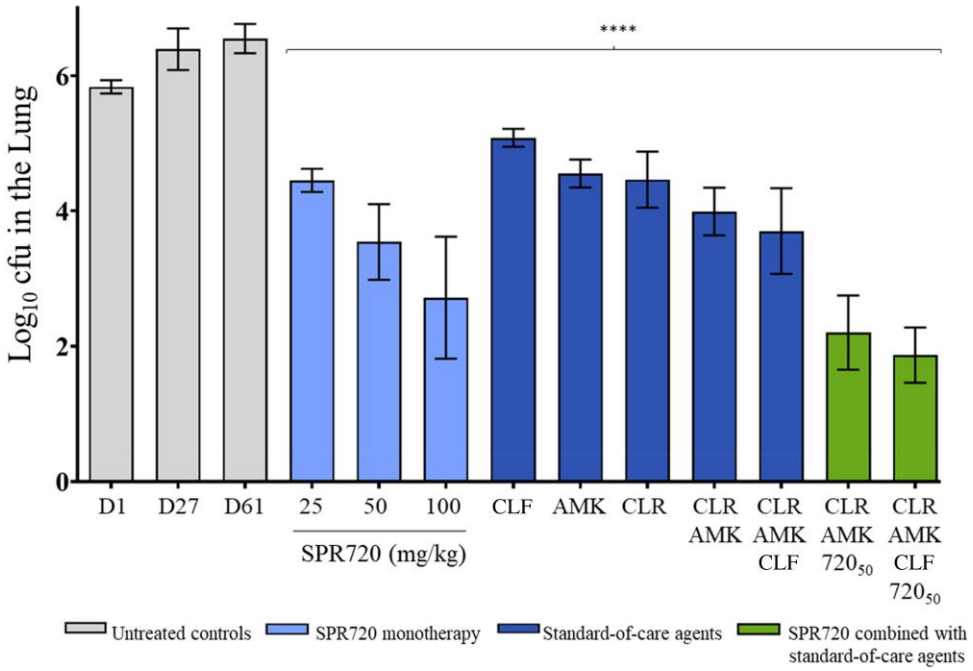

Figure 2. Pulmonary bacterial burden (log_{10} cfu/lung) of MDR *M. abscessus* in SCID mice after treatment with oral SPR720 alone and in combination with clarithromycin (CLR), amikacin (AMK) and clofazimine (CLF) in a chronic murine infection model. ****$P < 0.0001$ relative to the Day (D) 61 untreated control group. The addition of SPR720 to the CLR + AMK and CLR + AMK + CLF treatment regimens further reduced bacterial burden in the lung ($P \leq 0.0001$). Dosing regimens: AMK, subcutaneously, q24h at 150 mg/kg; CLR, PO, q24h at 250 mg/kg; CLF, PO, q24h at 20 mg/kg; SPR720, PO, q24h at 25, 50 and 100 mg/kg; SPR720$_{50}$ indicates SPR720 was dosed at 50 mg/kg, PO, q24h in combination with first-line agents.

the efficacy of SPR720, no regimens >100 mg/kg were evaluated, and no pharmacokinetic data were collected. Therefore, it was not possible to explore a sigmoid E_{max} dose–response relationship. As with the MAC infection model, the cfu reduction in the lung relative to the Day 61 untreated control was used to compare efficacy of SPR720 alone and in combination and the study was not designed to assess synergy or antagonism nor determine statistical differences in outcome between the different treatment regimens. Of note, *M. abscessus* is typically associated with inducible macrolide resistance as reflected by the high MIC of >8 mg/L, and the reduction in bacterial burden with 250 mg/kg clarithromycin monotherapy was somewhat modest when compared with 100 mg/kg SPR720 monotherapy. Treatment with clofazimine and amikacin monotherapies also resulted in moderate 1.2 to 1.9 log_{10} reductions in bacterial burden. In comparison, SPR720 as monotherapy exhibited a dose response with increasing reduction in bacterial burden of up to 3.7 log_{10} cfu as doses were increased from 25 to 100 mg/kg. The inclusion of SPR720 (50 mg/kg) to regimens containing clarithromycin and amikacin ± clofazimine further reduced pulmonary bacterial burden by an additional 1.8 log_{10} cfu. While the addition of SPR720 (50 mg/kg) to a regimen that included clarithromycin, amikacin and clofazimine showed the greatest reduction in bacterial burden in the lung of 4.5 log_{10} cfu ($P = 0.0001$ relative to the same regimen without SPR720), PK-PD studies with additional strains of *M. abscessus* will be needed to confirm these results and the PK-PD targets and magnitude required to support future dose justification strategies.

In both the C3HeB/FeJ and SCID models, no visible signs of discomfort or significant weight loss were observed in any of the treatment groups with SPR720 alone at the highest tested dose of 100 mg/kg or in combination. SPR720 is a new pharmacophore that has potential to be added to or substituted for existing SOC agents. Oral bioavailability in humans has been demonstrated[14] and the active moiety interacts with a bacterial target (DNA gyrase B)[23] that is not exploited by currently marketed antibiotics, with no cross-resistance to current SOC agents being observed. SPR720 is anticipated to be used clinically in combination therapy to mitigate resistance that would be unavoidable with monotherapy, although development of spontaneous resistance among *M. avium* was relatively low *in vitro*.[24]

Collectively, the results of this study highlight the antibacterial activity of SPR719 against commonly encountered NTM organisms, including those resistant to current antibiotics. SPR720 has demonstrated proof-of-concept efficacy by exhibiting dose response as a stand-alone agent and even greater reductions in bacterial burden when evaluated in combination with other SOC agents. These results support the ongoing clinical development of SPR720 as a new agent for the treatment of NTM-PD.

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Transparency declarations

The authors are current or former employees of Spero Therapeutics, Inc.

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