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Microbial Characterization Using Multiple Diagnostic Methods in the First Oral Phase 3 Community-Acquired Bacterial Pneumonia (CABP) Trial with Solithromycin

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Introduction and Objective

A Phase 3 community-acquired bacterial pneumonia (CABP) study of oral solithromycin (SOLI) versus oral moxifloxacin (MOXI) was successfully completed in 2015. This was the first oral CABP trial conducted under the new FDA and EMA guidances and also the first to use several molecular methods coupled with traditional culture and serology to identify pathogens. CABP is a significant cause of morbidity and mortality worldwide and is a serious infection requiring systemic antibiotic therapy. Antibacterial agents used to treat CABP include the penicillins, cephalosporins, fluoroquinolones, and macrolides. The current Infectious Diseases Society of America and American Thoracic Society (ATS) treatment guidelines generally recommend a β-lactam plus macrolide combination or a fluoroquinolone alone. Macrolides, such as clarithromycin and azithromycin, are well-tolerated and safe therapeutic agents and have been noted to have beneficial anti-inflammatory/immunomodulatory effects in addition to antibacterial effects.

The emergence and spread of respiratory pathogens resistant to macrolides and other classes of antibiotics have begun to limit therapeutic options for treatment of CABP, resulting in the need for newer agents that are active against resistant strains (File 2004; Niederman 2004). A macrolide with improved activity, a better safety profile and available in both intravenous (IV) and oral formulations would be a significant therapeutic advance in the treatment of CABP.

Solithromycin is a fourth-generation macrolide antibiotic, and the first fluoroketolide, that has completed two global Phase 3 trials in patients with CABP. The objective was to describe the diagnostic methods used, the variety of bacteria isolated and their susceptibilities in the global, oral Phase 3 CABP trial.

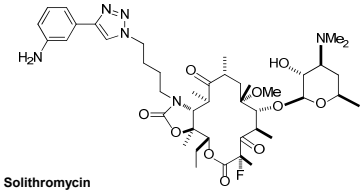

Figure 1: Solithromycin

Methods

This randomized, double-blind Phase 3 trial was conducted in 16 countries on 4 continents with 860 patients dosed once a day for 5 days with oral solithromycin or 7 days with oral moxifloxacin. The severity of pneumonia in the enrolled patients was as follows: 50% PORT II, 50% PORT III/IV.

Pathogen Identification: Multiple microbiological and molecular methods were used to identify causative CABP pathogens. Every effort was made to collect the following specimens: (1) Sputum, pleural or bronchial lavage fluids for Gram stain and culture; (2) Sputum for PCR detection of pathogens by the Curetis Unyvero™ LRT Application (Curetis, AG; Holzgerlingen, Germany). The pneumococcal positives were confirmed by a required positive result from a confirmatory singleplex PCR targeting the pneumococcal surface antigen (*psaA*); (3) nasopharyngeal (NP) swabs for pneumococcal culture and a quantitative real-time PCR (qPCR) assay utilizing primers that target the pneumococcal single copy *lytA* gene was performed in the laboratory of Dr. Jorge Vidal (Emory University) to quantify the pneumococcal bacterial load in nasopharyngeal swab specimens. This assay was established by Albrich et al. (Albrich 2012) that a *lytA* qPCR density ≥8000 copies/mL (equivalent to CFU/mL) distinguished between pneumococcal colonization and lower respiratory tract infection in patients presenting with CABP. This assay was modified to reduce the threshold to ≥1000 copies/mL for diagnosis as the original threshold was in immunocompromised patients with HIV. Compared to the former threshold, a cutoff of 1000 copies/mL provided greater sensitivity to detect pneumococcal pneumonia (55.93% vs. 38.98%) with retention of high specificity (86.44% vs. 91.86%); (4) Oropharyngeal (OP) swabs for Mycoplasma culture and PCR identification (University of Alabama, Birmingham, Diagnostic Mycoplasma Laboratory). Presumptive positive based on colony morphology or pH color change of broth were subjected to qPCR analysis utilizing primers that target the repetitive element repMp1 (Dumke 2007), to differentiate *M. pneumoniae* from commensal *Mycoplasma* species. Additionally, DNA extracted directly from the transport media inoculated by OP swab specimens was subjected to repMp1 qPCR analysis to detect and diagnose *M. pneumoniae*; (5) Blood for culture; (6) Blood for Legionella serology. Due to the high rate of Legionella detection by serology, only a four fold rise in titer ≥128 from acute to convalescent phase was accepted as positive; (7) Blood for mycoplasma serology (4-fold rise in IgG titer); (8) Urine for detection of *S. pneumoniae* (Alere BinaxNOW®); and (9) Urine for *Legionella pneumophila* serogroup 1 antigen detection (Alere BinaxNOW®).

Any pathogens isolated at the local laboratories were identified whenever possible to the genus and species level and submitted to the central microbiology laboratory for confirmatory identification and antibiotic susceptibility testing.

Results

Using all of the diagnostic methods defined in the Methods section the mITT pathogen detection rate was 54% of ITT (459/856). These results exceed the previously published 27% in the only other CABP trial that was conducted recently, i.e. for ceftaroline (File 2010).

The distribution of pathogens identified at baseline from Blood Specimens, Respiratory Specimens, Urinary Antigen Tests, and/or Serology are shown below (**Table 1**).

Baseline Pathogen	mITT Solithromycin 800/400 mg QD N=235 n (%)	mITT Moxifloxacin 400 mg QD N=224 n (%)
Gram-positive bacteria (aerobes)	116 (49.4)	111 (49.6)
Staphylococcus aureus	22 (9.4)	14 (6.3)
MRSA	0	0
MSSA	17	9
Macrolide-resistant	2	1
Quinolone-resistant	0	0
Streptococcus pneumoniae	96 (40.9)	101 (45.1)
Positive via urinary antigen	15	13
Positive via respiratory/blood specimen	84	95
MDRSP	13	10
PSSP	29	43
PISP	13	11
PRSP	8	4
Macrolide-resistant	12	8
Beta-Haemolytic streptococcus	3 (1.3)	4 (1.8)
Gram-negative bacteria (aerobes)	111 (47.2)	83 (37.1)
Haemophilus influenzae	80 (34.0)	55 (24.6)
Haemophilus parainfluenzae	6 (2.6)	5 (2.2)
Moraxella catarrhalis	28 (11.9)	23 (10.3)
Bordetella bronchiseptica	1 (0.4)	0
Enterobacter cloacae	1 (0.4)	0
Escherichia coli	0	3 (1.3)
Klebsiella oxytoca	0	1 (0.4)
Klebsiella pneumoniae	7 (3.0)	5 (2.2)
Morganella morganii	0	1 (0.4)
Salmonella spp.	0	1 (0.4)
Serratia liquefaciens	1 (0.4)	0
Serratia marcescens	1 (0.4)	1 (0.4)
Pseudomonas aeruginosa	3 (1.3)	1 (0.4)
Atypical Pathogens	91 (38.7)	99 (44.2)
Mycoplasma pneumoniae	37 (15.7)	41 (18.3)
Positive via respiratory specimen	21	27
Macrolide-resistant	0	2
Positive via serology	23	33
Legionella pneumophila	61 (26.0)	63 (28.1)
Positive via respiratory specimen	3	2
Positive via serology	56	62
Positive via urinary antigen	5	0
Legionella dumoffii	0	1 (0.4)

The primary endpoint was early clinical response (ECR) at 72 hours in the Intent to Treat (ITT) population and secondary endpoints included clinical response at the short-term follow-up visit (SFU), 5-10 days after the last dose of study drug in the ITT and clinically evaluable (CE-SFU) populations (results recently published by Barrera et al. 2016).

Another key secondary endpoint was the ECR rate in the microbiological Intent to Treat (mITT) population (patients with an identified pathogen). Outcomes were also assessed in the mITT-2 population, comprised mostly of patients with culture-based microbiological diagnoses. These data demonstrate that both study drugs had equivalent efficacy for treatment of CABP in these microbiologically defined populations (shown in **Table 2** below).

Early Clinical Response in Patient Population with Microbial Pathogen	Statistic	Solithromycin 800/400 mg QD N=235 n (%)	Moxifloxacin 400 mg QD N=224 n (%)
Total mITT (n)		235	224
ECR- mITT	n (%)	176 (74.9)	176 (78.6)
	95% CI	-3.68 (-11.8, 4.5)	
Total mITT-2 (n)		119	106
ECR- mITT-2	n (%)	92 (77.3)	89 (78.3)
		-0.99 (-12.8.10.8)	

When pathogen diagnosis was restricted to culture of blood, good quality respiratory specimens, and Legionella urinary antigen (the mITT-2 population), the microbial detection rate fell to 27% of the ITT population (225/856). Thus, use of serology data and molecular methods significantly increased diagnosis rates. Populations by study drug are shown in the Table below (**Table 3**).

Study Population	Solithromycin 800/400 mg QD n (%)	Moxifloxacin 400 mg QD n (%)
ITT (Total study population)	425 (100)	431 (100)
mITT	235 (55.3)	224(52.0)
mITT-2	119 (28.0)	106 (24.6)

The most frequently identified pathogens were *S. pneumoniae* (23%), *H. influenzae* (16%), *L. pneumophila* (15%), *M. pneumoniae* (9%), *M. catarrhalis* (6%), and *S. aureus* (4%) (**Table 1, ITT**). Macrolide resistance was 19% in *S. pneumoniae* while SOLI MICs were ≤0.5 mg/L (MIC90 = 0.015 mg/L).

MICs (μg/mL) of solithromycin, moxifloxacin and azithromycin for pathogens identified at baseline (mITT Population) are shown in the Table below (**Table 4**). Although moxifloxacin was used as the comparator in the trial, the susceptibility to azithromycin was also tested in vitro as it is frequently used to treat CABP in spite of pneumococcal resistance and is the most commonly used macrolide in treating respiratory tract infections.

Susceptibility of the Most Frequently Identified CABP Pathogens (μg/ml)

Pathogen (#)	Solithromycin		Moxifloxacin		Azithromycin	
	MIC90	Range	MIC90	Range	MIC90	Range
S. pneumoniae (107)	0.015	0.002 - 0.50	0.25	≤0.008 – 0.5	>32	0.06 - >32
H. influenzae (64)	4	0.25 - 4	0.06	0.008 - 1	4	0.25 - 8
M. pneumoniae (26)	≤0.000032	≤0.000032 - 0.25	0.125	0.031 - 0.125	0.0005	0.00006 - 32
S. aureus (24)	0.12	0.06 - 0.12	0.06	0.015 - 0.12	8	1 - >32
M. catarrhalis (17)	0.25	0.06 - 0.25	0.06	0.03 – 0.12	0.12	≤0.015 - 0.12
Legionella spp. (6)	NA	≤ 0.00024 - 0.016	NA	0.016 – 0.031	NA	0.004 - 0.125

Pathogen Susceptibility after Treatment if Isolated:

In the mITT population, no pathogens identified in solithromycin patients experienced decreasing susceptibility (defined as a change in MIC of >1 dilution). Apparent selection for resistance was observed for *H. parainfluenzae* (sputum specimen) in one patient who received moxifloxacin. The baseline *H. parainfluenzae* isolate had a moxifloxacin MIC of 0.12 g/mL (susceptible) and the Day 7 isolate had an MIC of 4.0 g/mL (non-susceptible). The solithromycin MIC of these isolates did not increase (4 μg/mL at baseline and 2 μg/mL on Day 7).

Conclusions

- In recent years, CABP caused by multi-drug resistant pathogens has been observed more frequently. Pneumococcal macrolide resistance, in particular, is now approximately 50% in the US (Morrissey 2014; Jones 2013). Therefore, there is an urgent need for additional antibacterial drugs with activity against these pathogens. The need for oral agents that can be administered in outpatient settings is particularly important.
- This Phase 3, randomized, double-blind, multicenter, non-inferiority efficacy and safety study was conducted to evaluate oral solithromycin compared with oral moxifloxacin in the treatment of adult patients with CABP. Solithromycin demonstrated NI to moxifloxacin and a comparable treatment effect to moxifloxacin against the most frequently observed CABP pathogens.
- Using traditional and molecular methods, 54% of patients in the trial had a bacterial pathogen identified. Culture and serology confirmed many of the results from molecular methods, which supplemented the overall bacterial identification in this oral CABP trial, the first to use the new FDA guidance. Allowance of many diagnostic methods enhanced the quality of the mITT population.
- Given its activity against common typical and atypical CABP pathogens including those with macrolide-resistance, solithromycin has the potential to restore macrolide monotherapy for the effective treatment of CABP.

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Disclosures

This phase 3 CABP trial was conducted and funded by Cempra, Inc. Kara Keedy, Brian Jamieson, Kay Clark, Amanda Sheets, Prabhavathi Fernandes, Evan Martens and David Oldach are employees of Cempra. M. Nitu, J. Flores, B. Rewerska, C. Tanaseanu, A. Das, and D. Hardy are consultants to Cempra, Inc.