Abstract
Nontuberculous mycobacteria (NTM) are troublesome pathogens that can cause significant pulmonary disease in patients with cystic fibrosis (CF). Diagnosis can be difficult in the setting of underlying CF and treatment regimens are burdensome on both patients and providers. Recent consensus guidelines for treatment of NTM in CF have provided a guide for the CF community, however research is lagging regarding accuracy of our diagnostic abilities and treatment efficacy. In this review, we provide new insights into the complexity of NTM from emerging whole genome sequencing data, a summary of current NTM diagnosis and treatment guidelines, highlight new treatment options, and discuss future research projects which aim to better define which patients to treat and timing and duration of treatment.

KEYWORDS
cystic fibrosis, mycobacteria, Mycobacterium abscessus, Mycobacterium avium, nontuberculous, transmission

1 | INTRODUCTION
Nontuberculous mycobacteria (NTM) are well-recognized pathogens in the cystic fibrosis (CF) patient population, and are widely viewed as one of the most challenging infectious complications of the disease.¹,² Patients with CF have the highest prevalence of pulmonary NTM cases compared to other disease states.³,⁴ The true rate of NTM detection is difficult to determine due to variability in screening practices, culture techniques, and data collection, however, in largest studies the overall prevalence was estimated at 6-13%.⁵⁻¹⁰ Longitudinal data from U.S. CF Patient Care Registry reveals that 19% of patients who were cultured over a 4-year span (2011-2015) had one or more NTM species isolated.¹¹ While the impaired mucociliary clearance and altered host defense innate to the CF lung provides an environment that is at especially high risk for NTM, it appears only a subpopulation of CF patients are vulnerable.¹² Reaching a diagnosis of NTM pulmonary disease in the setting of CF is uniquely complex, as a single positive culture is not synonymous with disease and the majority of patients have only transient or indolent infection.¹²⁻¹⁴ There is significant overlap of clinical symptoms of NTM infection with those of underlying CF disease and more typical bacterial infections, thus clinicians often struggle with how to proceed clinically and how best to counsel patients and families.¹³,¹⁵ Importantly, the complexity, duration, side effects, and unpredictable response associated with NTM treatment pose a tremendous burden on patients and providers.¹²,¹³,¹⁶ Despite exciting achievements in the care of patients with CF, including successful development of CFTR modulators which directly target the underlying protein dysfunction in many patients with CF,¹⁷,¹⁸ the problem of NTM infection continues to grow, and fundamental aspects of the disease pathogenesis are not yet understood.

2 | RISK FACTORS FOR NTM IN CF
In CF patients in the United States, the most common NTM species isolated in respiratory cultures are those of the slow-growing Mycobacterium avium complex (MAC), which includes the species
**M. avium**, **Mycobacterium intracellulare**, and **Mycobacterium chimaera** among others.12,13 The next most common are the **Mycobacterium abscessus complex** (MABSC), which include three subspecies, **M. abscessus subsp. abscessus**, **M. abscessus subsp. massiliense**, and **M. abscessus subsp. bolletii**. MABSC are classified as “rapid growers,” and are genetically quite distinct from MAC.12,13 Due to unknown mechanisms, CFTR dysfunction alone may predispose to patients to NTM infection since rates of CF-carrier status are high (30-50%) within the non-CF pulmonary NTM disease population.19,20 Among the NTM species, patients infected with MABSC are often younger, and may include children, with relatively more severe lung disease.9,21 MAC is more commonly seen in older, adult-diagnosed CF patients with a less severe phenotype, often due to presence of a residual function CFTR mutation.21 There are, of course many exceptions to this observation, and CF patients of any age can develop NTM disease from either MAC or MABSC.

Of central concern within the CF community is identifying factors that place individuals at risk for infection. In CF (and non-CF) patients, the source(s) of NTM infection and modes of bacterial transmission are not clearly understood. NTM are generally thought to be acquired from the environment, as they reside in biofilms within plumbing and various water delivery systems,24–28 as well as aquatic-type environments and soil.29,30 However, a limited number of studies have genetically linked environmental and patient isolates.31–34 Recently, studies of CF patient isolates from suspected outbreaks, suggest the potential for direct or indirect person-to-person transmission in CF clinics.35,36 In general, disease transmission studies require two types of data: genetic matching of paired isolates (patient vs environmental or patient vs patient) and epidemiological evidence of exposure and potential cross-infection. Genetic matching entails comparisons of DNA fingerprints generated via rep-PCR, pulsed field gel electrophoresis (PFGE) or whole genome sequencing (WGS). There are, however, no standardized thresholds of genetic relatedness by which two isolates are considered “the same” resulting in varied interpretations of the data. Moreover, genetically matched isolates on their own are not enough to prove disease transmission as there must also be convincing epidemiological evidence supporting cross-infection.

Three studies from Europe have utilized WGS and epidemiological analyses to assess the potential for person-to-person transmission among CF patients. First, a retrospective study at the Papworth hospital in the United Kingdom (UK) examined the genetic relatedness of 168 MABSC isolates from 31 adult CF patients.36 Using core genome single nucleotide polymorphism (SNP) analysis, they identified distinct genetic clusters and observed three categories of genetic similarity between isolates: highly similar (<25 SNPs), loosely clustered (50-200 SNPs) and non-clustered (>10,000 SNPs). The highly similar pairs included longitudinally sampled **M. abscessus subsp. massiliense** isolates from multiple patients living in distinct geographic areas, but who had opportunities for cross-infection via overlapping clinic visits. Taken together with the lack of a clear environmental source, the authors concluded that person-to-person transmission of **M. abscessus subsp. massiliense** within the hospital was plausible. A second study at the Great Ormond Street Hospital in the UK37 employed a similar approach to test for potential cross-infection among a cohort of 20 pediatric CF patients. They performed WGS on 27 MABSC isolates, and observed distinct genetic clusters, but within **M. abscessus subsp. abscessus** rather than **M. abscessus subsp. massiliense**. High genetic similarities (<25 SNPs) were observed between isolates from four different patients suggesting the potential for transmission. Their epidemiological data, however, did not identify opportunities for cross-infection among patients, expect for one sibling pair who lived in the same household. The study did not include environmental sampling and, therefore, could not rule out a common source of infection. Their conclusion was that the collective data did not suggest cross-transmission among pediatric CF patients. Finally, a recent study from Italy analyzed WGS of 162 MABSC isolates from 48 patients across four geographically diverse CF centers.38 Using a genetic similarity threshold of <30 SNPs, they identified isolate clusters in all three MABSC subspecies and seven “possible transmission” episodes. In only three out of the seven cases, epidemiological evidence suggested the potential for cross-infection between patients. Moreover, their analysis ruled out any major outbreaks over the past 12 years in the four centers studied suggesting minimal risk of inter-human transmission. Additional studies using both genetic and epidemiological analyses will be needed to provide clarity on the possibility and frequency of cross-infection between CF patients in the clinic setting or otherwise.

Beyond transmission studies, WGS of NTM has resulted in significant advancements in the understanding of NTM species diversity. A recent study of MABSC utilized WGS to analyze the global population structure of 1080 isolates derived from 510 CF patients from various clinics across Europe and in the United States and Australia.39 The study identified three predominant genotypes or “dominant circulating clones” (DCC) in the CF-NTM isolate population as well as many other genetically diverse genotypes that infect CF patients. Curiously, the DCCs were identified in all CF clinics and countries sampled and correspond to the same isolate genotypes observed in non-CF populations40 and in a nationwide epidemic of soft tissue infections in Brazil.41–43 The widespread geographic diversity and differing disease etiologies of the DCCs raise questions of whether the strains have been spread globally through inter-continental transmission or are simply resident genotypes in the environment that are evolutionarily fit for human infection. Recent evidence of long-term survival of MABSC on fomite particles is potentially supportive of either hypothesis.39,44 A global population study of environmental NTM isolates in comparison to clinical strains from CF and non-CF patients has yet to be performed, but could reveal the risk of environmental exposure of these genotypes to various patient communities.45 Additional corresponding studies of CF patient isolates for MAC species are critically needed, as they are the most prevalent species in many CF patient populations. The CF Foundation is supporting this evolving research in part through support of the Colorado CF Research and Development Program.

Further benefits of WGS of NTM are new insights into the complexity of disease. While previously, a patient may have been diagnosed with recurrence of MAC or MABSC, with WGS, recurrence of prior disease can conclusively be distinguished from acquisition of a new NTM species or strain-type within the same species. WGS also
provides the ability to detect multi-species and multi-strain infections within a single patient and can identify the presence or emergence of drug resistance mutations. Finally, as more clinical isolates are sequenced and phenotyped in laboratory disease models, investigators will ultimately be able to elucidate genetic determinants of virulence that will, in turn, provide rapid molecular diagnostics and potential targets for novel therapeutics.

3 NTM PULMONARY DISEASE DIAGNOSIS

NTM pulmonary disease diagnostic criteria are the same for patients with and without CF based on recommendations from the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA). Included are both microbiologic and clinical criteria with appropriate exclusion of other diagnoses (Table 1). The extensive overlap of both clinical features and radiographic manifestations of NTM infection with underlying manifestations of CF requires that the clinician pay strict attention to ensure disease due to typical CF co-pathogens and co-morbidities are adequately treated. NTM almost universally appears in patients with underlying pulmonary disease and chronic airway infection with more typical bacteria such as Pseudomonas aeruginosa and Staphylococcus aureus. In this setting, it is challenging to assess the role of NTM in an individual patient. Generally, one should suspect NTM pulmonary disease in a CF patient with positive NTM cultures and increased constitutional or respiratory symptoms above baseline, unexpected increased decline in lung function, or progressive radiographic disease, that are not responsive to augmented airway clearance therapy and aggressive antibiotic treatment of typical CF co-pathogens. Making an accurate diagnosis in those patients with true NTM disease is vital. Adding treatment in patients with indolent infection and no clinical decline will not result in clinical benefit and places the patient at risk from treatment-related side effects. In the setting of therapeutic research trials, mistakenly including patients with only transient or indolent infection could directly affect study outcomes, potentially reducing or preventing detection of benefit from a useful therapy. On the other hand, inability to make a timely and accurate diagnosis can delay treatment when need, resulting in further clinical decline. Though there are historic reports that have shown no clinical impact of NTM, there is compelling evidence that NTM disease is associated with worse clinical outcomes. Specifically, Esther et al, showed that CF patients infected with MABSC have more rapid decline in lung function compared to those uninfected (2.52% per year vs 1.64% per year). In a retrospective study in Colorado, we showed that in patients with NTM disease, a significantly increased rate in decline in pulmonary function actually occurs in the year prior to the first isolation of NTM, highlighting that NTM culture positivity is often a later sign of disease. Unfortunately, in current practice, the presence of MABSC nearly always results in exclusion from lung transplant candidacy in the setting of failure to eradicate the bacteria, although transplant of patients with MABSC has not been associated with increased mortality.

Even with guidance from ATS/IDSA and the CF Foundation and European CF Society (CFF/ECFS) consensus statements, the diagnostic criteria are still somewhat subjective and variably interpreted by providers. The CFF has recognized this unmet need, and are sponsoring the PRospective Evaluation of nontuberculous mycobacterial Disease In Cystic Fibrosis (PREDICT) trial. PREDICT has been ongoing at the adult and pediatric programs within the Colorado CF Center since December 2013 (NCT02073409), with the primary objective of developing a user-friendly, evidence-based protocol for the diagnosis of NTM disease to be used for all CF patients. The secondary aims are to define an expected rate of development of NTM disease for patients with a positive NTM culture and to identify clinical features associated with development of disease. To date, the PREDICT trial has enrolled close to 50 CF subjects from Colorado. Currently about one third of patients with at least one positive NTM culture have met diagnostic criteria for NTM pulmonary disease and there is emerging evidence that specific clinical assessments may help delineate those patients who will have transient or indolent infection versus those who develop disease. Planned expansion to seven additional, geographically diverse CF Care Center sites is expected in early 2018.

4 TREATMENT OF NTM PULMONARY DISEASE

In 2016, CFF/ECFS published consensus recommendations for the management of NTM in CF. It important to note that data

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**TABLE 1** ATS/IDSA clinical and microbiologic criteria for diagnosis of NTM pulmonary disease

Clinical criteria (both required):

1. Pulmonary symptoms, nodular or cavitory opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules.

2. Appropriate exclusion of other diagnoses.

Microbiologic criteria (one of the following required):

1. Positive culture results from at least two separate expectorated sputum samples.

2. Positive culture result from at least one bronchial wash or lavage.

3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM.

Adapted from Floto et al.
reviewed came from studies in patients without CF and to date there have been no randomized control trials of NTM treatment in the CF population.52 Optimal treatment duration is unknown, but current recommendations are to achieve 12 consecutive months of negative cultures.13 An example of a typical MAC and MABSC treatment schedule from the CFF/ECFS guidelines is shown in Figure 1. For patients with a clarithromycin-sensitive MAC species, treatment should include a daily oral antibiotic regimen containing a macrolide (preferably azithromycin), rifampin, and ethambutol. Intermittent therapy (ie, thrice weekly) is not recommended due to concerns about abnormal absorption of drugs and altered pharmacokinetics in CF.13 Addition of a 1 to 3-month course of intravenous amikacin may be beneficial in patients with evidence of more severe infection suggested by AFB smear positivity, cavitary disease on chest radiography, or systemic signs of illness. For patients with clarithromycin-resistant MAC, CFF/ECFS guidelines suggest management in collaboration with experts in the treatment of NTM and CF.13 In addition to standard treatment, these patients may benefit from addition of intravenous amikacin and/or other oral alternatives. Notably, rifampin is a strong CYP3A4 inducer and will significantly decrease the serum concentration of ivacaftor, thus concomitant use should be avoided. In a CF patient receiving ivacaftor or lumacaftor/ivacaftor, alternative treatment options include holding the CFTR modulator during MAC treatment or replacement of rifampin with an alternative MAC agent, including: inhaled amikacin, or oral clofazimine or moxifloxacin.

With various treatment regimens among patients with and without CF, clearance of sputum in patients with MAC pulmonary disease is reported to range from 45% to 75%.13,14,53 Macrolide resistance is associated with a poor prognosis and sputum culture conversion rates as low as 5-15% in patients without CF.53,54 Risk factors for development of macrolide-resistant MAC are prior macrolide monotherapy and macrolide therapy with inadequate accompanying drugs.53

![Figure 1](image-url)

**FIGURE 1** Typical treatment schedules for individuals with CF with Mycobacterium abscessus or MAC pulmonary disease. (A) M. abscessus treatment is divided into an initial intensive phase with an oral macrolide (preferably azithromycin) and intravenous amikacin with one or more additional intravenous antibiotics (tigecycline, imipenem, cefoxitin) for 3-12 weeks (depending on severity of infection, response to treatment, and the tolerability of the regimen), followed by a continuation phase of oral macrolide (preferably azithromycin) and inhaled amikacin with 2-3 additional antibiotics (minocycline, clindamycin, moxifloxacin, linezolid). Antibiotic choices should be guided but not dictated by drug susceptibility testing. Baseline and interval testing for drug toxicity is essential (B). MAC treatment (for clarithromycin-resistant disease) should be with a daily oral macrolide (preferably azithromycin), rifampin and ethambutol. An initial course of injectable amikacin or streptomycin should be considered in the presence of (i) AFB smear positive respiratory tract samples; (ii) radiological evidence of lung cavitation or severe infection; and (iii) systemic signs of illness. Baseline and interval testing for drug toxicity is essential (AFB, acid-fast bacilli; CF, cystic fibrosis; HRCT, high-resolution CT; MAC, Mycobacterium avium complex). Adapted from Thorax 2016;71:i1-i22.
Treatment of pulmonary disease due to MABSC is complicated because of the high level of in vitro resistance, need for intravenous antibiotics, high frequency of adverse reactions, and generally lower rates of successful treatment compared to MAC. MABSC culture conversion rates are typically reported in the 40-50% range. Among MABSC subspecies, *M. abscessus* infection rates are higher than *M. abscessus* subsp. *abscessus*. In a study from France, a macrolide-based regimen resulted in 100% culture conversion in CF patients with *M. abscessus* subsp. *massiliense* compared with 27% when infected with *M. abscessus* subsp. *abscessus*. These differences are presumably related to the presence of a functional erm(41) gene in *M. abscessus* that results in inducible macrolide resistance, whereas in *M. abscessus* subsp. *massiliense* the gene is nonfunctional and generally associated with susceptibility to macrolides. The CFF/ECFS guidelines recommend that MABSC should be sub-spectated to assist in treatment decisions and prognosis.

MABSC treatment involves an intensive phase of therapy followed by a continuation phase. The intensive phase is used to rapidly reduce bacterial load and should include 3-12 weeks of intravenous amikacin plus one or more of the following agents: intravenous tigecycline, imipenem, or cefoxitin, plus a macrolide (preferably azithromycin), plus one to two additional oral drugs. Inclusion of a macrolide in the treatment of *M. abscessus* subsp. *abscessus* or *M. abscessus* subsp. *bolletii* has been debated due to presence of an erm(41) gene in most strains and potential for inducible macrolide resistance but still is included in the treatment plan outlined in the CFF/ECFS guidelines at this time. The duration of the intensive phase is determined by severity of disease, response to therapy, and tolerability of the regimen.

After the intensive phase, patients should continue into a prolonged chronic suppressive phase. The continuation phase treatments should include inhaled amikacin in conjunction with 2-3 of the following oral antibiotics which have shown historic in vitro activity: linezolid, clofazimine, ciprofloxacin, moxifloxacin, or minocycline. If macrolides were used initially, they should be continued throughout the continuation phase. Although azithromycin has recently been shown to reduce macrophage autophagy of MABSC and may have potential to impair host defense independent of its antibiotic properties, this potential detriment has not been identified in patients with NTM disease and guidelines have not yet changed. A plan for monitoring of drug toxicity is required and should be set in place at the initiation of treatment. Changes to the treatment regimen are common and can be prompted by evidence of lack of treatment response assessed by culture conversion or clinical and radiographic response, or drug intolerances or side effects. CF patients with MABSC should generally managed in collaboration with an expert in the treatment of NTM disease.

As noted, several classes of drugs have been used to treat NTM infections including fluoroquinolones, clofazimine, linezolid, inhaled amikacin, and bedaquiline. The fluoroquinolones are widely available and have variable in vitro and in vivo activity against MAC and typically poor activity against MABSC. However, in refractory disease, there may be an indication for use. In one study of 41 non-CF patients with MAC refractory to a macrolide-based three-drug regimen, 29% achieved negative sputum cultures after addition of moxifloxacin. Clofazimine has also been used with success as an alternative agent for patients. In a retrospective review, sputum conversion rates were 100% among 90 adult patients with MAC who were treated with clofazimine, ethambutol, and a macrolide. In another study of 112 adult and pediatric patients, primarily with refractory disease and 20% of whom had CF, culture conversion was achieved in 42% of patients with MAC, 45% with MABSC, and 33% in patients with more than one NTM species. In both studies, the drug was tolerated well with 6-14% of patients having to stop clofazimine because of drug-related intolerance.

Inhaled amikacin has been used for many years in patients intolerant to parenteral aminoglycosides or as an adjuvant to oral therapy, but adverse effects may limit its use. In a phase II randomized placebo-controlled multicenter trial examining the utility of inhaled liposomal amikacin when added to a standard three-drug regimen in patients with refractory MAC, approximately 32% of those assigned to amikacin achieved culture conversion to negative versus 9% in the placebo group. In this study, 16% of patients receiving inhaled liposomal amikacin stopped the drug due to adverse events including: bronchiectasis exacerbation, dyspnea, other respiratory events with oropharyngeal pain, and allergic alveolitis, compared with none in the placebo group. A phase III trial of inhaled liposomal amikacin has completed enrollment and study results should be available soon. Linezolid is an oxazolidinone with broad antimycobacterial activity that has been used to treat multidrug-resistant tuberculosis as well as NTM. However, the use of the drug has been limited by high rates of adverse reactions including peripheral neuropathy, optic neuritis, and cytopenias. Bedaquiline is a diarylquinoline approved for the treatment of multidrug-resistant tuberculosis in adults. The drug is an ATP synthase inhibitor with broad antimycobacterial activity with MICs for MAC ranging from 0.008 to 0.03 μg/mL. Six non-CF patients with refractory MAC and four with MABSC were treated with bedaquiline as salvage therapy in addition to other antimycobacterial drugs. On treatment, 90% had symptom improvement and there was evidence of microbiologic response with 50% of patients achieving at least one negative culture after 6 months.

Relapse following primary treatment of NTM is reported in up to 35% for MAC and 23% for MABSC. Importantly, in patients with CF previously infected with NTM, the presence of a future, second NTM species is common, reported in up to 26% of patients at 5 years and 36% at 10 years following the first NTM species cultured. With treatment, stabilization, or improvement in clinical symptoms of NTM pulmonary disease, including cough, sputum production, and fatigue, as well as radiographic measures have been shown. Longitudinal follow-up of cases of CF patients with NTM have also shown evidence of stabilization of pulmonary function decline.

Despite guidelines, questions remain as to what is the most appropriate treatment regimen for NTM in CF patients, especially in the setting of co-infection. The CFF is supporting the Prospective Algorithm for the TrEatment of NTM in CF (PATIENCE) trial (NCT02419989) with the primary goal to develop an evidence-based
treatment protocol to be used for first-time NTM treatment of CF patients. Additional objectives are to define an expected rate of response and tolerance to treatment using the current CFF/ECFS guidelines. Preliminary data from the Colorado CF center are promising and there is planned expansion in early January 2018. Establishing an expected rate of response to current therapies in the CF population is the first step toward assessing the impact of new therapies or testing alternative treatment strategies, including the potential for an early eradication approach or shortened antibiotic courses. Ideally, in the future we will have culture-independent identification of infection, as well as biomarkers of pathogen virulence or host susceptibility to disease to combine with standard clinical assessments to better guide who and when to treat, as well as duration of therapy.

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