Characterization of Resistance to DNA Gyrase Inhibitor SPR719 in *Mycobacterium avium*

Wassihun Wedajo Aragaw,[a] Nicole Cotroneo,[b] Ian Critchley,[b] Suzanne Stokes,[b] Michael Pucci,[b] Martin Gengenbacher,[a,c] Thomas Dick[a,c,d]

[a]Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ, USA; [b]Spero Therapeutics, Cambridge, MA, USA; [c]Department of Medical Sciences, Hackensack Meridian School of Medicine, Nutley, NJ, USA; [d]Department of Microbiology and Immunology, Georgetown University, Washington, DC, USA

Background

- Lung disease caused by Non-tuberculous Mycobacteria (NTMs) is increasing. *Mycobacterium avium* is the most common cause of NTM infections in the United States.[1]
- *M. avium* disease is usually treated with a macrolide-containing multidrug regimen. However, treatment times are lengthy and complicated by the emergence of drug resistance.[2] Thus new, and more efficacious drugs are needed. Recently, the aminobenzimidazole SPR720 entered development for the treatment of M. avium disease.[3]
- SPR720 is a phosphate prodrug which is converted to SPR719 as its bioactive component. SPR719 inhibits mycobacterial DNA gyrase by inhibiting the ATPase of the enzyme located on its GyrB subunit.[3]
- Here, we report spontaneous in vitro resistance frequencies and the mechanism of resistance to the novel DNA gyrase inhibitor in *M. avium* subspecies hominissuis.

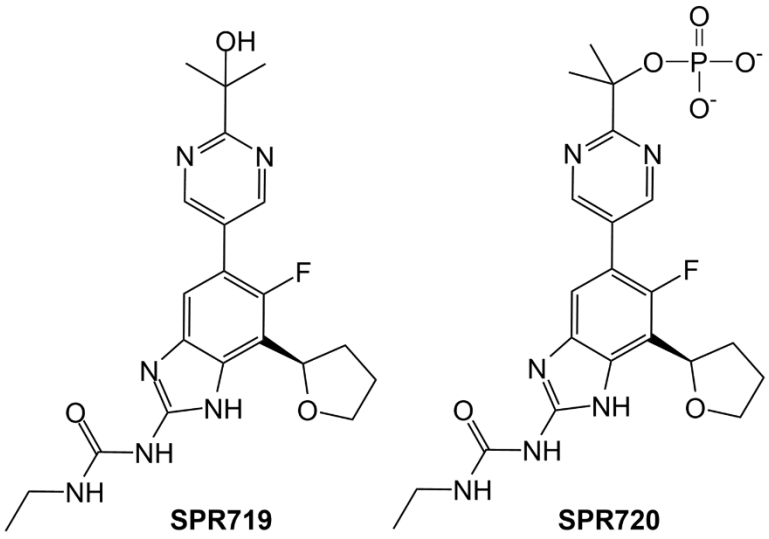

Figure 1. Structure of SPR719 and its phosphate prodrug SPR720

Methods

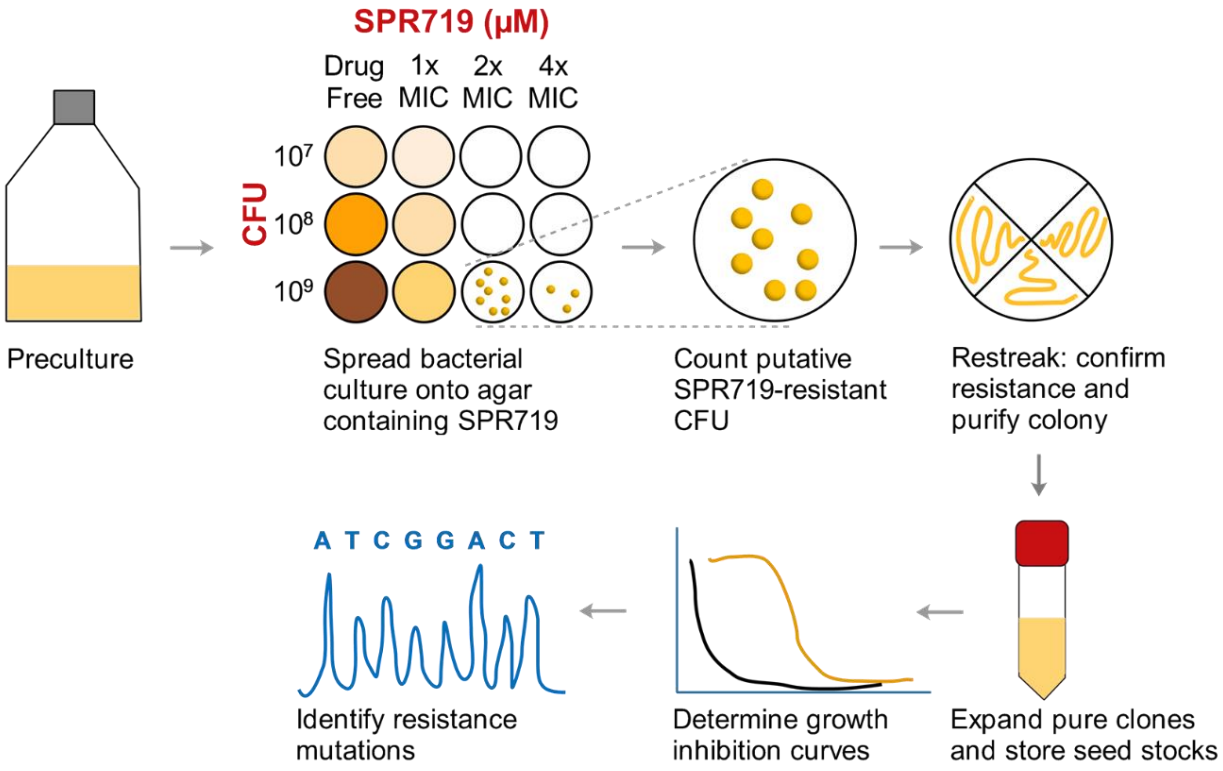

Figure 2. Schematic representation of the selection of spontaneous SPR719-resistant mutants using *M. avium* 109 and *M. avium* ATCC 700898. Bacterial inocula were spread onto Middlebrook 7H10 agar supplemented with a range of concentrations of SPR719. Putative resistant mutants were picked from the lowest compound concentration which prevented the growth of wildtype bacteria. To confirm the resistance, colonies were restreaked on agar containing corresponding concentrations of SPRR719 on which they were originally selected. The frequency of spontaneous resistance was determined by the ratio of the number of confirmed SPR719-resistant colonies to the total number of plated bacteria. Resistant strains were subjected to whole-genome sequencing to identified resistance mutations.

Results

Table 1. Characterization of SPR719-resistant *M. avium* 109 mutants

Exp.	Strain	MIC (µM)			*gyrB* (nt/aa)	*gyrA* (nt/aa)	*gyrB* mutation type
		SPR719	CLR	MXF			
	Mav wt	6	2	6	wt	wt	na
1	Spr^{r}1.1	>25*	2	6	T518C/I173T	wt	Missense
	Spr^{r}1.2	>25*	2	6	T518C/I173T	wt	Missense
2	Spr^{r}2.1	>25*	2	6	T518C/I173T	wt	Missense
	Spr^{r}2.2	>25*	2	6	T518C/I173T	wt	Missense
3	Spr^{r}3.1	>25*	2	6	T518C/I173T	wt	Missense
	Spr^{r}3.2	>25*	2	6	T518C/I173T	wt	Missense

Exp., experiment number; Mav, Mycobacterium avium subsp. hominissuis 109; wt, wildtype; Spr^r, SPR719 resistant; CLR, clarithromycin; MXF, moxifloxacin; na, not applicable; nt/aa, observed nucleotide polymorphism and associated amino acid substitution; >25*, MIC was greater than 25 µM. Concentrations were only tested up to 25 µM as at higher concentrations precipitation of the compound was observed. At 25 µM significant inhibition of growth was observed (~60%). The MIC experiments were carried out three times independently and the results are displayed as mean values.

Conclusion

- *In vitro* resistance analyses of SPR719 in *M. avium* revealed a frequency of resistance of 10^{-9} to 10^{-8}/CFU and associated polymorphism mapped to the ATPase domain of the GyrB subunit of DNA gyrase.
- These results demonstrate a low propensity for the development of resistance and confirm the mechanism of action of the novel DNA gyrase inhibitor in *M. avium*.

References

[1]Winthrop, K. L. et al., (2020). Annals of the American Thoracic Society 17(2): 178-185.
[2]Daley, C. L. et al., (2020). Clinical Infectious Diseases 71(4): e1-e36.
[3]Stokes, S. S., R. Vemula and M. J. Pucci (2020). ACS infectious diseases 6(6): 1323-1331.