CHEST

Open-Label Trial of Amikacin Liposome Inhalation Suspension in *Mycobacterium abscessus* Lung Disease

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BACKGROUND: *Mycobacterium abscessus* is the second most common nontuberculous mycobacterium respiratory pathogen and shows in vitro resistance to nearly all oral antimicrobials. *M abscessus* treatment success is low in the presence of macrolide resistance.

RESEARCH QUESTION: Does treatment with amikacin liposome inhalation suspension (ALIS) improve culture conversion in patients with *M abscessus* pulmonary disease who are treatment naive or who have treatment-refractory disease?

STUDY DESIGN AND METHODS: In an open-label protocol, patients were given ALIS (590 mg) added to background multidrug therapy for 12 months. The primary outcome was sputum culture conversion defined as three consecutive monthly sputum cultures showing negative results. The secondary end point included development of amikacin resistance.

RESULTS: Of 33 patients (36 isolates) who started ALIS with a mean age of 64 years (range, 14-81 years), 24 patients (73%) were female, 10 patients (30%) had cystic fibrosis, and nine patients (27%) had cavitary disease. Three patients (9%) could not be evaluated for the microbiologic end point because of early withdrawal. All pretreatment isolates were amikacin susceptible and only six isolates (17%) were macrolide susceptible. Eleven patients (33%) were given parenteral antibiotics. Twelve patients (40%) received clofazimine with or without azithromycin as companion therapy. Fifteen patients (50%) with evaluable longitudinal microbiologic data demonstrated culture conversion, and 10 patients (67%) sustained conversion through month 12. Six of the 33 patients (18%) demonstrated mutational amikacin resistance. All were patients using clofazimine or clofazimine plus azithromycin as companion medication(s). Few serious adverse events occurred for ALIS users; however, reduction of dosing to three times weekly was common (52%).

INTERPRETATION: In a cohort of patients primarily with macrolide-resistant *M abscessus*, one-half of the patients using ALIS showed sputum culture conversion to negative findings. The emergence of mutational amikacin resistance was not uncommon and occurred with the use of clofazimine monotherapy.

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KEY WORDS: amikacin liposome inhalation suspension; *Mycobacterium abscessus*; nontuberculous mycobacteria lung disease

Take-home Points

Study Question: Does treatment with amikacin liposome inhalation suspension improve culture conversion in patients with *M abscessus* pulmonary disease who are treatment naive or who have treatment-refractory disease?

Results: Fifteen patients (50%) with evaluable longitudinal microbiologic data demonstrated culture conversion, and 10 patients (67%) sustained conversion through month 12.

Interpretation: In a cohort of patients primarily with macrolide-resistant *M abscessus*, half of the patients using amikacin liposome inhalation suspension showed sputum culture conversion to negative findings.

Nontuberculous mycobacterium (NTM) lung disease is increasing in incidence worldwide.[1-3] *Mycobacterium abscessus* is the second most common cause of NTM lung disease in North America and Asia.[2,3] *M abscessus* comprises three subspecies: *M abscessus* subspecies *abscessus* (Mab-A), *M abscessus* subspecies *bolletii*, and *M abscessus* subspecies *massiliense* (Mab-M).[4,5] Mab-A is the most common cause of *M abscessus* lung disease in North America and is the most highly drug resistant of the rapidly growing mycobacteria.[1,2,6,7]

The critical element for treatment success of all *M abscessus* subspecies is macrolide susceptibility. Mab-A and *M abscessus* subspecies *bolletii* have two known mechanisms of macrolide resistance.[8-10] The most common is an *erm* gene that confers inducible macrolide resistance.[8,9] With rare exception, Mab-M isolates have a truncated, nonfunctional *erm* gene.[4,8] Approximately 20% of Mab-A isolates have a mutation in the *erm* gene that inactivates it, thereby maintaining macrolide susceptibility.[11] The second and less common mechanism for macrolide resistance, pertinent to all *M abscessus* subspecies, is acquired macrolide resistance associated with mutations in the 23S ribosomal RNA gene.[10,12] Culture-conversion rates with treatment for macrolide-resistant *M abscessus* subspecies have ranged from 25% to 42%, whereas conversion rates with macrolide-susceptible *M abscessus* subspecies predictably and significantly are much better, ranging from 82% to 96%.[13]

After macrolides, the most important antimicrobial for treating *M abscessus* subspecies is amikacin.[13,14] Currently no other antibiotics, parenteral or oral, are available individually or in combination that unambiguously have shown predictably favorable treatment outcomes for *M abscessus* other than the macrolides and amikacin.[7,13-15] Most untreated *M abscessus* isolates have minimum inhibitory concentration (MIC) tests consistent with susceptibility to either IV or inhaled amikacin.[16,17] A major constraint for amikacin is the necessity for IV administration with the attendant potential for serious systemic side effects including nephrotoxicity and ototoxicity that limit the duration of IV amikacin exposure.[6,7,13,15] *M abscessus* subspecies also are vulnerable to acquired amikacin resistance associated with a mutation in the 16S ribosomal RNA gene.[16]

Amikacin liposome inhalation suspension (ALIS) is a liposomal formulation of amikacin recently shown to improve microbiologic outcomes in patients with treatment-refractory *Mycobacterium avium* complex lung disease.[18,19] The high airway concentrations and penetration of biofilms and macrophages by this compound provide the rationale for also evaluating its use in the treatment of *M abscessus*.[20] Recent NTM treatment guidelines recommend an initial regimen of at least four drugs against *M abscessus*, including two IV medications, based on in vitro susceptibility results.[15] Long-term therapy frequently is unsuccessful because of poor tolerance of parenteral therapy combined with the lack of activity of most currently available oral agents. Alternative treatment options including inhaled therapies are needed urgently. Accordingly, we sought to add ALIS to treatment regimens for patients starting or currently receiving multidrug therapy for *M abscessus* lung disease. Herein we report the results of this open-label evaluation of ALIS.

ABBREVIATIONS: 6MWT = 6-min walk test; AE = adverse event; ALIS = amikacin liposome inhalation suspension; CF = cystic fibrosis; Mab-A = *Mycobacterium abscessus* subspecies *abscessus*; Mab-M = *Mycobacterium abscessus* subspecies *massiliense*; MIC = minimum inhibitory concentration; NTM = nontuberculous mycobacterium; NTM-NET = Nontuberculous Mycobacterial Network European Trials group; QOL-B = Quality of Life Questionnaire-Bronchiectasis

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Study Design and Methods

Study Design

This phase 2 open-label pragmatic study was conducted at two sites in North America: Oregon Health and Science University and the University of Texas Health Science Center at Tyler. Each site's institutional review board approved the protocol, and patient informed consent was obtained.

Patients aged 12 years or older with a diagnosis of *M abscessus* lung disease (resulting from any *M abscessus* subspecies) as defined by 2007 diagnostic criteria who showed positive culture results at the time of screening were eligible for the study.[21] Patients were enrolled regardless of whether they had received prior *M abscessus* therapy. Key exclusion criteria were a history of lung transplantation, active pulmonary tuberculosis, those treated with inhaled or IV amikacin within 14 days before baseline evaluation, or those with isolates known to be amikacin resistant.

Patients received ALIS (590 mg) once daily added to their companion regimen for 12 months. Companion antimicrobials were selected independently by investigators at each site. No predetermined criteria such as in vitro susceptibility results were used in the selection of companion antibiotics. Patients were managed according to standard of care for efficacy and toxicity monitoring at each site. We attempted to obtain two sputum culture samples monthly from all patients. If expectorated sputum could not be obtained, sputum was induced at each site with nebulized hypertonic (7%) saline. Over the course of the study, 12 patients were unable to produce sputum during at least one visit. The largest number of visits without sputum production was in one individual who was unable to produce sputum at five visits, all toward the beginning of the ALIS plus companion regimen.

Participants who received one or more doses of study drug were divided into two treatment groups. Those who received < 4 months of study drug were considered nonevaluable for long-term microbiologic treatment outcome, but evaluable for adverse events (AEs) and amikacin resistance development.[18,19] Those who received > 4 months of study drug were evaluable for all outcome measures. Patients also were assessed as to whether they required change in study drug dosage or frequency of administration, including discontinuation of the study drug.

Primary and Secondary End Points

The primary outcome measure was culture conversion without reversion over the 12-month treatment period. Conversion to negative results was defined as showing negative results for sputum acid-fast bacillus cultures for 3 consecutive months. Patients with initial mixed infection with macrolide-susceptible and macrolide-resistant *M abscessus* strains were classified as being macrolide resistant. Culture reversion was defined as showing one or more *M abscessus* cultures with positive results obtained during the study period after culture conversion.

Secondary microbiologic end points included the time to culture conversion defined as the date of the first of three consecutive negative culture findings, the proportion of patients showing negative culture results by month 12, the number of patients who achieved culture conversion, the proportion of patients showing sustained culture conversion through month 12 who continued to show negative culture results without therapy for 3 months after stopping therapy, and the emergence of amikacin-resistant *M abscessus* isolates during the study period.

Other secondary end points included 6-min walk test (6MWT) results and patient quality of life as assessed by the Quality of Life Questionnaire-Bronchiectasis (QOL-B) and NTM Module. The 6MWT was performed as described previously.[18,19] The distance walked at baseline was compared with distances walked at month 6, month 12 (end of treatment), and month 15 (3 months without study drug). The QOL-B and NTM Module were administered at baseline and at months 6 and 12. Baseline item-level scores were compared with those obtained at months 6 and 12.

Microbiologic Assessment

Sputum specimens were processed with standard concentration and decontamination methods for acid-fast bacilli (e-Appendix 1).[22]

Antimicrobial Susceptibility Testing

Pretreatment *M abscessus* subspecies isolates (screening, n = 6; baseline, n = 27) were tested for in vitro antibiotic susceptibility by the Clinical and Laboratory Standards Institute-recommended method of broth microdilution in cation-adjusted Mueller Hinton broth using doubling dilutions of antimicrobials in microtiter panels manufactured by Thermofisher (e-Appendix 1).[16,23-25]

M abscessus *Strain Genotyping*

We compared *M abscessus* genotypes between baseline and later isolates in patients who did not achieve culture conversion (the last positive culture results were used) and in patients who did achieve culture conversion, but later reverted to positive culture results (the first new positive results were used). We performed *erm* (41) gene typing and pulsed-field gel electrophoresis as described previously (e-Table 1).[26]

Tolerability and Safety

At each monthly visit or reminder call, treatment-emergent AEs were discussed and then evaluated for their severity and relatedness to the study drug. Audiologic data were collected before initiation of ALIS, at month 6, and at month 12. We described treatment-emergent AEs and audiologic testing results for all 33 study participants.

Results

Patient Disposition and Demographic Features

Thirty-three patients enrolled in the study, of whom three discontinued because study drug-related AEs before month 4 and were considered nonevaluable for microbiologic outcomes (ie, culture conversion). All enrolled patients were included in AE and amikacin resistance analyses (Fig 1). The mean age at enrollment was 64 years (range, 14-81 years), 21 patients (70%) were female, 27 patients (90%) were White, 25 patients (85%) reported non-Hispanic or Latino ethnicity, and 22 patients (73%) had never used tobacco. For the 30 remaining patients evaluable for microbiologic outcome, 27 patients (90%) completed 12 months of therapy, whereas three patients (10%) discontinued therapy at months 5, 6, and 7 for respiratory treatment-emergent AEs. Ten

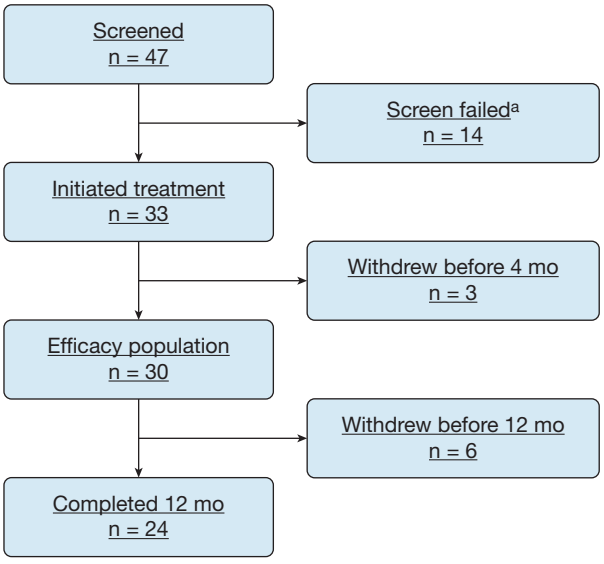

Figure 1 – *Consolidated Standards of Reporting Trials (CONSORT) diagram showing progression of patients in the study.* [a]*All 14 screening failures were the result of no positive culture findings for* M abscessus *during the screening visit.*

evaluable patients (33%), including the one patient with Mab-M, had cystic fibrosis (CF) (Table 1).

Antimicrobial Susceptibility Testing

At the initiation of therapy, 25 participants' culture samples (83%) showed *M abscessus* isolates with macrolide-inducible resistance (ie, a functional *erm* [41] gene). Six patients showed *M abscessus* isolates susceptible to macrolide before starting therapy (e-Table 2). One patient showed sequevar II Mab-A (30) only with a nonfunctional *erm* (41) gene. One patient with CF showed Mab-M only. One patient demonstrated a mixed infection with Mab-A with a functional *erm* (41) gene and sequevar II Mab-A[27] with a nonfunctional *erm* (41) gene. The remaining three patients showed mixed infections with Mab-A and Mab-M. For treatment outcome purposes, these latter three patients were included in the analysis of macrolide-resistant isolates.

Linezolid, tigecycline, and imipenem MICs were obtained, although no resistance breakpoints for *M abscessus* currently are approved for tigecycline (note that MICs for all antimicrobials other than for macrolide and for amikacin *M abscessus* have unproven clinical significance).[13,15] All seven patients (23%) who began linezolid therapy showed susceptible or intermediate MICs (≤ 16 μg/mL), whereas the seven patients (23%) who began tigecycline therapy showed low MICs (< 0.5 μg/mL), in the range of most wild-type (untreated)

TABLE 1] Demographic and Clinical Characteristics of Study Participants Beginning ALIS Therapy (n = 33)

Characteristic	Data
Age, y	64 ± 24
Female sex	24 (73)
Race	
White	29 (88)
Asian	2 (6)
American Indian or Alaska Native	1 (3)
Other (Arabic)	1 (3)
Ethnicity	
Hispanic or Latino	5 (15)
Non-Hispanic or Latino	28 (85)
Cystic fibrosis diagnosis	10 (30)
Tobacco use status	
Never	24 (73)
Former	9 (27)
M abscessus subspecies[a]	
Abscessus	32 (97)
Massiliense	4[a] (12)
Historic positive culture results[b]	3.8 ± 3.4
Patients with historic positive culture results[b]	31 (94)
Cavitary disease at baseline	9 (27)
Treatment naive at time of ALIS initiation	18 (55)
Average time of historic treatment, mo	24 ± 26

Data are presented as No. (%) or mean ± SD. ALIS = amikacin liposome inhalation suspension.
[a]Three patients with *M abscessus* subspecies *massiliense* demonstrated mixed infections with *M abscessus* subspecies *abscessus*. One patient showed *M abscessus* subspecies *massiliense* alone.
[b]Number of *M abscessus* species positive respiratory culture results in 12 months before enrollment.

isolates.[27] Of the seven patients (23%) who began imipenem therapy, five patients (71%) showed MICs of 16 μg/mL (intermediate) and two patients (29%) showed MICs of 32 μg/mL (resistant).[17] Clofazimine MICs were not obtained because no resistance breakpoints for NTM currently are approved and no MIC panels with this agent were available.[17]

Baseline Multidrug Therapy

Twenty-four patients (80%) received azithromycin, 23 patients (77%) received clofazimine, seven patients (23%) received linezolid, seven patients (23%) received imipenem, and seven patients (23%) received tigecycline. Notably, four of seven patients (57%) who received tigecycline (usually 50 mg tid) did so for < 2 months. Overall, only 11 of 30 patients (37%) received

TABLE 2] Concomitant Antimicrobials Used With ALIS Therapy Among Those Patients With Longitudinal Evaluable Microbiologic Data (n = 30)

Concomitant Antimicrobials	No. of Patients
Oral only	19
Azithromycin plus clofazimine	12
Azithromycin plus clofazimine plus ethambutol	1
Clofazimine plus tedizolid	1
Azithromycin plus linezolid	2
Clofazimine alone	1
Linezolid alone	1
Azithromycin plus linezolid plus tedizolid	1
Oral and parenteral	11
Azithromycin plus clofazimine plus tigecycline	3
Azithromycin plus clofazimine plus tigecycline plus imipenem	2
Azithromycin plus clofazimine plus imipenem	2
Clofazimine plus linezolid plus tigecycline	1
Azithromycin plus ethambutol plus linezolid plus imipenem	1
Linezolid plus imipenem	1
Tigecycline plus imipenem	1

ALIS = amikacin liposome inhalation suspension.

IV medications and seven of 11 patients (64%) completed 3 months of IV therapy (Table 2).

Microbiologic Outcomes

Of the 30 evaluable patients, 23 patients (77%) showed one or more negative culture results during months 1 through 12. Fifteen patients (50%) achieved sputum conversion to negative findings during the study, including 12 of 24 patients (50%) with macrolide-resistant *M abscessus* (Table 3). Treatment was considered to have failed in patients who demonstrated mixed culture results with conversion in the susceptible genotype, but failure in the resistant genotype, because the culture results were still positive.

Six patients showed macrolide-susceptible *M abscessus* isolates at the start of therapy (e-Table 2). The two patients with only macrolide-susceptible *M abscessus* at treatment initiation achieved sputum conversion to negative findings with therapy. Three patients with mixed macrolide-susceptible and macrolide-resistant *M abscessus* isolates achieved conversion to negative findings for the macrolide-susceptible *M abscessus* isolate, but not the macrolide-resistant *M abscessus* isolate. One Mab-M isolate recovered before treatment from a patient with CF became mutationally resistant to macrolide and 2 months later became amikacin resistant, with persistence of the macrolide-resistant *M abscessus* with therapy. Overall, one patient (isolate) was nonevaluable, and four of five patients (80%) with evaluable macrolide-susceptible isolates from the beginning of therapy achieved conversion to negative findings during therapy, compared with 10 of 24 patients (42%) with macrolide-resistant isolates.

Seven of 10 patients with CF (70%) achieved sputum conversion. Six of nine patients (67%) with cavitary disease achieved sputum conversion, four of six patients (67%) continued to show negative results through month 12. Eight of 11 patients (73%) who received parenteral antibiotics achieved sputum culture conversion. Eleven of 27 patients (41%) who completed 12 months of therapy continued to show negative culture findings through month 12.

Patients who sustained the conversion vs those who reverted were of similar proportions for use of parenteral agents (71% vs 50%), but showed a difference in proportions for those patients with a diagnosis of CF compared with those without (14% vs 38%). Overall, 75% of patients with cavitary disease achieved conversion, as compared with 45% of those without cavitary disease. Prior amikacin therapy was a factor for conversion. Of those with previous amikacin use, eight of 11 patients (73%) achieved conversion compared with seven of 19 patients (37%) who did not have a history of amikacin use. This difference may be attributed to concomitant parenteral therapy use between those who achieved conversion who previously used amikacin and those who were naive to amikacin therapy (63% vs 43%). Of the 10 patients showing sustained microbiologic conversion at the end of treatment (month 12), five patients maintained the conversion status through month 15. The percentage of patients who maintained microbiologic conversion for 3 months without therapy was similar for those with cavitary disease (60% vs 40%), but varied by CF status (40% vs 0%) for those who did not maintain conversion status. The percentage of those who used of parental therapy was 80% for those who sustained microbiologic conversion compared with 20% for those who did not.

TABLE 3] Patients Who Achieved Conversion Before Month 12

Patient No.	Inducible Clarithromycin MIC	CF	Cavity	Amikacin Before Study	Amikacin MIC, μg/mL		Genotype		Companion Antibiotics at Conversion	Month of Conversion	Month of Reversion	Relapse or Reinfection	
					Screening	Baseline	Screening	Baseline				Genotype	Reversion Amikacin MIC, μg/mL
A01-003	S	Yes	No	No	16	16	mass	mass	Azithromycin	1	NA	NA	NA
									Clofazimine				
									Tigecycline				
A01-006	R	No	Yes	Yes	8	8	VII	VII	Azithromycin	1	4 and 5	VII	16
									Clofazimine				
A01-008	R	No	No	No	8	ND	VI	ND	Azithromycin	1	15[a]	VI	8
	R				8	ND	I	ND	Clofazimine			I	8
A01-010	R	Yes	No	Yes	16	16	I	I	Azithromycin	2	12	I	16
									Clofazimine				
A01-013	R	Yes	No	No	8	16	VIII[b]	VIII	Azithromycin	9	NA	NA	NA
									Clofazimine				
									Imipenem				
									Tigecycline				
A01-020	S	No	Yes	Yes	16	Negative	mass	Negative	Azithromycin	Baseline	11	I	8
									Clofazimine				
									Imipenem				
A01-021	R	Yes	Yes	No	ND	16	VIII	VIII	Azithromycin	1	7[c]	VIII	8
									Clofazimine				
									Imipenem				
									Tigecycline				
A01-026	R	No	Yes	Yes	16	Negative	VIII	Negative	Azithromycin	Baseline	NA	NA	NA
									Clofazimine				
									Tigecycline				
A01-027	R	Yes	No	No	ND	8	VI	VI	Azithromycin	9	13	VI	16
									Clofazimine				
									Imipenem				
A02-002[d]	S	No	No	No	8	8	Mab(30)	Mab(30)	Azithromycin	2	NA	NA	NA
	S				16	16	Mab(30)	Mab(30)	Clofazimine				
A02-008	R	No	No	Yes	16	16	I	I	Linezolid	1	5	I	32

(Continued)

TABLE 3] *(Continued)*

Patient No.	Inducible Clarithromycin MIC	CF	Cavity	Amikacin Before Study	Amikacin MIC, μg/mL		Genotype		Companion Antibiotics at Conversion	Month of Conversion	Month of Reversion	Relapse or Reinfection	
					Screening	Baseline	Screening	Baseline				Genotype	Reversion Amikacin MIC, μg/mL
A02-010	R	No	No	Yes	ND	16	X	X	Imipenem	3	NA	NA	NA
									Linezolid				
A02-013	R	No	No	No	ND	8	I	I	Azithromycin	1[e]	NA	NA	NA
									Linezolid				
A02-014	R	No	No	Yes	16	16	XIII	I	Imipenem	4[f]	NA	NA	NA
	R				4	16	XIII	I	Tigecycline				
A02-015	R	No	No	No	ND	8	VII	VII	Azithromycin	1	5	VII	ND
									Imipenem				
	R				ND	4	VII	VII	Linezolid			Mab(30)[g]	8

CF = cystic fibrosis; MIC = minimum inhibitory concentration; NA = not applicable; ND = not done; mass = massilene; S = suspectible; R = resistant.
[a]At 15 months showed positive culture findings (genotype VI) and amikacin MIC of 8 μg/mL.
[b]New infection (genotype I); no culture results were available for months 3, 5, 6, 12, or 14.
[c]Patient withdrew after month 7.
[d]Patient completed screening and enrollment visits twice, at two separate time points.
[e]No culture results were obtained at months 2 and 3, the culture results at month 4 were positive, and the culture results at months 5 through 15 were negative.
[f]Patient converted type I at month 4, XIII remained in culture findings, and the patient withdrew at month 6.
[g]Mab(30) was detected first at month 7, the patient converted Mab(30) at month 13, and *M avium* complex also was detected in culture samples for months 8 through 11.

Twenty-three of 30 patients (77%) underwent pulsed-field gel electrophoresis at baseline and later during the study (the last positive culture results in those who did not achieve conversion and the first new positive results in those who initially achieved conversion and then reverted). All four patients with reversion isolates harbored the same genotype of Mab-M or Mab-A as at initiation of therapy with positive culture findings that either were positive in broth only or showed fewer than five colonies on solid media. Two of 23 patients (9%) without sputum conversion demonstrated the appearance of new *erm* gene sequevars during therapy, whereas one patient demonstrated the appearance of a new *erm* sequevar after ALIS therapy was discontinued.

Pretreatment *M abscessus* isolates from all patients were amikacin susceptible in vitro and all had showed an MIC of $\leq$ 16 μg/mL, including nine patients known to have received IV amikacin before the study. During therapy, six of 33 patients (18%) demonstrated amikacin resistance with MICs of $>$ 64 μg/mL and the 16S ribosomal RNA mutation at base pair position 1408 (Table 4). Of those who demonstrated amikacin resistance during the study, three patients were receiving ALIS with azithromycin and clofazimine, and three patients were receiving ALIS and clofazimine only.

Functional and Quality-of-Life Assessments

6MWT Outcomes: The overall mean distances walked in the 6MWT throughout the time points were similar, ranging from 470 to 490 m. Baseline mean $\pm$ SD distance walked for the 6MWT was similar between those individuals who achieved conversion before month 12 and those who did not (489.2 $\pm$ 127.0 m and 489.7 $\pm$ 107.5 m, respectively). At 12 months, those who achieved conversion showed a larger decrease of distance walked as compared with those who did not achieve conversion. At 15 months, those who achieved conversion showed an increase in distance walked from baseline as compared with those who did not achieve conversion who were still walking distances shorter than the baseline distance walked.

QOL-B and NTM Module: Quality-of-life assessments were similar between patients who achieved conversion and those who did not before month 12. Both the NTM symptoms and respiratory symptoms domains showed borderline improvement. The NTM symptoms domain comparison from baseline to month 6 found that both those who did not achieve conversion and those who did achieve conversion experienced an improvement in NTM symptoms, but only those who did not achieve conversion sustained this improvement by month 12 (they were more likely to be ranked as improved or stable than worsened when compared with those who achieved conversion: 19-point increase vs 4-point increase, respectively). The respiratory symptom domain showed that those who did not achieve conversion experienced an increase in symptoms that was maintained over the course of the study, whereas those who achieved conversion experienced an improvement in symptoms at month 6, but returned to baseline levels at month 12 (3-point decrease at month 12 vs 1-point increase at month 12, respectively). No other domain approached significance at either 6 or 12 months when comparing those who achieved conversion with those who did not.

AEs (Tolerability and Safety)

Of the 33 patients initiating ALIS therapy, three patients withdrew before 4 months of being enrolled in the study and were nonevaluable microbiologically because of study drug intolerance: two patients because of dysphonia, sore throat, and cough, and one patient because of fatigue, abdominal pain, diarrhea, and weight loss. Of the 30 patients treated beyond month 4, three patients dropped out before completing 12 months of ALIS therapy, one patient after month 5 (because of intermittent malaise), one patient at month 6 (because of worsening of NTM disease and receiving a lung transplant), and one patient at month 7 (because of moving out of state). The most common AEs were pulmonary exacerbations (88.9% of patients with CF and 16.7% of patients without CF) followed by dysphonia (22.2% of patients with CF and 16.7% of patients without CF) (Table 5).

Hearing loss was considered an AE of special interest. Two of 30 patients (7%) experienced a grade 1 AE while receiving ALIS therapy. One patient experienced a grade 1 AE at month 6, although this AE resolved by the 12-month mark. The second patient experienced both grade 1 (right ear) and grade 3 (left ear) hearing loss AEs at the 6-month visit. This individual withdrew from the study at this visit. For the remaining participants, audiology results did not vary over the course of the protocol. The average decibel shifts for right (0.32 and –0.8 dB) and left (0.46 and 1.08 dB) ears were similar at months 6 and 12, respectively.

Twenty-seven of 30 patients (90%) reported missing some study drug doses, whereas 21 patients (70%) reported missing five or more doses, mostly because of AEs. Sixteen of 30 patients (53%) underwent dosage

TABLE 4] Patients With Amikacin-Resistant Isolates

Patient No.	CF Status	Subspecies or Sequevar	Isolate Time of Culture	Amikacin Before Study	Companion Antibiotics	Amikacin MIC, μg/mL	Amikacin 16S Mutation Present	3-Day Clarithromycin MIC, μg/mL	Extended (Inducible) Clarithromycin MIC
A01-009	No	Subspecies	Baseline	Yes[a]	Clofazimine	16	NP	0.5	R
	...	*abscessus*	Month 4	...	...	> 64	Yes	≤ 0.06	R
	...	(Sequevar 1)	Month 7	...	...	> 64	Yes	0.25	R
	...	...	Month 9	...	...	> 64	Yes	0.12	R
A01-022	No	Subspecies	Baseline	No	Azithromycin	8	NP	0.25	R
	...	...	...	...	Clofazimine	...	...	...	...
	...	*abscessus*	Month 6	...	...	> 64	Yes	0.5	R
	...	Sequevar VI	Month 10	...	...	> 64	Yes	0.25	R
	...	...	Month 11	...	...	> 64	Yes	0.12	R
	...	...	Month 15	...	...	> 64	Yes	0.25	R
A01-028	No	Subspecies *abscessus* (sequevar XIV)[b]	Screening	Yes	Clofazimine	NP	NP	NP	NP
	...	...	Baseline	...	...	8	NA	≤ 0.06	R
	...	...	Months 2-4	...	...	NP	NP	NP	NP
	...	...	Month 6	...	...	> 64	NP	≤ 0.06	R
	...	...	Month 11	...	...	> 64	Yes	0.5	R
	...	...	Month 12	...	...	> 64	Yes	≤ 0.06	R
A01-029	Yes	Subspecies[c]	Screening 1	No	Azithromycin	8	NP	≤ 0.06	S
		...	...	...	Clofazimine	...	...	...	...
	...	*massiliense*	Screening 2	...	...	16	NP	0.5	S
	...	...	Month 1	...	...	NP	NP	0.12	S
	...	...	Month 6	...	...	32	NP	> 16	NA
	...	...	Month 7	...	...	16	NP	0.12	S
	...	...	Month 8	...	...	> 64	Yes	> 16[b]	NA
A02-006	No	Subspecies	Screening	No	Azithromycin	32	NP	1	R
	...	...	...	...	Clofazimine	...	...	...	...
	...	*abscessus*	Baseline	...	...	16	NP	0.5	R
	...	(sequevar 1)	Month 2	...	...	> 64	Yes	0.25	R
	...	...	Month 4	...	...	64	NP	1	R

(Continued)

TABLE 4] *(Continued)*

Patient No.	CF Status	Subspecies or Sequevar	Isolate Time of Culture	Amikacin Before Study	Companion Antibiotics	Amikacin MIC, μg/mL	Amikacin 16S Mutation Present	3-Day Clarithromycin MIC, μg/mL	Extended (Inducible) Clarithromycin MIC
A02-011	No	Subspecies	Screening 1	Yes	Azithromycin	16	NP	0.5	R
...	...	...	...	...	Clofazimine	...	...	...	...
...	...	*abscessus*	Screening 2	...	...	16	NP	0.5	R
...	...	(sequevar 1)	Month 2	...	...	> 64	Yes	0.12	R
...	...	...	Month 6	...	...	> 64	Yes	0.25	R
...	...	...	Month 9	...	...	> 64	Yes	0.12	R

CF = cystic fibrosis; MIC = minimum inhibitory concentration; NA = not applicable; NP = not performed.
[a]Duration of amikacin therapy before study unknown.
[b]Months 7, 9, and 10 culture results were NA; month 8 culture results were negative.
[c]Clarithromycin mutation in the 23S ribosomal RNA also was detected in this isolate amikacin resistance ≥ 64 μg/mL.[28]

TABLE 5] AEs Occurring in Two or More Patients

AE	Patients With AE, No. (%)
Dysphonia	18 (55)
Infective pulmonary exacerbation of cystic fibrosis	18 (55)
Worsening cough	9 (27)
Fatigue	8 (24)
Hemoptysis	8 (24)
Infective pulmonary exacerbation of bronchiectasis	8 (24)
Vaginal yeast infection	8 (24)
Nausea	7 (21)
Upper respiratory infection	7 (21)
Oral thrush	5 (15)
Dyspnea	4 (12)
Generalized weakness	4 (12)
Progression of NTM lung disease	4 (12)
Worsening dyspnea	4 (12)
Chest pressure or heaviness	3 (9)
Diarrhea	3 (9)
Emesis	3 (9)
Influenza A	3 (9)
Pneumonia	3 (9)
Sinusitis	3 (9)
Wheezing	3 (9)
Anorexia	2 (6)
Contact dermatitis	2 (6)
Elevated C-reactive protein	2 (6)
Elevated liver function test results	2 (6)
GI disturbance	2 (6)
Intermittent nausea	2 (6)
Ototoxicity, grade 1	2 (6)
Rash	2 (6)
Sore throat	2 (6)
Tinnitus	2 (6)
Worsening pulmonary function test results	2 (6)

AE = adverse event; NTM = nontuberculous mycobacterium.

frequency reduction from daily to three times weekly. Thirteen of 30 patients (43%) completed the study with less than a daily dosing regimen. No apparent negative impact on microbiologic outcome (ie, culture conversion) was found in patients who underwent dose frequency adjustment compared with those without adjustment (42% vs 50%, respectively). No participant in this study experienced a life-threatening AE, and no study-related deaths occurred.

Discussion

We evaluated the usefulness of ALIS in the treatment of a cohort with macrolide-resistant *M abscessus* lung disease. Most importantly, one-half of patients achieved sputum conversion to negative results during the trial, and most demonstrated one or more negative culture results during the study. This was despite the very limited use of active companion medications in this study. Perhaps equally important, we observed the emergence of amikacin resistance in a small but not inconsequential proportion of individuals. Our study suggested that ALIS may contribute to the treatment of *M abscessus* lung disease. Moreover, our results highlighted the need for concomitant antibiotics that protect against the emergence of amikacin resistance.

The culture conversion results are similar to those of Zweijpfenning et al[29]: 44% of the patient population, because exposure to ALIS was longer term (eg, ≥ 12 months). The Nontuberculous Mycobacterial Network European Trials group (NTM-NET) protocol noted that all of the participants with *M abscessus* showed no difference in culture conversion between macrolide-susceptible and macrolide-resistant strains. The Olivier et al[18] study showed a lower rate of conversion for this patient population, < 5% by month 3. The difference is to be expected, because these individuals were exposed to ALIS for a shorter period compared with patients in our study and the NTM-NET protocol.[18,29] Both the NTM-NET and Olivier et al[18] studies showed large portions of patients refractory to treatment as compared with our study, although it is unclear if individuals who have refractory *M abscessus* infection are less likely achieve a culture-conversion response to ALIS therapy.

It is notable that none of the patients who received initial parenteral tigecycline or imipenem demonstrated amikacin resistance and that all of the patients who demonstrated amikacin-resistant isolates were receiving clofazimine alone with ALIS or clofazimine and azithromycin with ALIS and did not respond to therapy. In all but one patient, the isolate showed inducible macrolide resistance, such that these patients would have had clofazimine as potentially the only active drug alongside ALIS. In vitro studies have suggested that clofazimine and amikacin show synergy against *M abscessus*; however, our findings suggest that such a two-drug combination is not adequate to prevent the emergence of mutational amikacin resistance.[30] It is possible that amikacin and clofazimine are in different compartments. ALIS distribution largely is limited to the airway with little systemic absorption, whereas oral clofazimine is unlikely to build up substantial levels inside the airway. Attempts at developing clofazimine as an inhaled compound[28] might alleviate this potential concern, and future studies of such a combination still would be considered despite our findings.

In general, ALIS was tolerated well, but as noted, most patients (87%) reported missing some study drug doses, mostly because of AEs, and one-half of the patients underwent dosage frequency reduction (daily to thrice weekly) because of tolerability issues. Hearing loss occurred in a small group of the population, with one individual experiencing bilateral hearing loss. The patient had a history of IV amikacin treatment before initiating this protocol, which may be a contributing factor. No apparent negative impact on microbiologic outcome was found in patients with dose frequency adjustment compared with those without. It is apparent that for many patients, dosage interruptions and modification in dosage frequency are necessary for patients to work through AEs and to continue the drug long term. No participant in this study experienced a life-threatening AE, and no study-related deaths occurred.

This study also demonstrated the heterogeneity of *M abscessus* sequevars both before and during treatment. Three patients demonstrated mixed macrolide-susceptible *M abscessus* sequevars cultured at initiation of therapy, one with Mab-A plus Mab-M and two with Mab-A and sequevar II Mab30-A. The macrolide-susceptible *M abscessus* isolates that included macrolide were eliminated during therapy. Unfortunately, the tools to identify different *M abscessus* sequevars are not available in many laboratories. Our data suggest that some patients with macrolide-resistant isolates also may harbor susceptible strains concomitantly, thereby providing rationale for use of a macrolide in the treatment of *M abscessus* lung disease even in the case that a resistant strain is detected.

Major limitations of this proof-of-concept study include the heterogeneity of the background therapy to which ALIS was added, minimal use of IV drugs, reliance on companion drugs (including clofazimine) with weak or no activity against Mab-A, the observational nature of its design with no control group, and the small number of participants. Beyond microbiologic outcomes, neither functional assessment by 6MWT nor quality-of-life measures by the QOL-B and NTM Module showed consistent trends in the study. We were limited in assessing these

outcomes given the low number of study participants and the variability in patient treatment regimens, disease severity, and prior disease course.

However, the study does provide important data in support of designing future trials of ALIS against *M abscessus*. Clearly, building regimens with more active companion medications will be important to lessen the risk of resistance emergence. Although this may not be possible for some highly drug-resistant isolates, it is likely that the initial addition of IV therapy using active agents, such as imipenem, tigecycline, or both, or related newer tetracyclines (omadacycline and eravacycline) will be necessary to diminish the risk of amikacin resistance developing. Importantly, our study suggested that ALIS has activity and a potential role in multidrug regimens against *M abscessus*, especially macrolide-resistant *M abscessus*. The study also suggested that decreased dosing frequencies resulting from drug intolerance may not result in diminished efficacy. Further, the ability to use ALIS with limited or no potential for systemic aminoglycoside toxicity relative to IV amikacin should not be understated. We believe our data support a role for ALIS in the multidrug treatment of *M abscessus* pulmonary disease.

Interpretation

In summary, our findings support further evaluation of the use of ALIS as part of a treatment regimen for *M abscessus* pulmonary disease. This study found that a high percentage of patients with macrolide-resistant *M abscessus* using ALIS achieved sputum culture conversion to negative results within 12 months, although reversion to positive culture findings was not uncommon. Drug withdrawal was rare, although many patients decreased the dosage frequency because of tolerability issues. Individuals receiving clofazimine monotherapy alongside ALIS were more likely to experience the emergence of mutational amikacin resistance.

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