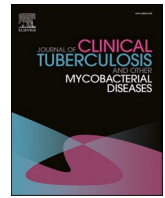




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Sex, ancestry, senescence, and aging (SAnSA) are stark drivers of nontuberculous mycobacterial pulmonary disease

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ABSTRACT

Nontuberculous mycobacterial (NTM) pulmonary disease (PD) disproportionately affects otherwise healthy, older, Caucasian females. The reasons behind this are likely multifactorial involving several conspiring factors. A variety of factors are thought to contribute to increased susceptibility to NTM in the older adult including exposure to various environmental conditions and contaminants across the lifespan, genetic risk factors, hormonal changes, and immunodeficiency. Independent of sex and ancestry, respiratory muscle atrophy intensifies with age and an aging immune system can show functional decline of macrophages, poor lung migration and homing of dendritic cells, promotion of aberrant pro-inflammatory responses, acceleration of inflammation related to aging, and increased immunosenescence. The purpose of this review is to synthesize the current body of knowledge regarding the roles of sex, ancestry, senescence, and aging (SAnSA) in NTM acquisition and the possible mechanisms involved in NTM PD, highlighting age-related respiratory and immune system changes. We also summarize molecular tools and biomarkers of these fields and contextualize these into the study of NTM PD. Finally, we discuss the relevance of biomarkers described for senescence and aging and senolytic therapies as potentially new adjunctive strategies to reduce the burden of NTM PD.

1. Introduction

1.1. Sex, ancestry, senescence, and aging (SAnSA)

In this review, we apply the term “SAnSA” as the individual or collective roles of sex, ancestry, senescence, and aging to the development of a number of medical conditions. In the aftermath of the COVID-19 pandemic, it is increasingly important to understand the role of SAnSA in pulmonary diseases (PD). In regard to sex, autoimmune PD and lymphangioleiomyomatosis (LAM) are characteristically more common in females than males and rates of chronic obstructive PD (COPD) are rising rapidly in females [1]. Genealogical ancestry studies in PD is a burgeoning field, but remains a large and formidable unexplored territory. Cellular senescence is a broad term first used by Hayflick and Moorhead in 1961 referring to the irreversible loss of the proliferative activity of human somatic cells [1], but has since been applied to cells exposed to stressors including radiation, chemotherapeutics, and aging

[2]. While the body can undergo significant changes with advancing age, the immune system also concomitantly ages and shows reduced performance, referred as “immunosenescence,” affecting both the innate and adaptive arms of immunity [2]. In parallel, aging is a risk factor for the development of a number of medical conditions including PD and increased susceptibility to respiratory infections. Consequently, aging can cause the progressive accumulation of cellular metabolic products and increased DNA damage, resulting in the development of a low-grade inflammatory phenotype commonly referred as “inflammaging” [3–4]. While it is important to understand how SAnSA affects the ability of the pulmonary system to respond to infections, its role in the lung is limited. This review focuses on SAnSA in the context of nontuberculous mycobacterial (NTM) PD.

1.2. NTM is commonly a pulmonary disease of older, Caucasian females

PD caused by NTM is, in part, a SAnSA related disease. Caucasian

Abbreviations: NTM, nontuberculous mycobacteria; PD, pulmonary disease; SASP, senescence-associated secretory phenotype; ALF, alveolar lining fluid; SAnSA, Sex, ancestry, senescence, and aging.

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post-menopausal females who typically show taller and slender body morphotypes are a prominent NTM PD cohort compared to others in similar age groups [5]. A pair of studies reported that females were 1.4 times more likely to show NTM PD cases than males and NTM PD prevalence was two-fold higher among Asian/Pacific Islanders than Caucasians, clustering among persons 65 years of age or older [6–7]. Susceptibility also extends to individuals with prior lung infections, bronchiectasis, and genetic diseases such as cystic fibrosis, alpha-1 antitrypsin deficiency, and primary ciliary dyskinesia [8]. These vulnerabilities and other exposures have been previously reviewed in the literature [9–10].

In the United States (U.S.), the incidence of NTM infections has increased from 1.6 to 1.8 cases per 100,000 persons in the 1980's [11], to 8.7 cases per 100,000 persons in 2008, and to 13.9 cases per 100,000 persons in 2013 [12]. National studies of Medicare beneficiaries regarding disease frequency have also revealed NTM PD prevalence increasing annually at an average of 8.5% from 1997 to 2007, reaching a nation-wide average of 47 per 100,000 persons in 2007, with the highest rates seen in the West at 149 per 100,000 persons, driven primarily in California and Hawai'i [13]. By comparison, Europe shows lower incidence rates of NTM infections ranging from 0.2 to 2.9 cases per 100,000 persons depending on the geographic region. In Scotland, the mean incidence rate of NTM PD was 2.4 cases per 100,000 between 2000 and 2010, while 0.2 cases per 100,000 persons was reported in Croatia between 2006 and 2010 [14]. Among the pathogenic NTM, members of the *Mycobacterium avium* complex (MAC) or *Mycobacterium abscessus* group are the most common species associated with pulmonary infections [15].

1.3. The first “S” of SAnSA: The role of sex in NTM PD

Most studies have reported the preponderance of NTM PD in females. Within a subset of the U.S. population, females show 1.1–1.6 fold higher NTM prevalence rates than males [16]. Higher number of NTM PD in females is also observed among European, New Zealand, and Australian cohorts [17–19]. In a Japanese cross-sectional study of 11,034 individuals with NTM PD, the incidence of NTM was reported to be higher among females in all age groups except for those aged ≥ 80 years [20]. Of note, females are more likely to show more severe NTM PD disease than males, evidenced by more cavitation of the lung, lower body mass index, and extensive treatment history.

1.4. The “An” of SAnSA: The role of ancestry in NTM PD

Genealogical ancestry, or identifiable ancestors in a family tree, provides biogeographic history of genetic variation in a population (e.g., Asian, African, European ancestry) [21]. Ancestry can be further extrapolated into one's ethnicity (e.g., white, black, non-Hispanic white, etc...) [22]. In general, the role of ancestry in respiratory diseases has not been widely studied and will be inherently complex; however, the possible contribution of African ancestry among females and males to pulmonary function variables such as FEV₁, FVC, and the FEV₁:FVC ratio in the context of coronary artery risk and asthma has been reported [23]. Other genetic studies indicate that Amerindian ancestry influences susceptibility to PD such as COPD [24]. Genome wide-association studies (GWAS) have identified genetic variants associated with susceptibility to idiopathic pulmonary fibrosis (IPF) in people of European ancestry [25]. While the literature regarding the role of ancestry in NTM PD remains scant, NTM PD has been reported among families including a pair of siblings in Japan as well Korean, Caucasian, and Hispanic families in the U.S., suggesting there may be a heritable, genetic contribution to NTM PD [26–27]. While resident Asians in geographic hot spots for NTM like Hawai'i show the highest period prevalence of NTM PD, disease is lowest among Native Hawaiian and other Pacific Islanders [28].

Adding complexity to an already complicated situation, where

someone lives may also contribute to NTM exposures [29]. Still, other studies suggest exposure to hazardous aerosols such as air pollution can affect gene expression in individuals with respiratory diseases more than genetic ancestry [30]. Multiple GWAS studies using a multiethnic approach consisting of equal numbers of NTM PD and uninfected individuals from varied ancestral/ethnic backgrounds would be a welcome body of work to the literature.

1.5. The second “S” of SAnSA: The role of senescence in NTM PD

Cellular senescence is a widely recognized marker of aging, characterized as a state of irreversible cell-cycle arrest. Aging is a natural phenomena of every living organism that unfolds across the lifespan. The accumulation of senescent cells in aged animals result in tissue dysfunction, age-related diseases, and lifespan shortening. The consequence and functional role of senescent alveolar macrophages and airway epithelial cells in NTM PD are not known, but in other PD such as IPF the accumulation of these cells contribute to disease progression. Markers for cellular senescent cells include absence of proliferative makers such as Ki67, senescence-associated β -galactosidase (SA β GAL) activity, expression of tumor suppressors and cell-cycle inhibitors, and cells with enlarged flat morphology [31].

Senescent cells increase in number, show irreversible cell arrest across the aging process, but remain metabolically active, secreting a diverse array of biologically active molecules referred to as the senescence-associated secretory phenotype (SASP), first coined by Campisi *et al.*, in 2008 [32]. The SASP typically consists of inflammatory cytokines and chemokines, proteolytic factors, exosomes, miRNA, and other mediators that recruit immune cells responsible for clearance of damaged cells, wound healing, tissue regeneration, and tissue remodeling. Studies have not yet been performed to understand the SASP specific profile of NTM PD patients. However, from other generalized studies on senescence, an enhanced SASP response can be triggered by tumor suppressor p21 activation of p38 mitogen-activated protein kinase and Janus-activated kinases resulting in the activation of the proinflammatory transcription factor nuclear factor κ B (NF κ B) [33]. Upon NF κ B activation, multiple inflammatory proteins such as cytokines, chemokines, proteases, and growth factors increase particularly during chronic PD [34].

SASP components may also influence the development of mycobacterial PD in older adults. Alveolar lining fluid (ALF) from *Mycobacterium tuberculosis* infected older adults show a unique proteomic composition characterized by a pro-oxidative lung environment and surfactant protein dysfunction when compared to ALF of younger adults [47]. ALF from both older adults and older mice show increased amounts of surfactant proteins A and D (SP-A, SP-D) and complement C3b that opsonizes pathogens for destruction [50]. This literature advocates for new studies to study the role of SASP components in the development of NTM PD in older adults.

Similar to senescence of human cells, microbial senescence is overlooked in the context of respiratory infections. No matter the organism, senescence decreases overall fitness [35]. Even aged bacteria can and do undergo senescence that decreases the fitness of individual organisms [36]. The significance of exposure to aged microbes has been studied in the *Saccharomyces cerevisiae* yeast and for the bacterium *Caulobacter* [37]. Significantly more studies are needed to explore the role of immune lung cell and NTM senescence in the pathogenesis of NTM PD.

The majority of mycobacterial PD studies in aging have been reported in the context of *Mtb* [47–48] particularly in the context of “inflammaging” [50]. Inflammaging is an increase in basal inflammation driven by inflammatory cytokines including TNF- α , IL-6, and IL-1 β . ALF in aged mice and older human donors show increased levels of TNF α , IL-6, and IL-1 β in proteomic studies [39] and TNF α , IL-6, and IL-1 β levels are high in *Mtb*-infected older adults compared to uninfected older adults [49]. Elevated levels of TNF α , IL-6, and IL-1 β are produced by healthy adult macrophages infected with *M. avium* which may

negatively influence the ability of macrophages to control NTM [51]. A more comprehensive understanding of senescence versus inflammaging in the context of NTM PD remains to be assessed.

1.6. The last “A” of SAnSA: The role of aging in NTM PD

Aging, as a natural biological process, may progressively alter the physiological and molecular function of the respiratory system via multiple aging mechanisms. For example, FEV₁ and FVC (indicators of lung volume), peak at 25 years and decline slowly with age and lung capacity decreases with increasing age [38]. Decreased respiratory muscle strength, as much as 20%, is commonly observed in individuals over 70 years of age [39]. While alveoli numbers remain constant over the lifespan, their size can abnormally increase over time due to changes in the coiling structure of elastin and other fibers [38]. Physiological changes in the lung also occurs across the lifespan caused by exposure to common cellular stressors such as cigarette smoke or oxidative stress due to an imbalance of natural antioxidants and reactive oxygen species [40]. Depressed beating of lung cilia can start as early as age 40, reducing effective airway clearance and cough strength [39]. Depressed lung cilia beating results in impaired mucociliary clearance and trapping of particles and pathogens causing recurrent bacterial infections as observed with individuals with NTM lung infections [41].

It is well established that macrophage function also declines with age involving reduced expression of pattern-recognition receptors such as Toll-like receptors (TLR), diminished recognition of foreign opsonized pathogens, and reduced phagocytic ability [42]. Macrophages from older adults show reduced respiratory burst, resulting in ineffective intracellular bacterial killing and prolonged bacterial infections [43]. Monocyte-derived macrophages (MDM) from frail, older adults exhibit significant defects in controlling *Streptococcus pneumoniae* due to deficiencies in LC3-associated phagocytosis [44], but this needs elucidation in the context of MDM from older adults with NTM PD.

According to the U.S. Census Bureau, by the year 2050 21.4% of the total population of North America will be comprised of individuals aged 65 and older [45]; thus, expanding the population of individuals vulnerable to NTM. However, a study reported that age accounted for <25% of the total increase in NTM PD cases [46]. It may be reasonable to suspect that a combination of intersecting factors increase susceptibility to NTM PD, *i.e.*, SAnSA. NTM pulmonary infections have increased in number over the years, particularly in individuals over 50 years of age. Prevots *et al.* reported a higher increase in the annual prevalence of NTM in individuals aged ≥ 60 years than in those aged <60 years [16]. Adjusted odds ratio for NTM PD incidence in different age groups showed the lowest odds ratio (7.4; 95% CI, 2.9 to 19.3) for persons aged <18 years and highest odds ratio (106.4; 95% CI, 42.0 to 270.0) in individuals aged ≥ 65 years [28].

In a cardiac-specific study using aged female C57BL/6 mice (18 months), *M. avium* was shown to induce significant cardiac damage and dysfunction by surface electrocardiogram monitoring, histology, and gene expression immunoassays compared to young mice (3 months) [52]. Moreover, increased expression of chemokines and chemokine receptors were observed in aged mice compared to young mice. The implication of this study was that *M. avium* infection may escalate risk for cardiac failure, a common cause of hospitalization for older adults. A missing opportunity from this cardiac-specific study was that lung function was not concomitantly studied and NTM burden was not quantified from lung tissue. Future studies should explore potential physiological changes associated with aging in pulmonary NTM infected young and old mice. In a separate study, heme oxygenase-1 (HO-1) was implicated in protection against *M. avium* infection. HO-1 canonically functions as a key mediator of antioxidant/oxidant homeostasis in the prevention of inflammation-associated injury and was identified as an important modulator of granuloma formation and programmed cell death of macrophages through Bcl2 and necrosis pathways that protected young (4–6 month) and aged female and male (18–21 month)

C57BL/6J mice against *M. avium* infection [53].

NTM PD is generally rare in females younger than 50 years of age and it is surmised that menopausal status and hormone levels contribute to susceptibility to NTM infections. For example, deficiencies in estradiol, a predominant estrogen of reproductive years is associated with the development of NTM PD [54]. Aged mice have been leveraged in NTM studies to understand the role of hormones as drivers of infections. Tsuyuguchi *et al.*, utilized 6-week old ovariectomized DBA/2 female mice to study the role of estrogen *i.e.*, 17 β -estradiol (E2) in the pathogenesis of MAC PD, reporting estrogen protects mice from MAC infection through augmenting macrophage function via increased production of reactive nitrogen intermediates [55]. To investigate the opposing role of the male hormone testosterone in susceptibility to NTM infection, Yamamoto *et al.*, monitored survival, incidence of skin lesions due to *Mycobacterium marinum* (a slow-growing NTM responsible for water-associated skin lesions), and dissemination of *M. marinum* to visceral organs in 5–7 week castrated and non-castrated C3H/He, A/J, BALB/c, B10.A, DBA/2 and C57BL/6 mice [56]. While variations between mouse strains were observed, castrated mice with significantly reduced levels of testosterone showed similar capacity to defend against *M. marinum* infection as female mice, indicating host resistance to NTM may be related to sex. Follow-up studies using female and male aged mice and relevant NTM species important to PD such as MAC and *M. abscessus* are needed.

The changing lung microbiome with advanced age is an understudied area that may play a role in the susceptibility to or progression of NTM PD. Age-associated alterations in immune function may affect the lung microbiome, generating low-grade inflammation, a key contributor to arterial stiffness and declining lung function [57]. Lee *et al.*, compared the lung microbiome between healthy young and older adults and reported increased relative abundance of Firmicutes and decreased relative abundance of Proteobacteria in older adults [58], concluding that Firmicutes is positively associated with lung function and beneficial. In a microbiome study of oral washes, induced sputum, and bronchoalveolar lavage samples, *Mycobacterium* was identified in 27% of NTM positive samples tested along with other common oral commensals [59]. In a Korean cohort, lower microbial richness was observed among 11 NTM PD patients and 10 controls over the age of 57 years using bronchial washings [60]. Additional information about the lung microbiome of older adults are sure to come in the future and may reveal novel prognostic indicators of lung health status to forecast the likelihood of individuals developing NTM PD.

While innate immunity is important in aging, the adaptive immune system can also be consequentially affected. Studies of aged mice have shown B cell development declines with aging, specifically, lower numbers of pro-B, pre-B, and immature B cells that would develop into mature B cells [41]. Although the ability to produce antibodies remain intact, having fewer B cells results in diminished capacity for mounting strong antibody responses in older adults. Furthermore, the number of CD3+, CD4+, and CD8 + T effector cells is reduced in the older adults, which can extend the chronicity of lung infections (*e.g.*, NTM PD) [41].

A pictorial diagramming increased susceptibility of older females to NTM infection, possible exposure across a lifespan, and age-related immune system changes that may contribute to NTM acquisition are shown in Fig. 1.

2. Adapting established biomarkers to examine the role of SAnSA in NTM PD

Molecular biomarkers of NTM PD are an under-studied area of research, but perhaps three established molecular markers of senescence and a marker of aging can be applied including A) sirtuins, B) α -Klotho, and C) senescence marker protein-30 (SMP30) and mTOR, respectively.

A) Sirtuins (SIRT) were originally discovered to increase the life span of yeasts, nematodes, and flies [61] as promising regulators

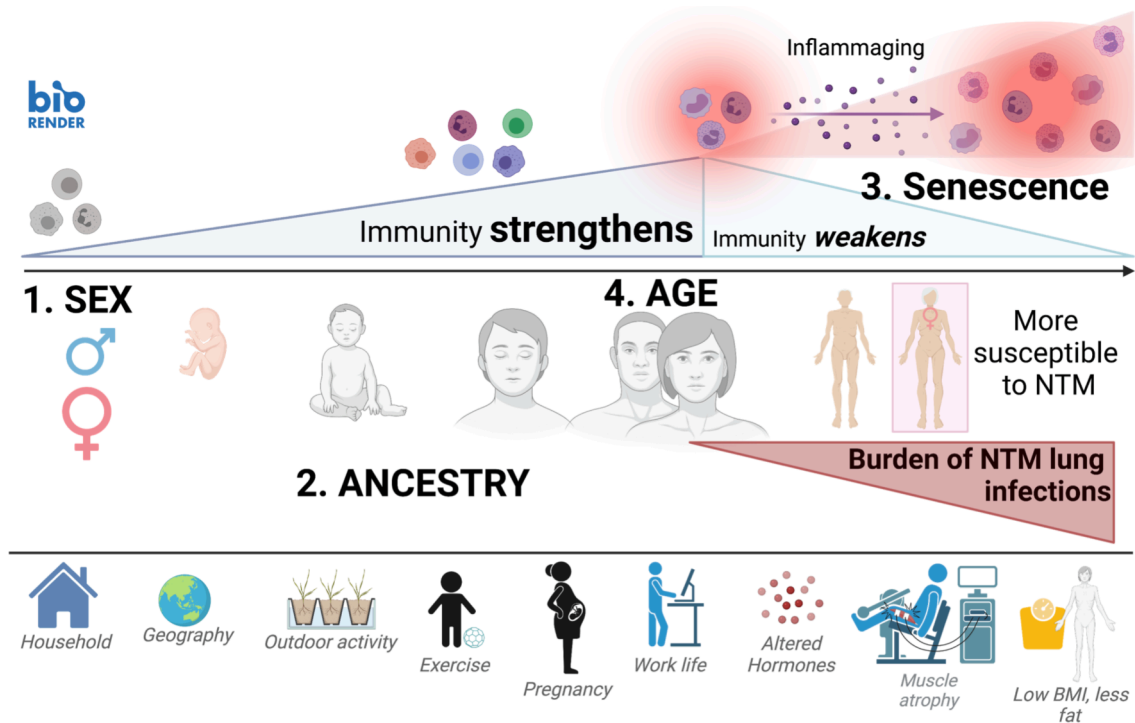


Fig. 1. SAnSA and NTM PD. Sex, ancestry, senescence, again and a variety of lifespan circumstances in the development of NTM PD. Right, middle: NTM infections occur more frequently in older females than males. A myriad of reasons may contribute to NTM infections at older age with influences across the lifespan including birth sex, ancestry, increased immuno senescence and the aging process. Top: Immune system changes with aging also include increased inflaming and immuno senescence. Bottom: independently, other factors may also contribute to the development of NTM infections including household exposures, geography, outdoor activity, exercise, pregnancy, worklife, altered hormones, muscle atrophy and BMI. Imaging created with BioRender.

of longevity [33]. SIRT1 regulates cellular senescence and longevity through acetylation and deacetylation of histones as well as non-histone proteins like p53 and NF κ B [62]. Over-expression of SIRT1 homologs increases the lifespan of mice and in human tissues, including lung tissue.

- B) α -Klotho functions as the receptor for fibroblast growth factor-23 involved in phosphate homeostasis. Decreases in α -Klotho levels accelerate with age correlating with phosphate toxicity, a feature of mammalian aging [63]. α -Klotho reduces cellular senescence by decreasing the biological activity of different Wnt family members. α -Klotho knockout mice have an accelerated aging phenotype similar to premature human aging [61,64]. These mice have a shorter life span as well as premature thymic involution and pulmonary emphysema; α -Klotho overexpression increases life span by 30% in both sexes and reduces the risk of age-related diseases. In studies using human serum, α -Klotho protein levels decrease with age [64]. A population-based study in Italy, involving 804 adults aged 65 and over, found that those with lower plasma α -Klotho levels showered a greater risk of death compared to those with higher levels [64].
- C) SMP30, also known as regucalcin, is an intracellular calcium signaling protein that confers protection against oxidative stress and chronic inflammation while regulating calcium homeostasis, chronic inflammatory processes, cell proliferation and cellular senescence [65]. SMP30 knockout mice show reduced weight and shorter lifespan compared to wild-type mice and elevated inflammatory markers with aging [66]. SMP30 deficient mice exposed to chronic smoke showed significant body weight loss, more lung protein oxidation, pulmonary emphysema, and significant lung parenchyma destruction as they aged compared to wild-type mice [66].
- D) mTOR is an important kinase protein that regulates biological processes related to longevity and aging and is involved in

autophagy, a cellular mechanism used for clearing damaged cells and protein aggregates [67]. Inhibition of the mTOR signaling pathway by rapamycin was initially discovered to increase the lifespan of yeast, nematodes, and flies but reduced symptoms associated with accelerated aging diseases, such as Hutchinson-Gilford progeria [61]. Hutchinson-Gilford progeria is a rare genetic disorder that causes newborn babies to rapidly age soon after birth [68]. The cause of premature aging in progeria is the dysregulation of the gene LMNA encoding for lamin A that accumulates in various tissues including the lung, skin, tongue, heart, liver, and skeletal muscles, and causes changes in the distribution and levels of heterochromatin as well as telomere shortening [68]. Telomere shortening in fibroblasts activates the production and expression of progerin, which rapidly induces telomerase dysfunction that leads to DNA damage in cellular structures. Progerin may be an important biomarker for studying the role of natural aging in the context of NTM infections, of which, no studies have been performed to date.

2.1. Role of anti-senescence and anti-aging treatments to reduce NTM PD progression

Current therapeutics for NTM PD are inefficient in controlling the progression of disease and senolytic therapies might be at the forefront of innovative drug therapies to slow down age-related PD. Senolytic drugs are among a class of small molecules under scrutiny as targeted therapies to induce the death of senescent cells and slow the aging process. Senolytic drugs induce apoptosis in senescent cells and reduce SASP proteins [34]. SIRT1 activators such as SRT1720 and SRT2104 have demonstrated to extend the lifespan of mice [61]. These animal studies have led to a number of human clinical trials employing SIRT1 activators [34,61]. Activation of SIRT1 also inhibits p53, and may be a

potential therapeutic strategy for aging-related diseases.

Finally, caloric restriction (CR), diet, and exercise could be the easiest ways to prevent age-related diseases and prolong lifespan. CR can inhibit mTOR signaling pathway, activate and increase the levels of SIRT1, and inhibit the intracellular insulin/IGF-1 signaling cascade [34]. An alternative to CR is intermittent fasting regimes and changes in diet regimes that give sufficient CR to activate anti-aging pathways. Such options are currently being explored in animal models, but the effectiveness of CR, diet changes, and increased exercise in age-related PD and NTM is yet to be realized.

3. Future directions

Expansion of knowledge regarding the roles of SAnSA in complex, chronic, recalcitrant infectious respiratory diseases such as NTM PD are needed and should be a global priority. Currently, more than 86,000 individuals with NTM PD in the U.S. alone [69]. These individuals await new information to alleviate their burden of NTM PD, but millions more worldwide also wait for these applicable answers for other similarly emerging respiratory pathogens such as COVID-19.

CRedit authorship contribution statement

Adrian Fifer: Data curation, Writing – original draft. **Karen Krukowski:** Manuscript writing and editing. **Jennifer R. Honda:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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