Milano, Monday April 15, 2024

Unimib

Corso laurea magistrale

Malattia Genetiche: dalla diagnosi alla malattia

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Università Vita-Salute San Raffaele, Milan, Italy

OSPEDALE
SAN RAFFAELE

Introduction

NTM-host interaction, DITID-San Raffaele Scientific Institute, Milan.

Emerging Bacterial Pathogens Unit

Project leader Nicola Lorè

NTM-host interaction:

- *Host Biomarkers in NTM-PD*
- *NTM-Host modellling infection (M. abscessus)*
- *Sequencing of M. abscessus clinical strains in CF*
- *New antimicrobials or therapeutics against Mabs*

Background

Genetic disease – Cystic Fibrosis CF

- Recessively inherited disorder caused by the presence of one mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (~ more than 1,500 possible mutations)
- incidence of clinical disease of 1 in 2,500 live births
- The mutations lead to the malfunction or loss-of-function of CFTR, a cyclic AMP-regulated chloride ion channel, resulting in defective chloride ion transport across epithelial cell surfaces.
- This decreases the volume of the periciliary fluid in the lower respiratory tract, which in turn interferes with the mucociliary clearance of inhaled microorganisms

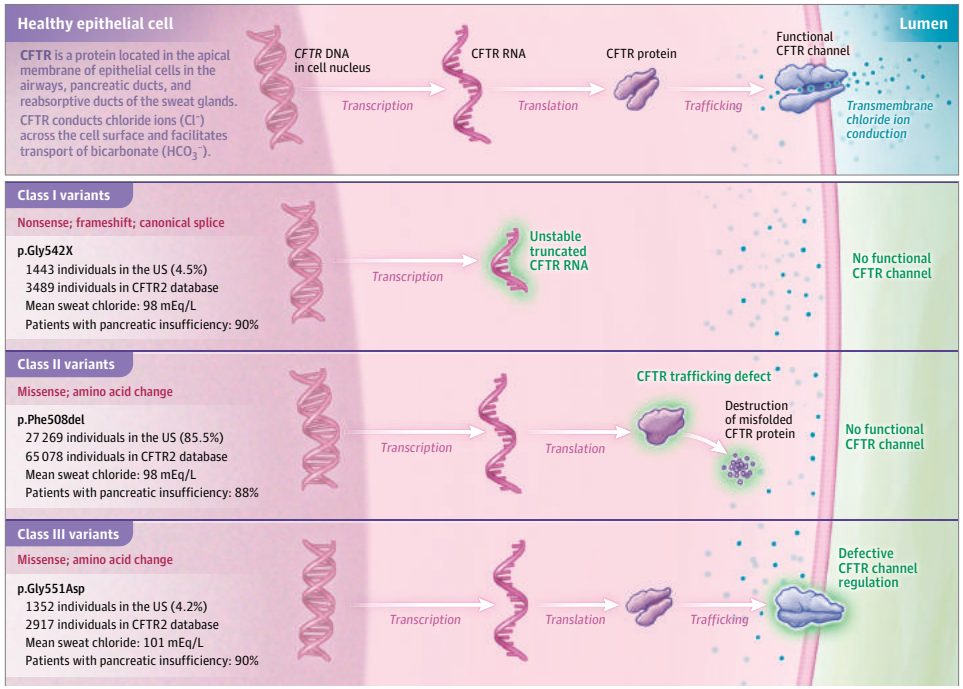
https://www.youtube.com/watch?v=YzjnxegMWfk&t=38s

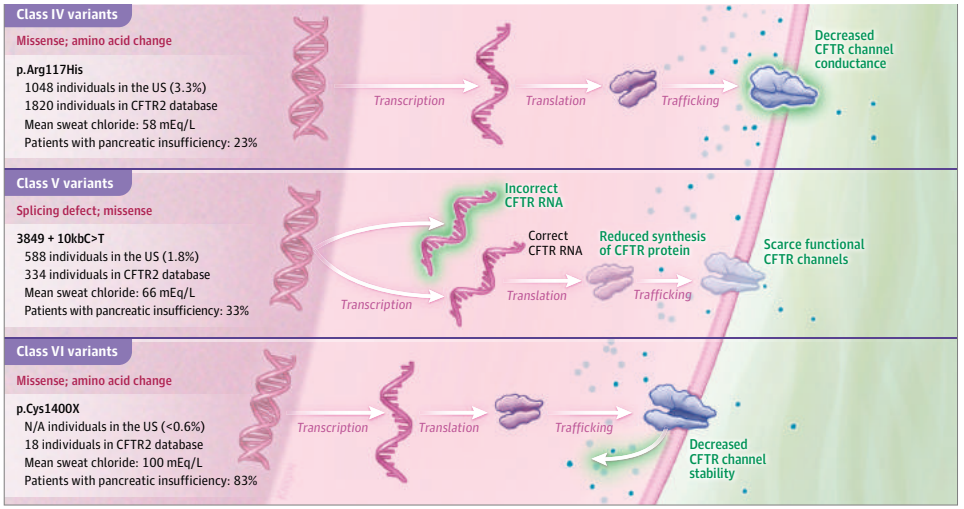
Folkesson A. et al Nat rev 2012
Rowe M.S. et al N engl j med 2005

Background

Genetic disease – Cystic Fibrosis CF

Figure 1. Cystic Fibrosis Transmembrane Conductance Regulator Variant Classes[1,5,6,19]

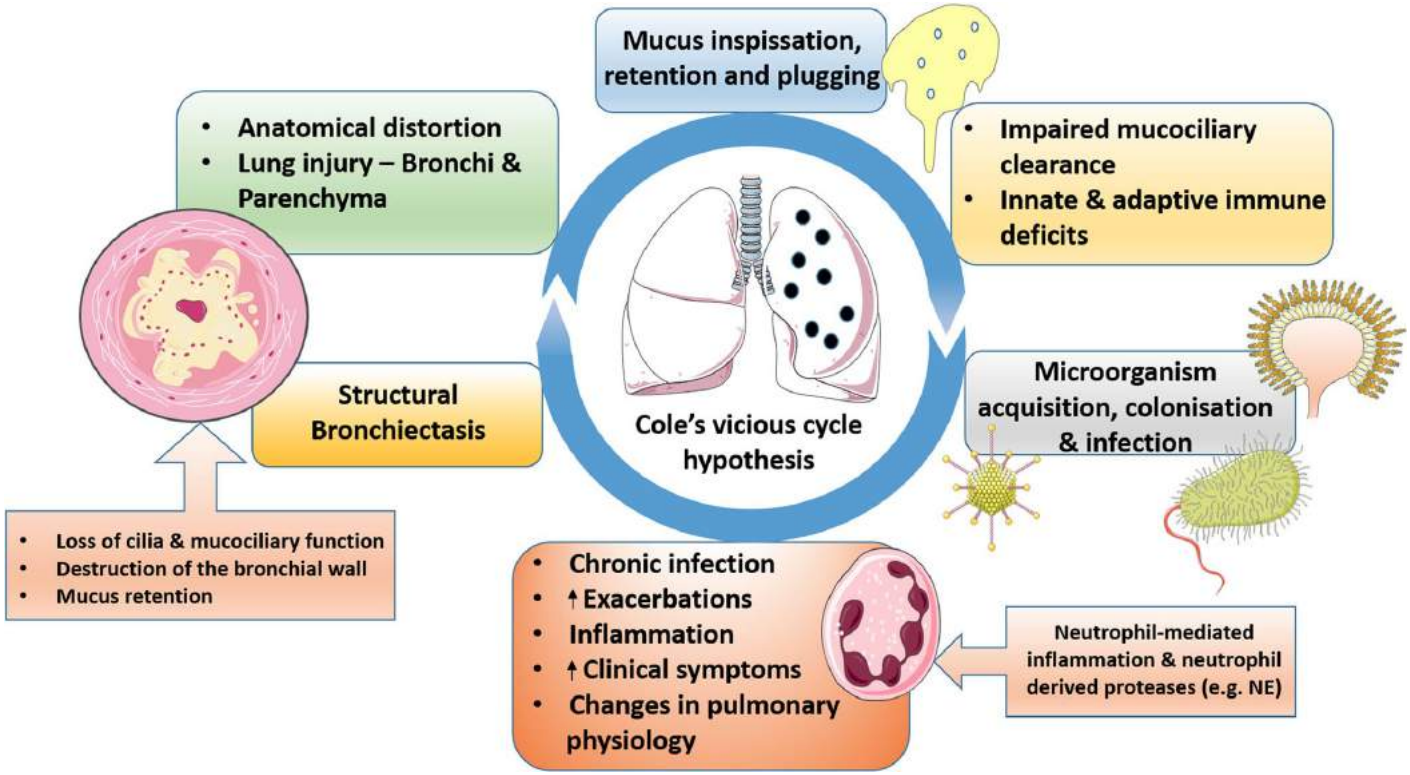




Cystic fibrosis transmembrane conductance regulator (*CFTR*) variants can be generally classified in 6 mechanistic classes based on how they alter CFTR RNA transcription, protein trafficking, channel function, and stability.[5,19] Reported prevalence, and clinical features (sweat chloride, pancreatic insufficiency) are summarized for exemplar variants per class.[1,6] The CFTR2 database provides information on all the *CFTR* variants and updates it as information becomes available.[6] The figure is adapted from Boyle and De Boeck.[5] N/A indicates number not available.

Rowe M.S. et al N engl j med 2005
Thida Ong, MD; Bonnie W. Ramsey, MD JAMA 2023

Cycles of infection/inflammation



Adapted from Chandrasekaran et al. BMC Pulmonary Medicine 2018

Background

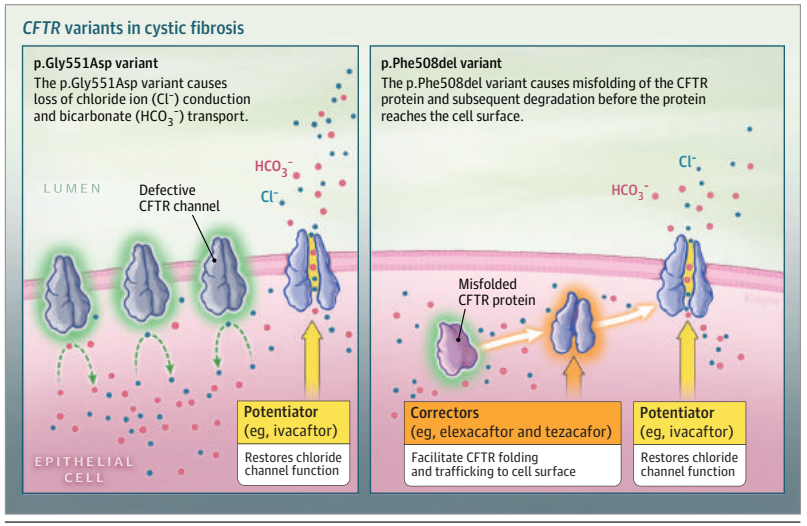
Cystic Fibrosis CF potentiator and Correctors

Thida Ong, MD; Bonnie W. Ramsey, MD JAMA 2023

Background

Cystic Fibrosis CF potentiator and Correctors

Figure 2. Cystic Fibrosis Transmembrane Conductance Regulator Modulator Therapy Functions[21-24]



Actions of cystic fibrosis transmembrane conductance regulator (*CFTR*) modulators as *correctors* and *potentiators*.[21,24] People with at least 1 copy of the F508del variant or 177 other variants are responsive to elexacaftor-tezacaftor-ivacaftor combination therapy.[23,78] Adapted from Cutting.[24]

p.Gly551Asp indicates glycine at residue 551 replaced by aspartic acid; and p.Phe508del, phenylalanine deleted at position 508.

https://www.youtube.com/watch?v=7WTjQY0V4qI

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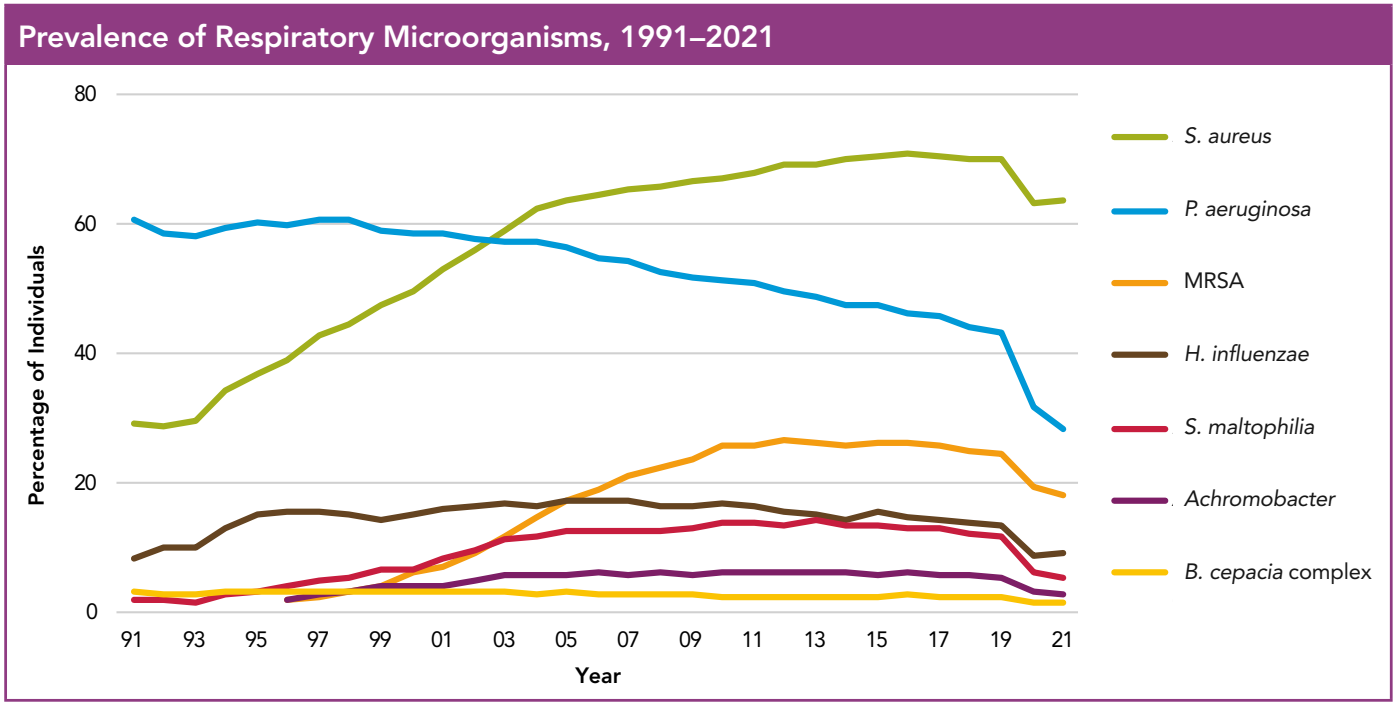
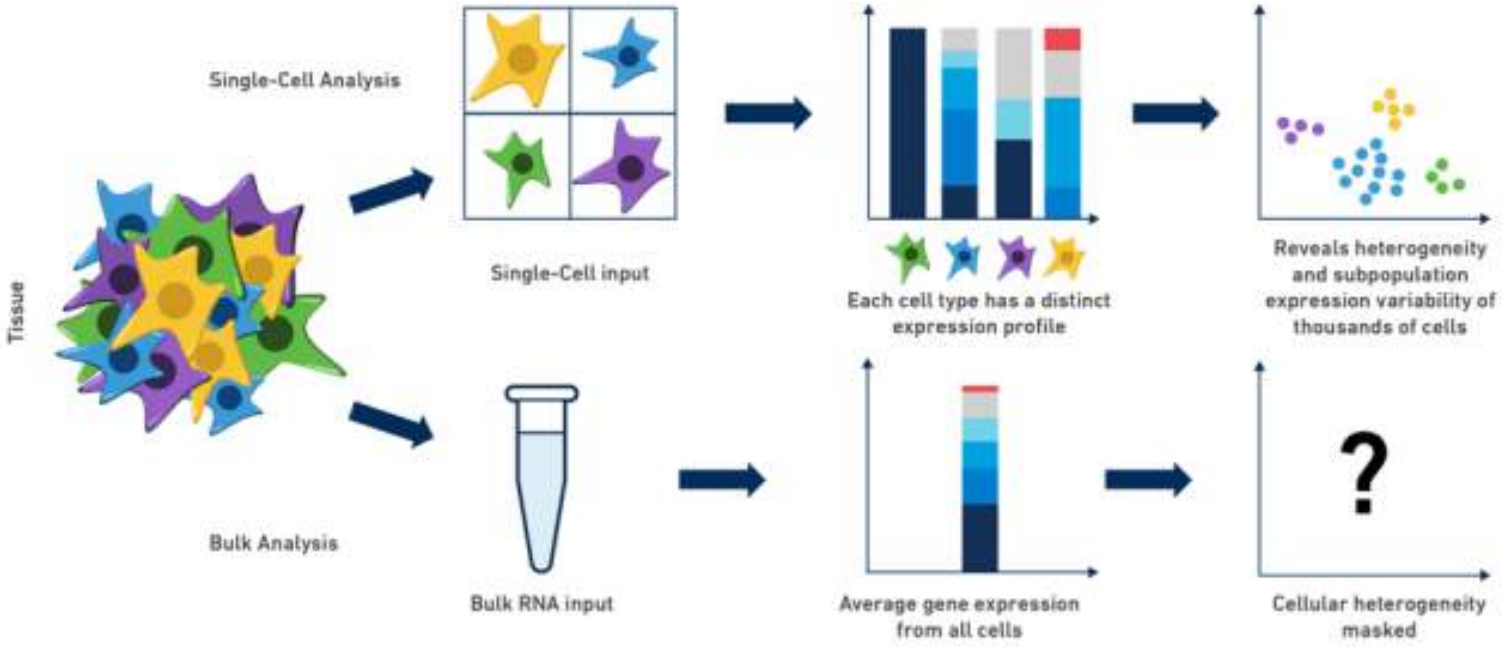
Background

Microbiology of CF lung disease



US CFF Registry 2021

Background | Single-cell RNA sequencing



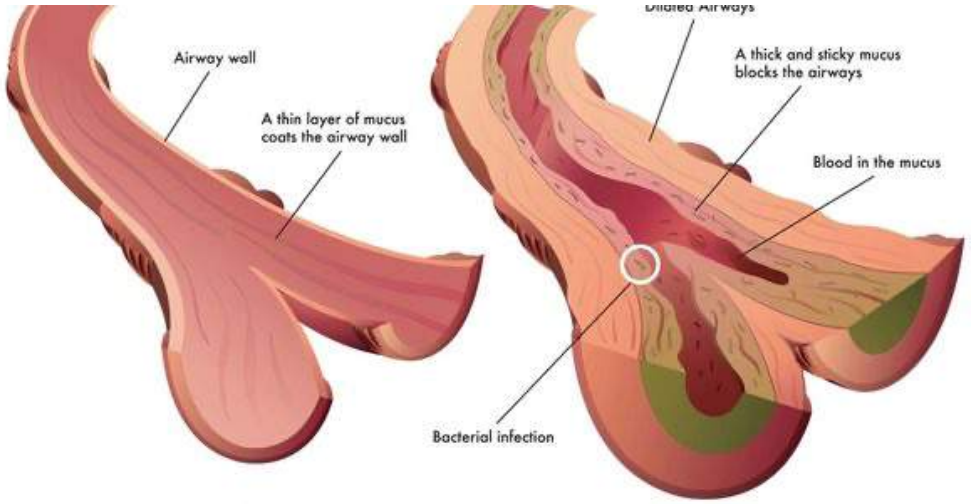
https://www.youtube.com/watch?v=6UVOdCc1Q7I

Question

How can we exploit Single-cell RNA sequencing techniques for a deeper understanding of Cystic Fibrosis disease?

Background

Single-cell RNA sequencing in Cystic Fibrosis



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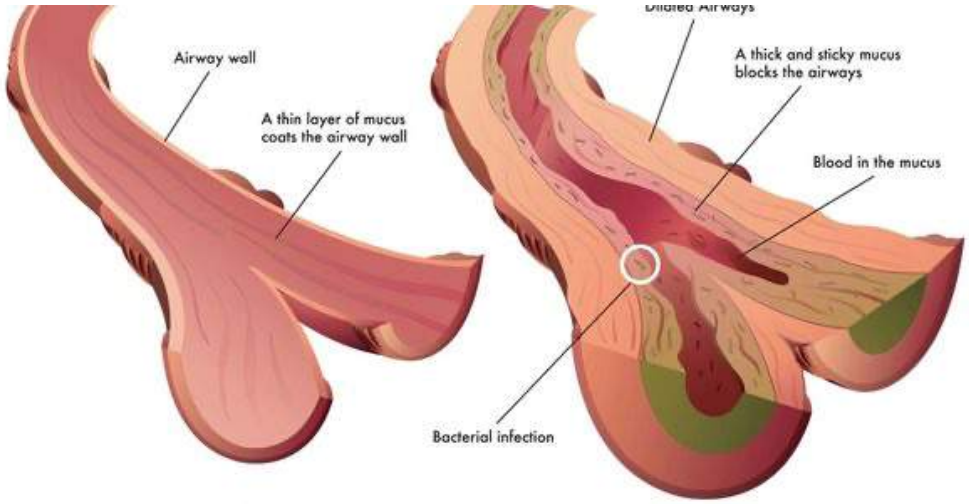
Background

Single-cell RNA sequencing in Cystic Fibrosis



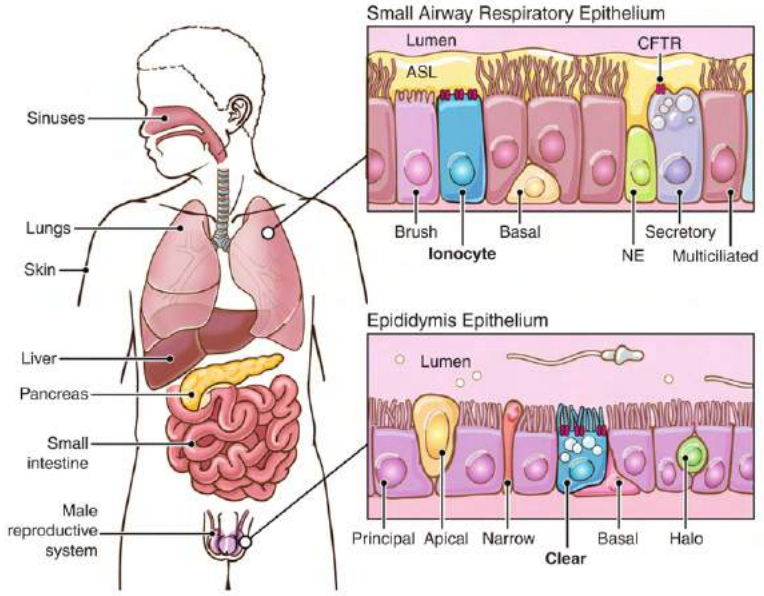


Figure 1. The organ systems most commonly affected in cystic fibrosis with representative small airway respiratory and epididymis epithelial cell types, including CFTR-expressing cell types. ASL = airway surface liquid; CFTR = cystic fibrosis transmembrane conductance regulator; NE = pulmonary neuroendocrine cell. Images reproduced and modified from Mount Sinai Health System with permission.

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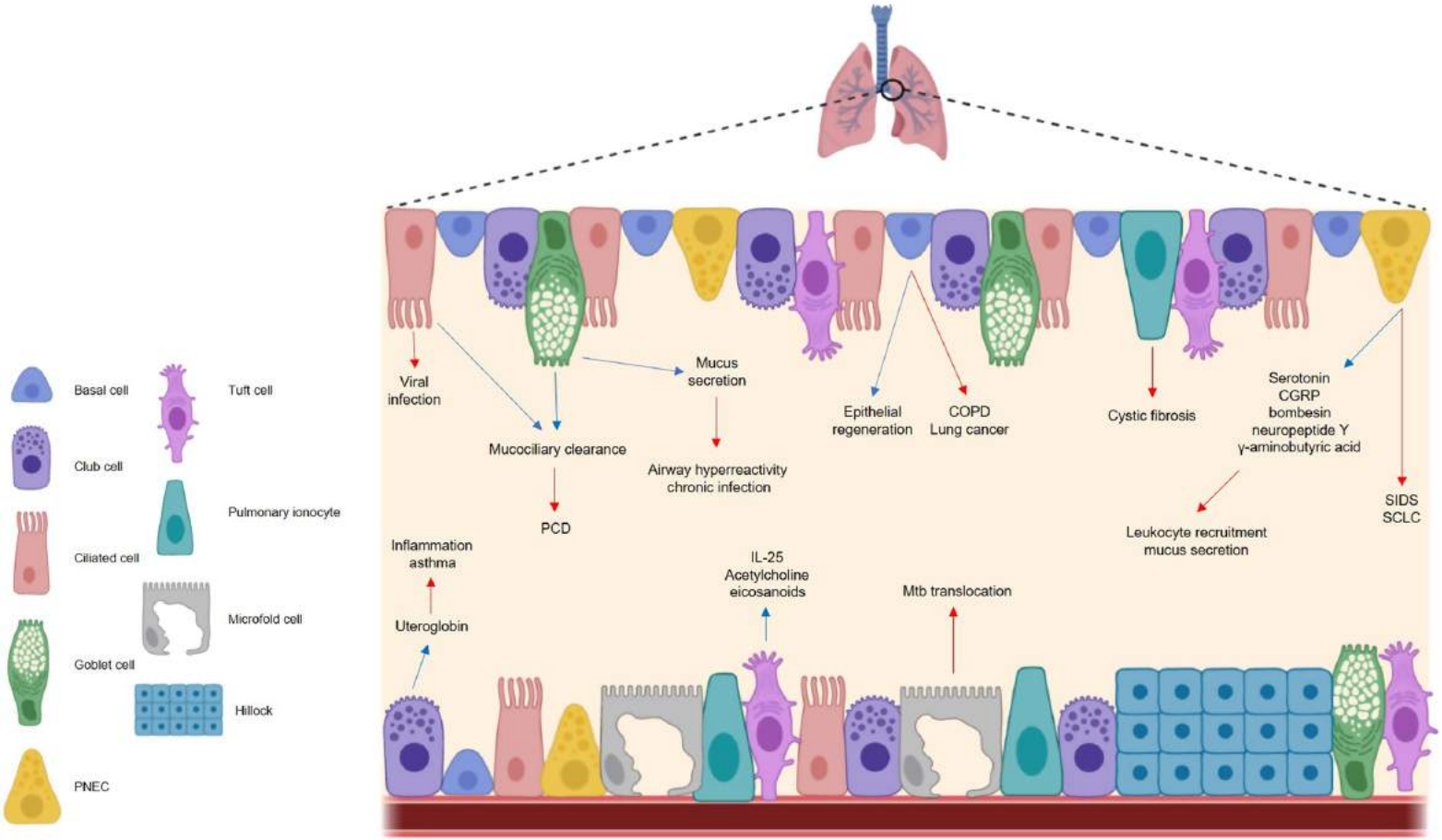
Background

Epithelial composition in the lung



Davis JD, et al Muc. Imm. 2021

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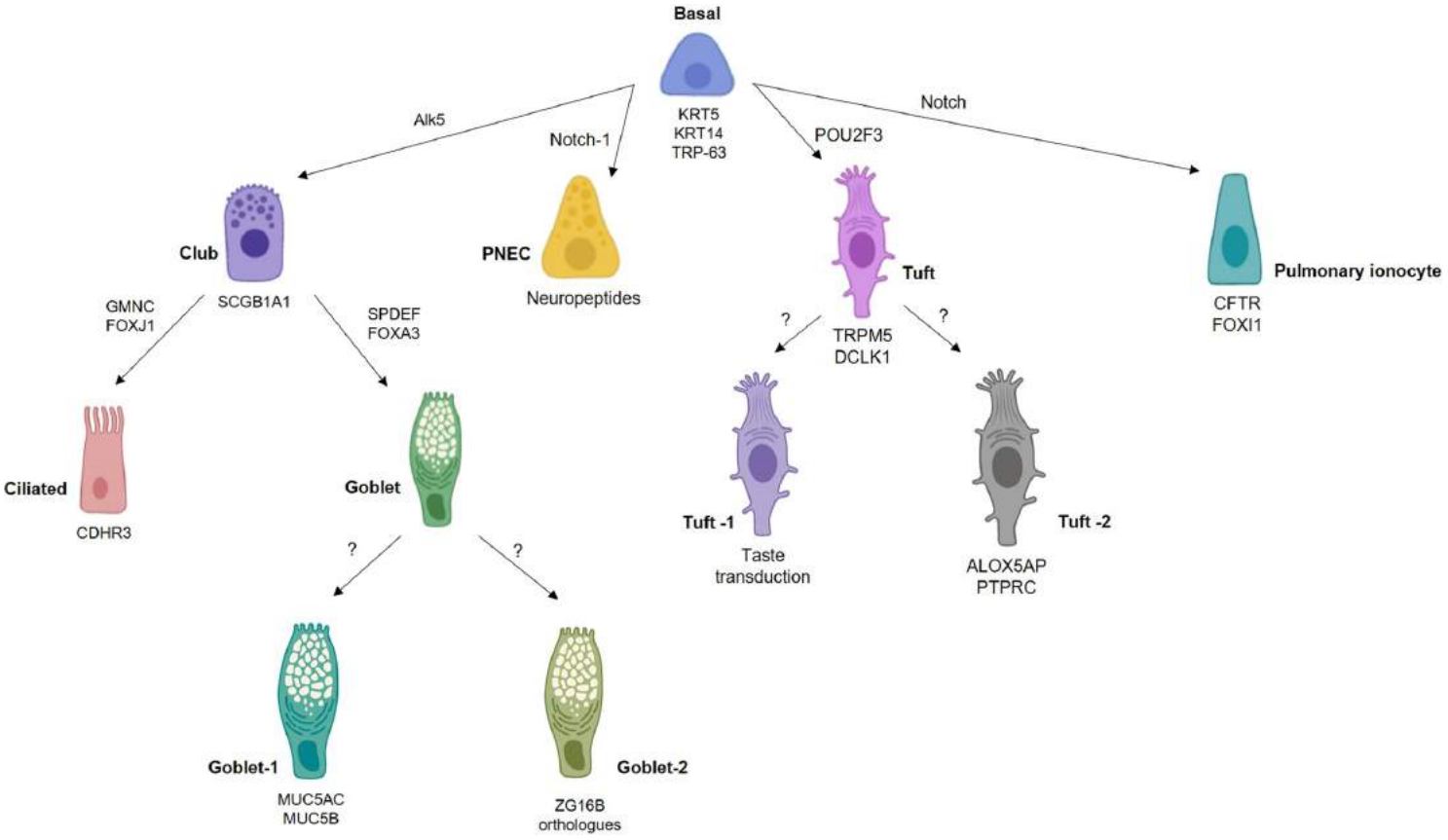

Davis JD, et al Muc. Imm. 2021

Methods

Sampling biological material for single-cell RNA sequencing in Cystic Fibrosis

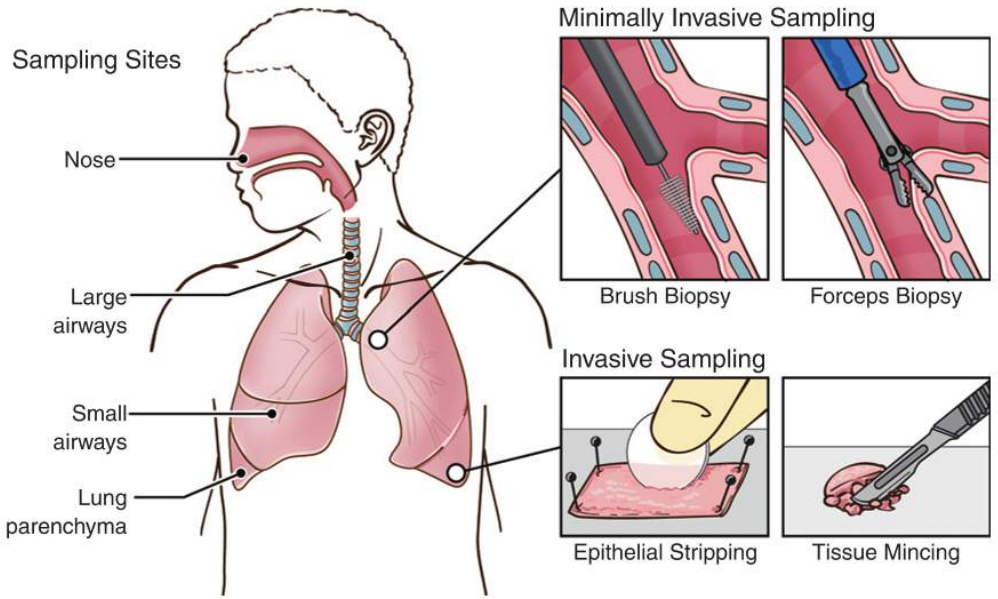

Figure 2. Sample sites and methods for obtaining respiratory epithelial cells. Images reproduced and modified from Mount Sinai Health System with permission.

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Results

Sampling biological material for single-cell RNA sequencing in Cystic Fibrosis

Table 1. Characteristics of Seven Single-Cell RNA Sequencing Data Sets from the Human Lung Describing the Cell Type–Specific Expression of *CFTR* Gene Transcripts

Authors	Data Set	Sampling Site	Sampling Method	Sequencing Technology	Reference
Montoro *et al.*	78,217 cells from one previously healthy donor	Large airways	—	10× Genomics	10
Deprez *et al.*	18,191 cells from seven healthy people	Nose	Brush and forceps biopsies	10× Genomics	14
	41,134 cells from nine healthy people	Large airways	Forceps biopsy	10× Genomics	
	18,644 cells from nine healthy people	Small airways	Brush biopsy	10× Genomics	
Okuda *et al.*	11,688 cells from eight previously healthy donors	Large airways	Epithelial stripping	10× Genomics	15
	4,955 cells from eight previously healthy donors	Small airways	Tissue mincing	10× Genomics	
	16,488 cells from four healthy people	Large airways	Brush biopsy	Drop-seq	
	9,831 cells from three previously healthy donors	Small airways	Tissue dissection	Drop-seq	
Carraro *et al.*	Ten donors with CF and 11 previously healthy donors	Large airways	Epithelial stripping and mincing	10× Genomics	21
	Nine donors with CF and eight previously healthy donors	Large airways	Epithelial stripping	Drop-seq	
Goldfarbmuren *et al.*	36,248 cells from 15 donors, including six never-smokers and six heavy smokers	Large airways	Epithelial stripping	10× Genomics	25
Habermann *et al.*	114,396 cells from 20 donors with pulmonary fibrosis and 10 previously healthy donors	Lung parenchyma	—	10× Genomics	40
Miller *et al.*	6,548 cells from two fetuses at 15–21 wk of gestation	Large airways	Epithelial stripping	10× Genomics	41
	11,829 cells from three fetuses at 11.5–18 wk of gestation	Small airways	Tissue mincing	10× Genomics	
	18,430 cells from three fetuses at 11.5–18 wk of gestation	Lung parenchyma	Tissue mincing	10× Genomics	

Definition of abbreviations: CF = cystic fibrosis; Drop-seq = droplet sequencing.

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Results

Sampling biological material for single-cell RNA sequencing in Cystic Fibrosis

Table 2. Characteristics of Five Single-Cell RNA-Sequencing Data Sets in Study of Cystic Fibrosis Lung

Authors	Species and Source	Data Set	Sampling Site	Sampling Method	Sequencing Technology	Reference
Carraro *et al.*	Human: superficial respiratory epithelial cells	Ten donors with CF and 11 previously healthy lung donors	Large airways	Epithelial stripping and tissue mincing	10× Genomics	21
		Nine donors with CF and eight previously healthy lung donors	Large airways	Epithelial stripping	Drop-seq	
Yu *et al.*	Pig: SMGs	14,561 cells from four *CFTR*$^{-/-}$ and four wild-type pigs	Large airways	Tissue dissection	10× Genomics	23
Schupp *et al.*	Human: sputum	12,494 cells from nine pwCF	Spontaneously expectorated sputum	Filtering	10× Genomics	44
		7,601 cells from five healthy people	Induced sputum	Filtering	10× Genomics	
Li *et al.*	Human: BALF	113,213 cells from three pwCF and four healthy people	BALF	Filtering	10× Genomics	46
Thurman *et al.*	Pig: lung	8,928 cells from five *CFTR*$^{-/-}$ and five wild-type pigs	Large airways	Epithelial stripping	10× Genomics	67
		17,773 cells from three *CFTR*$^{-/-}$ and four wild-type pigs	Small airways	Tissue dissection	10× Genomics	

Definition of abbreviations: BALF = BAL fluid; pwCF = people with cystic fibrosis; SMG = submucosal gland.

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Results | Single-Cell Transcriptional Archetypes of Respiratory epithelial/alveolar barrier

Published in final edited form as:
Nature. 2018 August ; 560(7718): 377–381. doi:10.1038/s41586-018-0394-6.

A single cell atlas of the tracheal epithelium reveals the CFTR-rich pulmonary ionocyte

Lindsey W. Plasschaert[#1], **Rapolas Žilionis**[#2,3], **Rayman Choo-Wing**[1], **Virginia Savova**[2], **Judith Knehr**[4], **Guglielmo Roma**[4], **Allon M. Klein**[2,†], and **Aron B. Jaffe**[1,†]

[1]Chemical Biology & Therapeutics, Novartis Institutes for BioMedical Research, Cambridge, Massachusetts 02139, USA. [2]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA. [3]Institute of Biotechnology, Vilnius University, Vilnius LT-10222, Lithuania [4]Chemical Biology & Therapeutics, Novartis Institutes for BioMedical Research, CH-4056 Basel, Switzerland.

[#] These authors contributed equally to this work.

The functions of epithelial tissues are dictated by the types, abundance, and distribution of the differentiated cells they contain. Attempts to restore tissue function after damage require

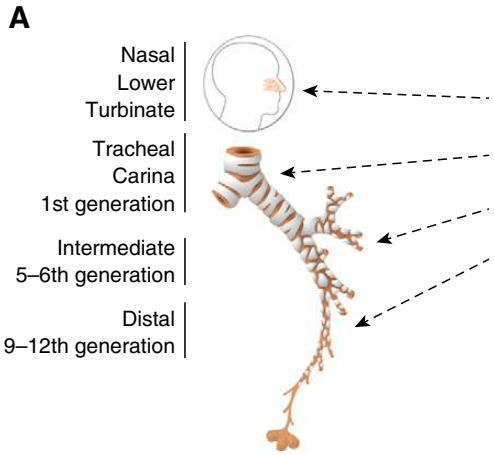
Published in final edited form as:
Nature. 2018 August ; 560(7718): 319–324. doi:10.1038/s41586-018-0393-7.

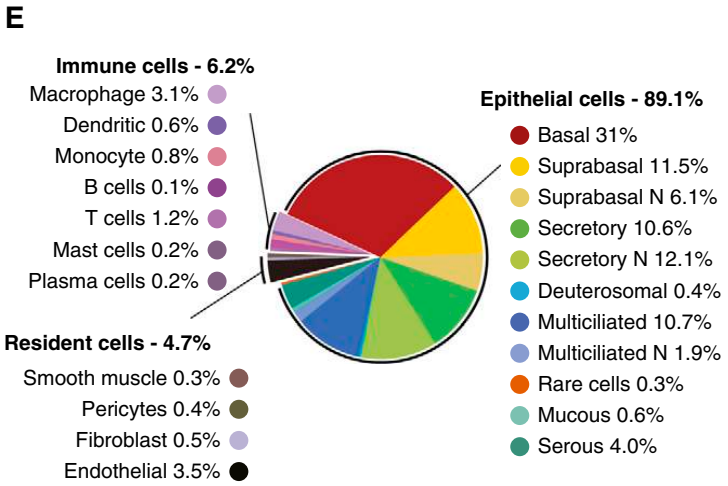
A revised airway epithelial hierarchy includes CFTR-expressing ionocytes

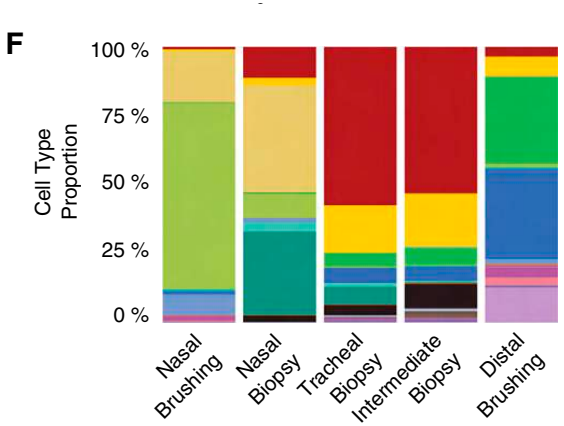
Daniel T. Montoro[#1,2,3], **Adam L. Haber**[#4], **Moshe Biton**[#4,5], **Vladimir Vinarsky**[1,2,3], **Brian Lin**[1,2,3], **Susan Birket**[6,7], **Feng Yuan**[8], **Sijia Chen**[9], **Hui Min Leung**[10,11], **Jorge Villoria**[1,2,3], **Noga Rogel**[4], **Grace Burgin**[4], **Alexander Tsankov**[4], **Avinash Waghray**[1,2,3], **Michal Slyper**[4], **Julia Waldmann**[4], **Lan Nguyen**[4], **Danielle Dionne**[4], **Orit Rozenblatt-Rosen**[4], **Purushothama Rao Tata**[12,13,14,15], **Hongmei Mou**[16,17], **Manjunatha Shivaraju**[1,2,3], **Hermann Bihler**[18], **Martin Mense**[18], **Guillermo J. Tearney**[10,11], **Steven M. Rowe**[6,7], **John F. Engelhardt**[8], **Aviv Regev**[4,19,§], and **Jayaraj Rajagopal**[1,2,3,§]

[1]Center for Regenerative Medicine, Massachusetts General Hospital, 185 Cambridge Street, Boston, Massachusetts 02114, USA [2]Departments of Internal Medicine and Pediatrics, Pulmonary and Critical Care Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA [3]Harvard Stem Cell Institute, Cambridge, Massachusetts 02138 [4]Klarman Cell Observatory, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA [5]Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston, MA, 02114, USA [6]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL. [7]Gregory Fleming James Cystic Fibrosis Research Center, Birmingham, AL. [8]Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA. [9]Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, The Netherlands [10]Department of Dermatology, Harvard Medical School, Boston, MA, USA [11]Wellman Center for Photomedicine, Boston, MA, USA [12]Department of Cell Biology, Duke University, Durham, NC 27710, USA [13]Duke Cancer Institute, Duke University, Durham, NC 27710, USA [14]Division of Pulmonary Critical Care, Department of Medicine, Duke University School of Medicine, Durham, NC 27710, USA [15]Regeneration Next, Duke University, Durham, NC 27710, USA [16]Department of Pediatrics, Massachusetts General Hospital, Boston, MA 02114, USA [17]Mucosal Immunology and Biology Research Center, Massachusetts General Hospital, Boston, MA 02114, USA [18]Cystic Fibrosis Foundation Therapeutics, Lexington, MA, USA

Results | Single-Cell Transcriptional Archetypes of Respiratory epithelial/alveolar barrier







Marie Deprez et al AJRCCM 2020

Results

Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis

1. Understand subject-specific immune dysfunction and its contribution to divergent clinical courses in CF.
2. As we progress toward personalized applications of therapeutic and genomic developments, this inflammation-profiling approach will enable further discoveries that change the natural history of CF lung disease.

Shupp C, et al AJRCCM 2020

Results

Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis

Rationale: Cystic fibrosis (CF) is a life-shortening, multisystem hereditary disease caused by abnormal chloride transport. CF lung disease is driven by innate immune dysfunction and exaggerated inflammatory responses that contribute to tissue injury. To define the transcriptional profile of this airway immune dysfunction, we performed the first single-cell transcriptome characterization of CF sputum.

Shupp C, et al AJRCCM 2020

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Objectives: To define the transcriptional profile of sputum cells and its implication in the pathogenesis of immune function and the development of CF lung disease.

Methods: We performed single-cell RNA sequencing of sputum cells from nine subjects with CF and five healthy control subjects. We applied novel computational approaches to define expression-based cell function and maturity profiles, herein called transcriptional archetypes.

Shupp C, et al AJRCCM 2020

Results | Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis

Table 1. Demographic Characteristics of Study Subjects from the Yale Adult Cystic Fibrosis Program and Healthy Control Subjects

Characteristics	HC Subjects (*n* = 5)	Subjects with CF (*n* = 9)
Age, yr		
Mean ± SD	35.4 ± 5.9	30.6 ± 6.5
Range	26–42	24–43
Sex, *n* (%)		
F	2 (40)	6 (67)
M	3 (60)	3 (33)
Mutation background, *n* (%)		
F508del/F508del	NA	7 (77.8)
F508del/other	NA	2 (22.2)
No *F508del* mutations	NA	0 (0)
FEV_1, L		
Mean ± SD	NA	1.9 ± 0.7
Range	NA	0.68–2.85
FEV_1, %		
Mean ± SD	NA	57 ± 21.5
Range	NA	19–84
BMI, kg/m^2		
Mean ± SD	NA	22.2 ± 2.1
Range	NA	19.11–25.73
CF comorbidities, *n* (%)		
Pancreatic exocrine insufficiency	NA	9 (100)
CF-related diabetes	NA	4 (44.4)
Liver disease	NA	1 (11.1)
Microbiology, *n* (%)		
P. aeruginosa colonization	NA	5 (55.6)
CFTR modulators, *n* (%)		
Ivacaftor/tezacaftor	NA	6 (66.7)
Ivacaftor/lumacaftor	NA	2 (22.2)
No modulator	NA	1 (11.1)

Definition of abbreviations: BMI = body mass index; CF = cystic fibrosis; CFTR= cystic fibrosis transmembrane conductance regulator; HC = healthy control; NA = not applicable; *P. aeruginosa* = *Pseudomonas aeruginosa*.

Shupp C, et al AJRCCM 2020

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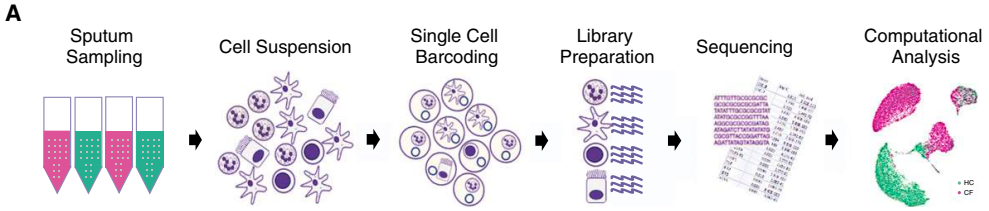
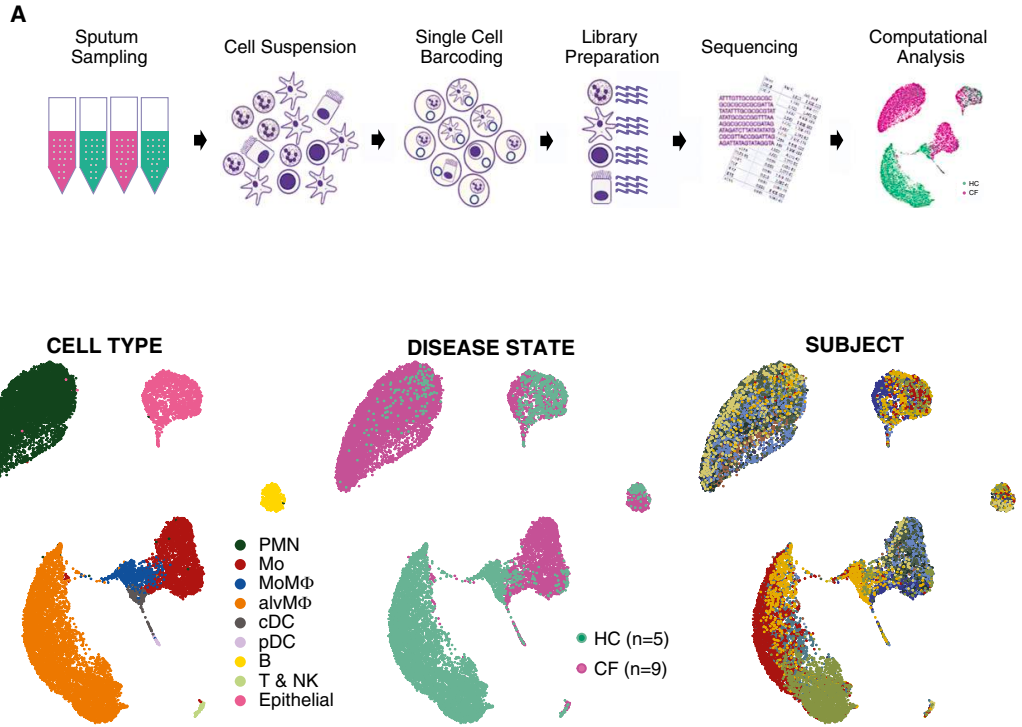


Shupp C, et al AJRCCM 2020

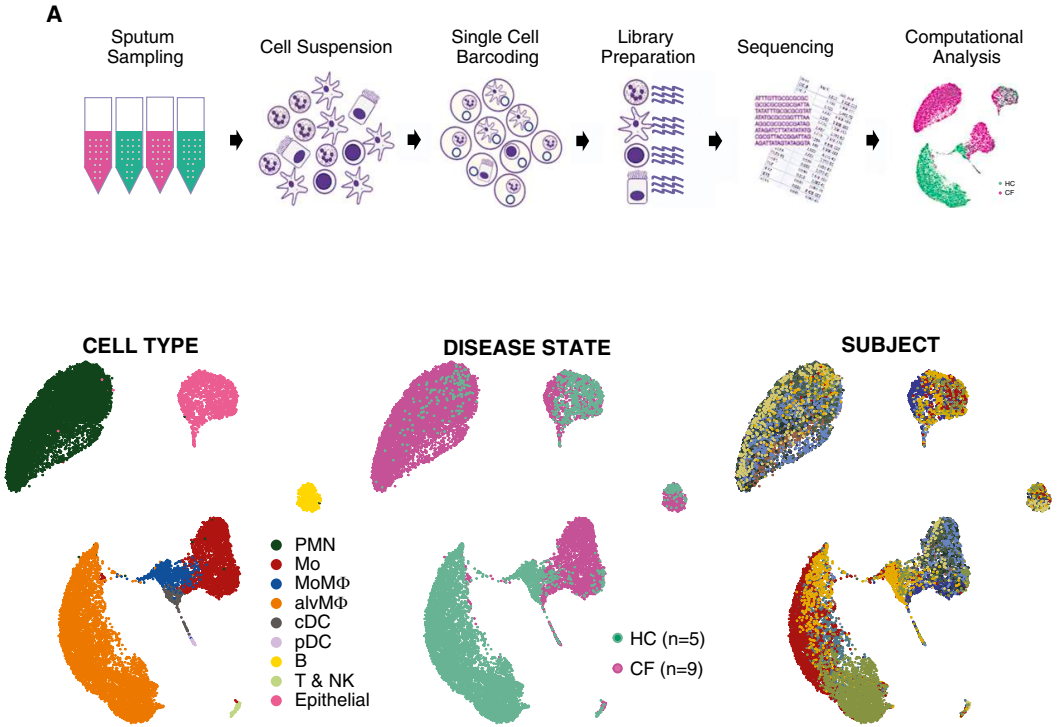
Results

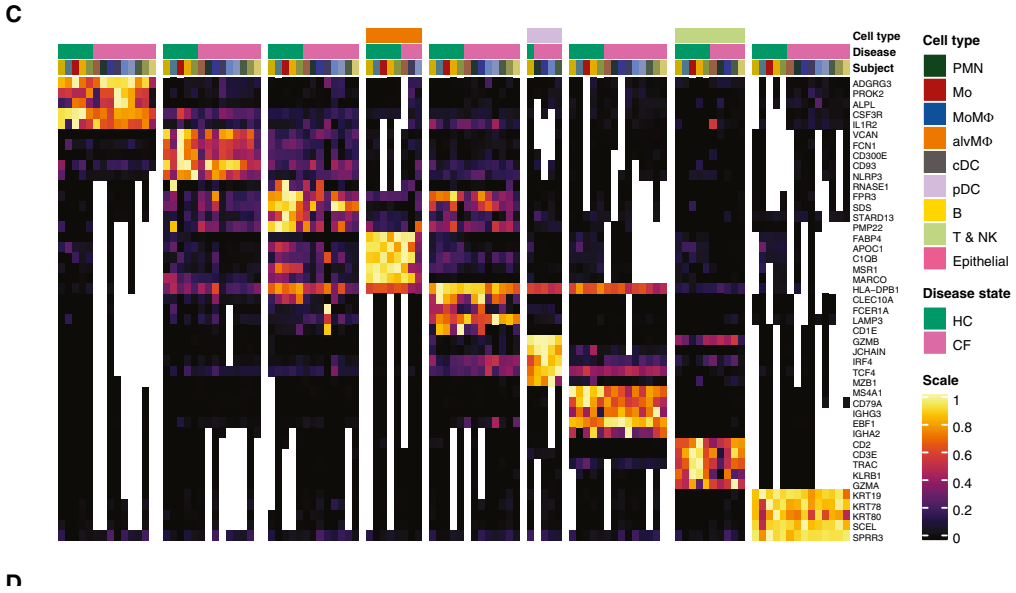
Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis



Shupp C, et al AJRCCM 2020

Results | Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis





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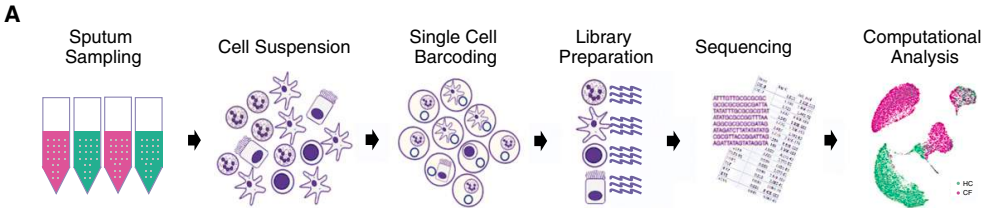

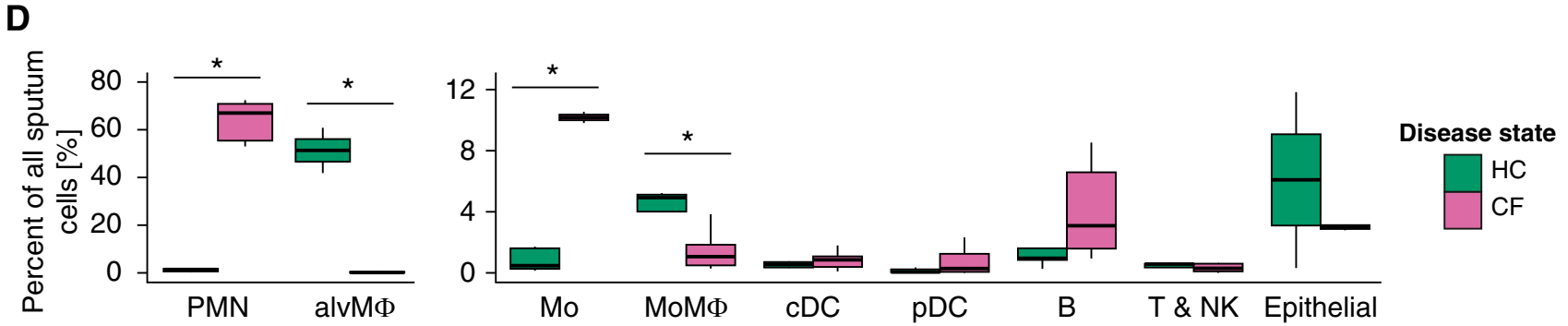

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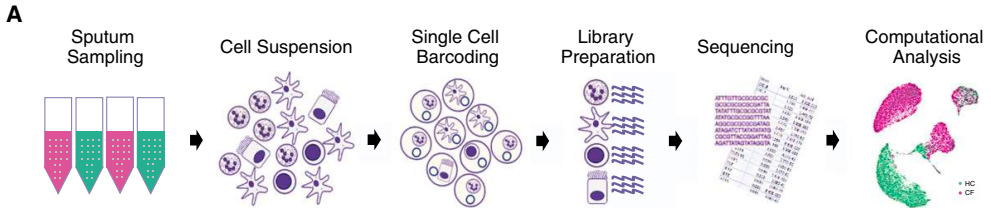
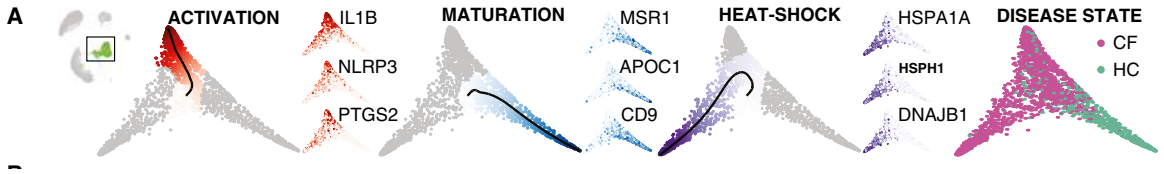
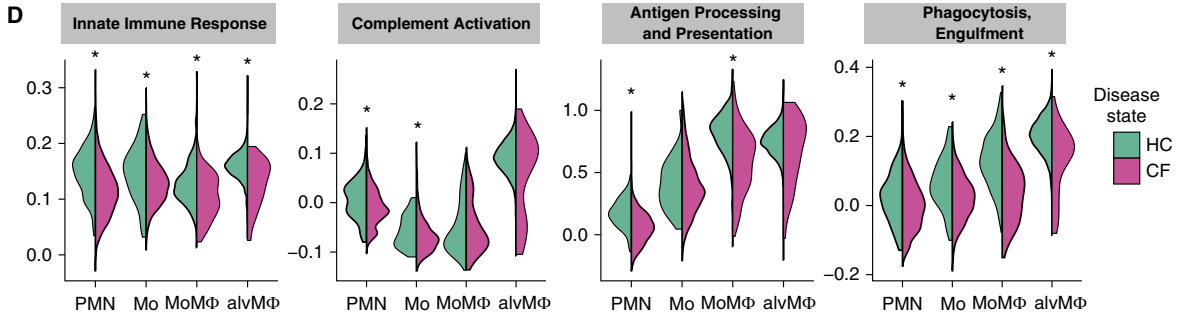
Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis







Shupp C, et al AJRCCM 2020

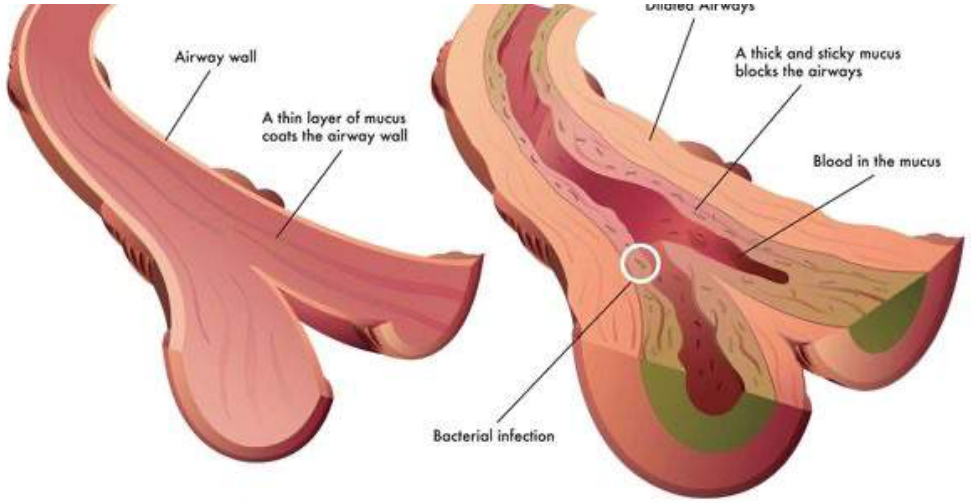
Results

Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis

1. Understand subject-specific immune dysfunction and its contribution to divergent clinical courses in CF.
2. As we progress toward personalized applications of therapeutic and genomic developments, this inflammation-profiling approach will enable further discoveries that change the natural history of CF lung disease.

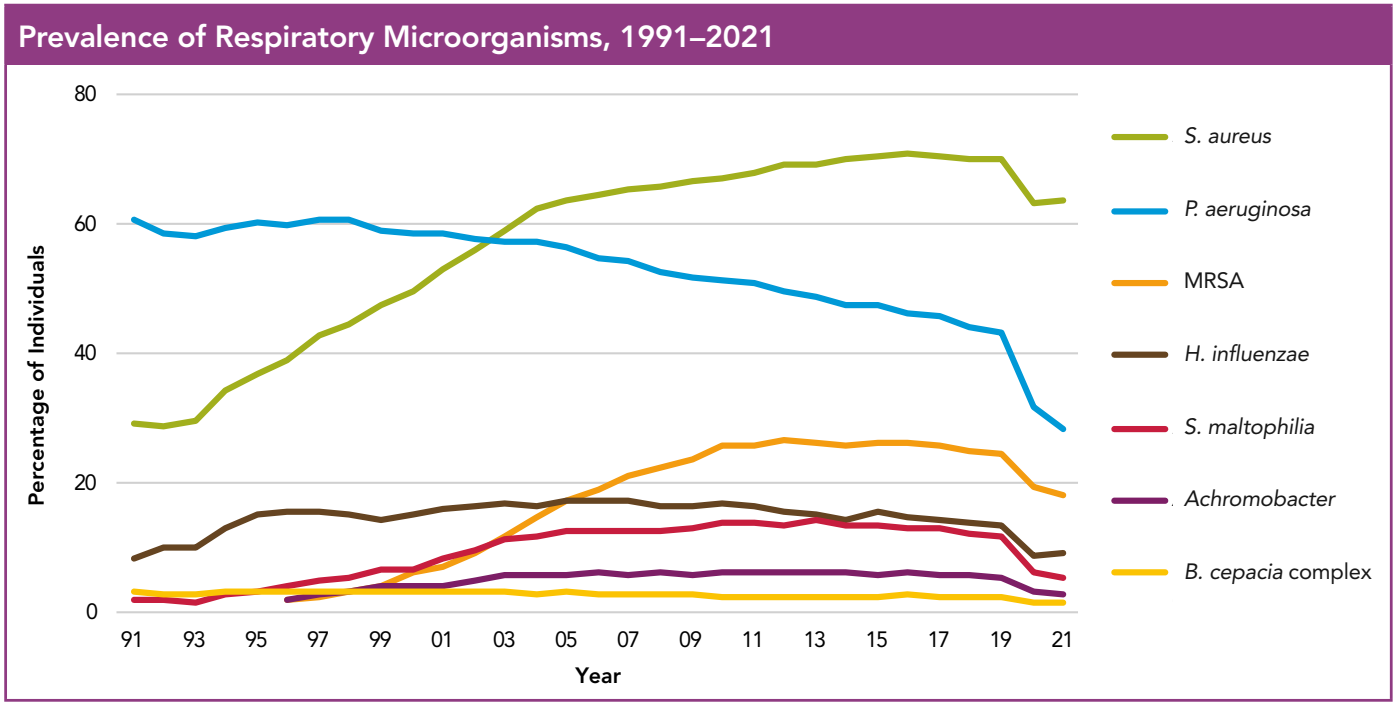
Shupp C, et al AJRCCM 2020

Infections in Cystic Fibrosis



Background

Microbiology of CF lung disease

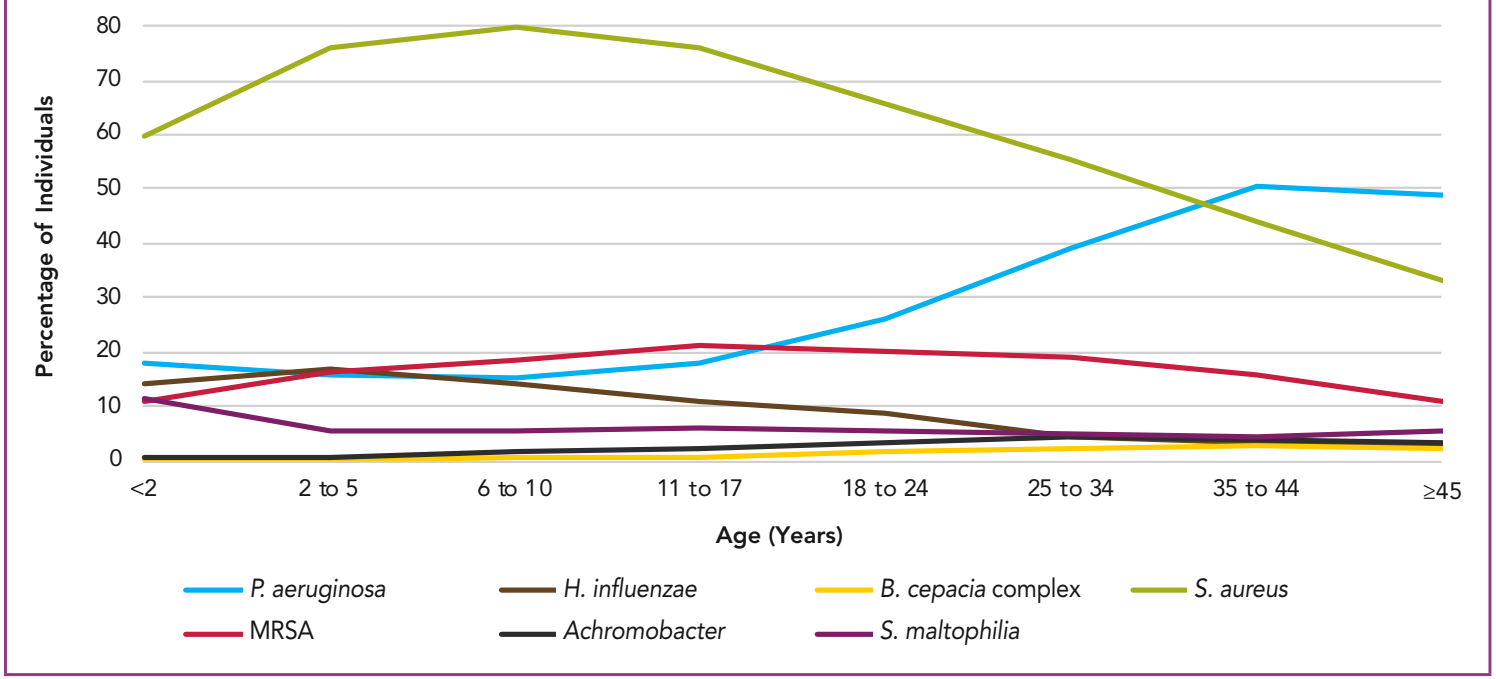


US CFF Registry 2021

Background

Microbiology of CF lung disease

Prevalence of Respiratory Microorganisms by Age Cohort, 2021



US CFF Registry 2021

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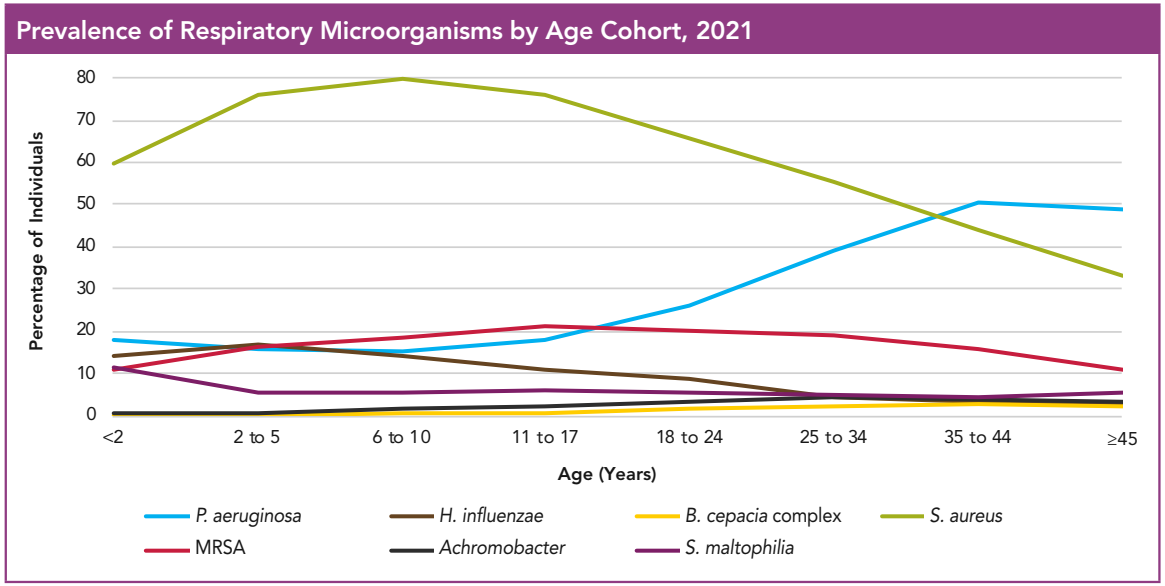
Microbiology of CF lung disease

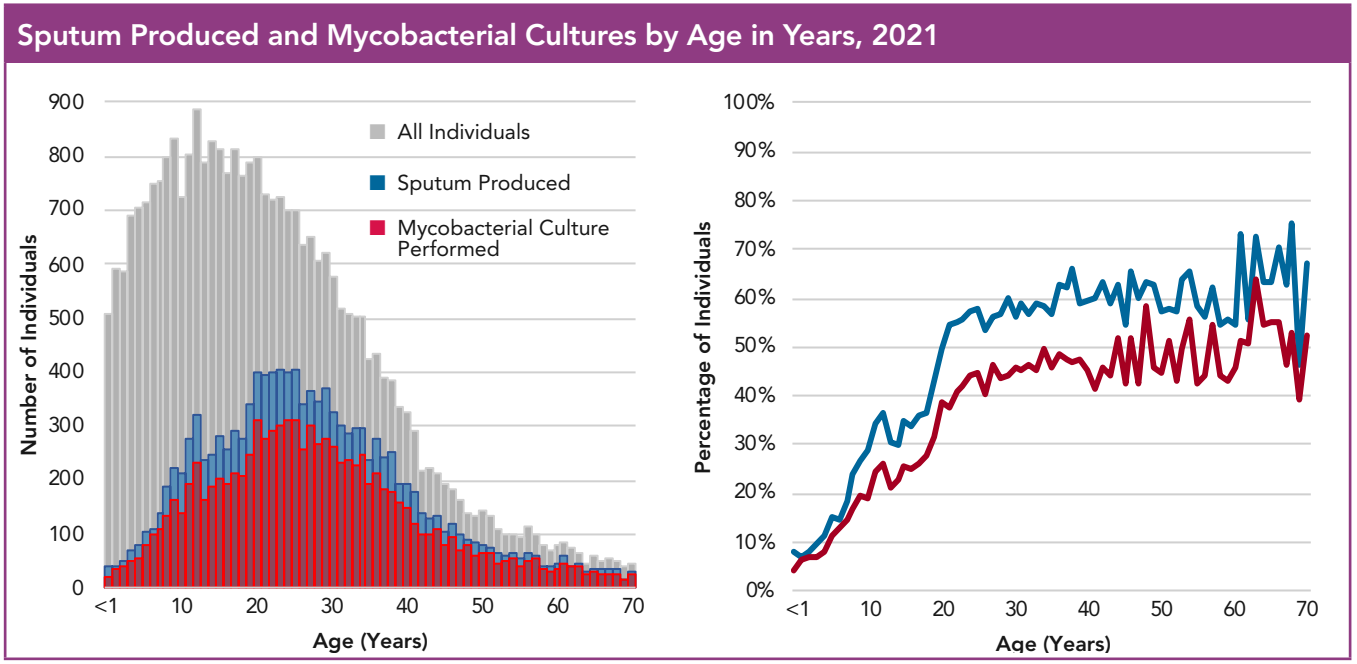


Bacteria	2018 Percent With Infection	2022 Percent With Infection
Pseudomonas aeruginosa	44%	26%
Stenotrophomonas maltophilia	12%	5%
Methicilin-resistant Staphylococcus aureus	25%	16%
Achromobacter xylosoxidans	6%	2%
Burkholderia cepacia complex	3%	1%
Nontuberculous mycobacteria	14%	10%

US CFF Registry 2021
US CFF Registry high 2022

Background

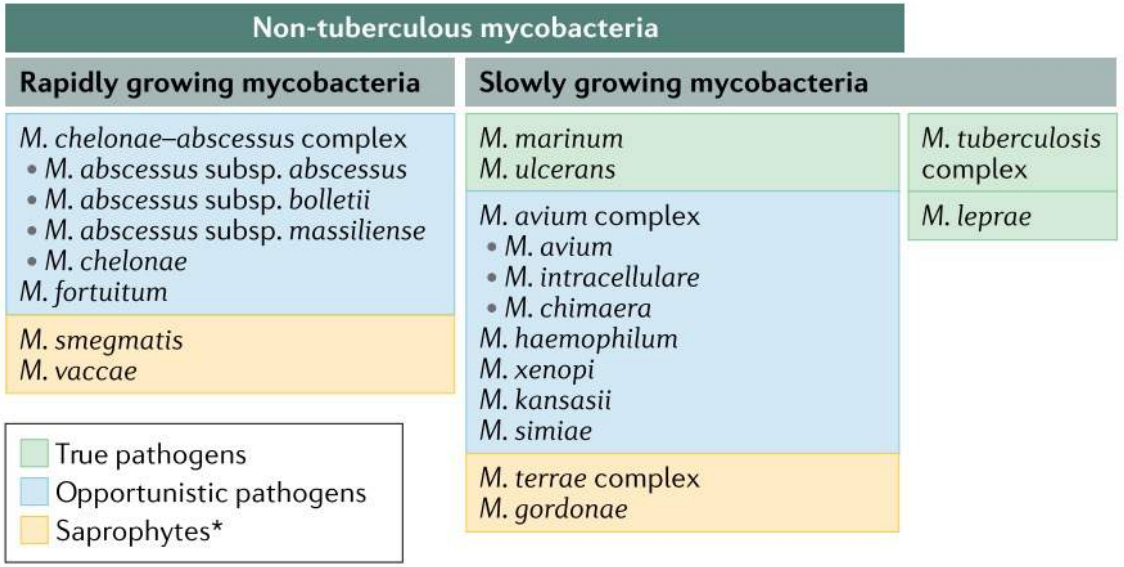
Nontuberculous mycobacteria (NTM) in cystic fibrosis



US CFF Registry 2021

Background

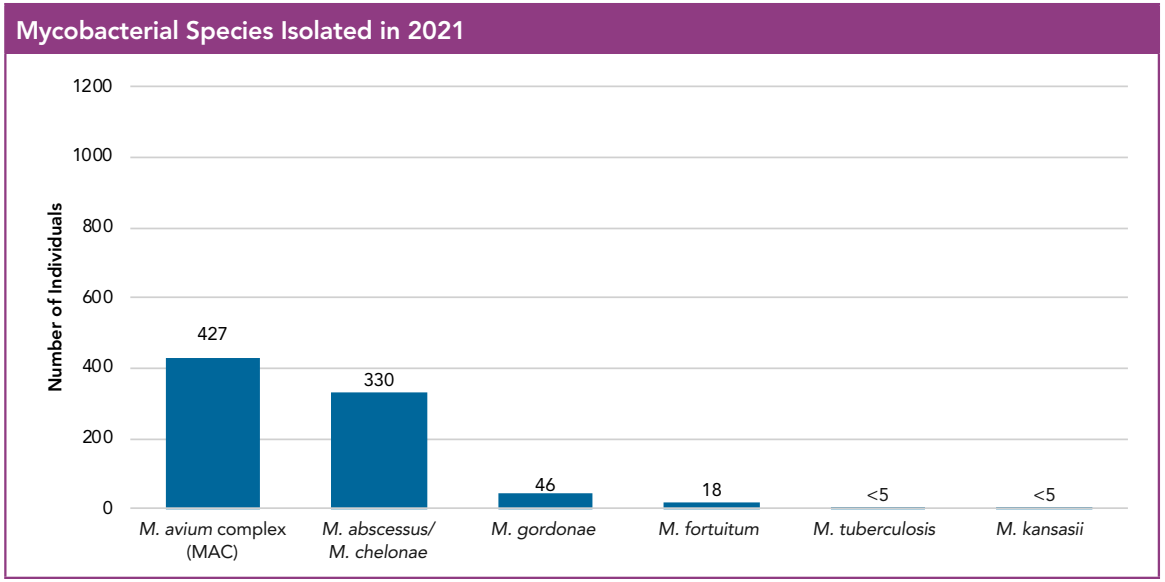
Nontuberculous mycobacteria (NTM)



Johansen MD et al, Nat Rev 2020
Tortoli et al., Infect Genet Evol. 2017
Ripoll et al., Plos one, 2009
Bernut et al., Cell Reports, 2019
Whang et al., Nature, 2017
Rhoades et al., J Immunol, 2009

Background

Nontuberculous mycobacteria (NTM) in cystic fibrosis

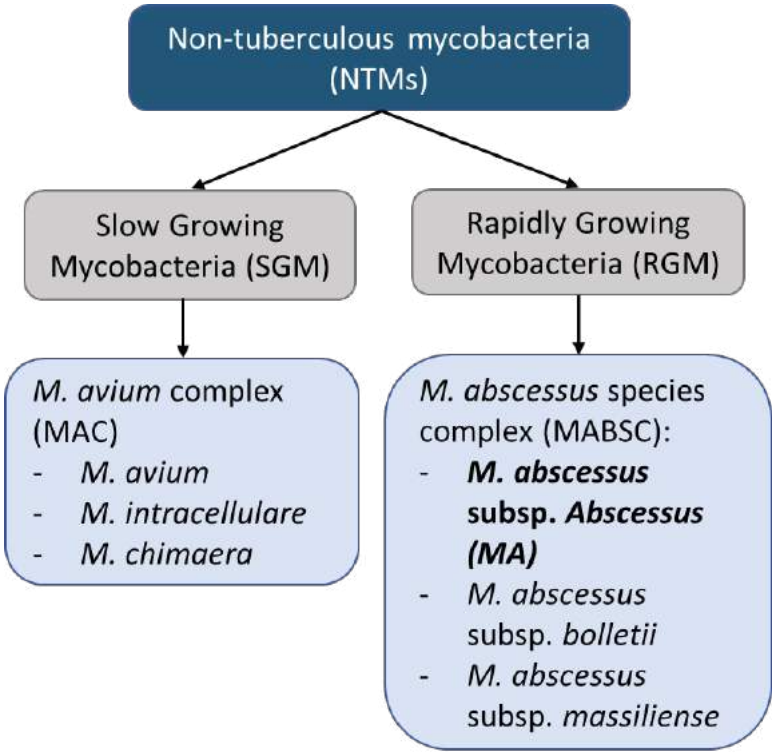


Data are not mutually exclusive. Some individuals had more than one species isolated in 2021.

US CFF Registry 2021

Background

Nontuberculous mycobacteria (NTM)



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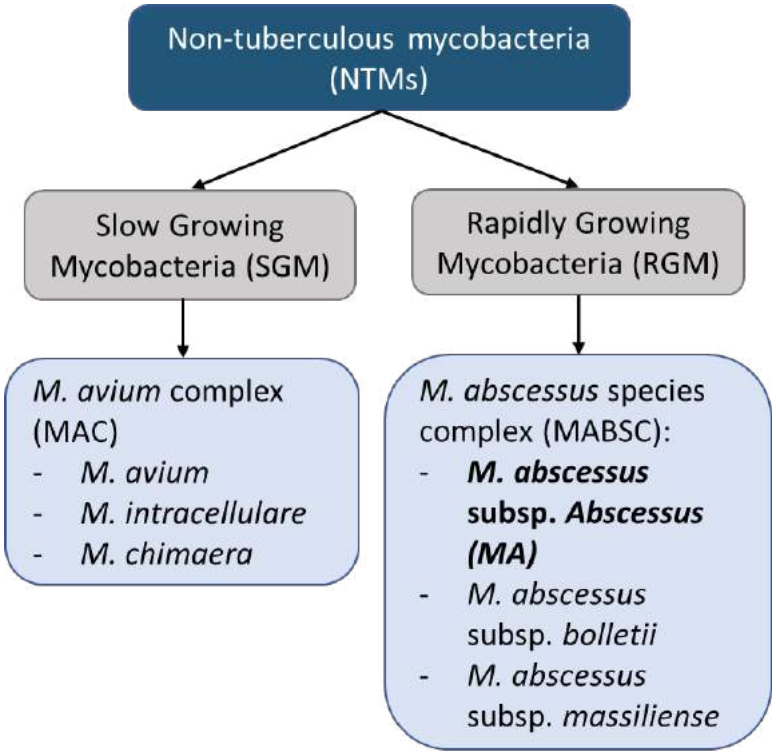
Background

Nontuberculous mycobacteria (NTM) lung disease in cystic fibrosis



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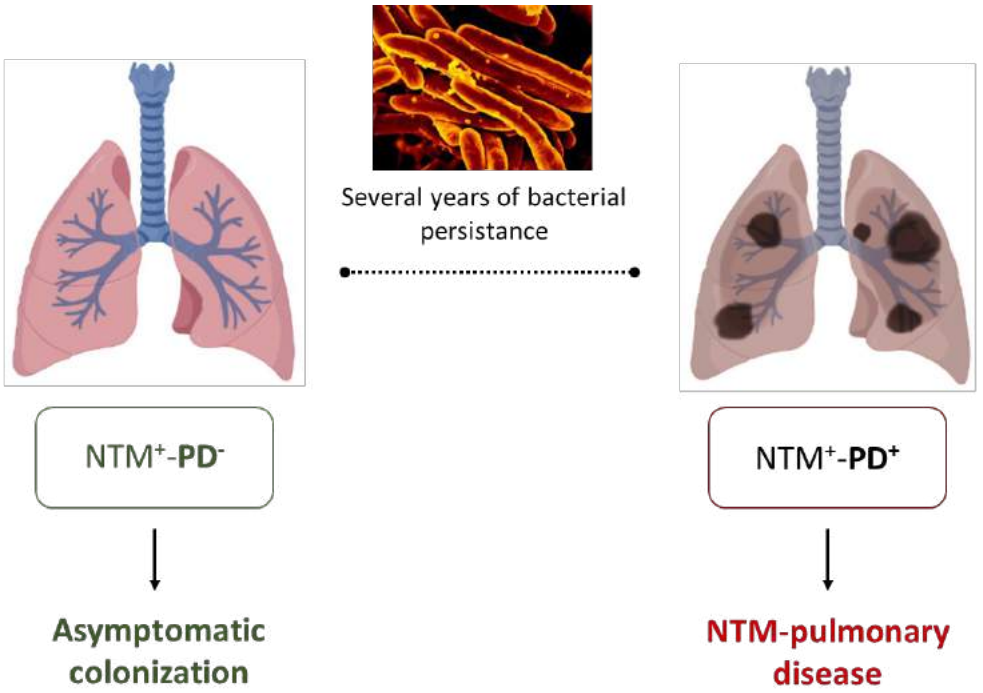
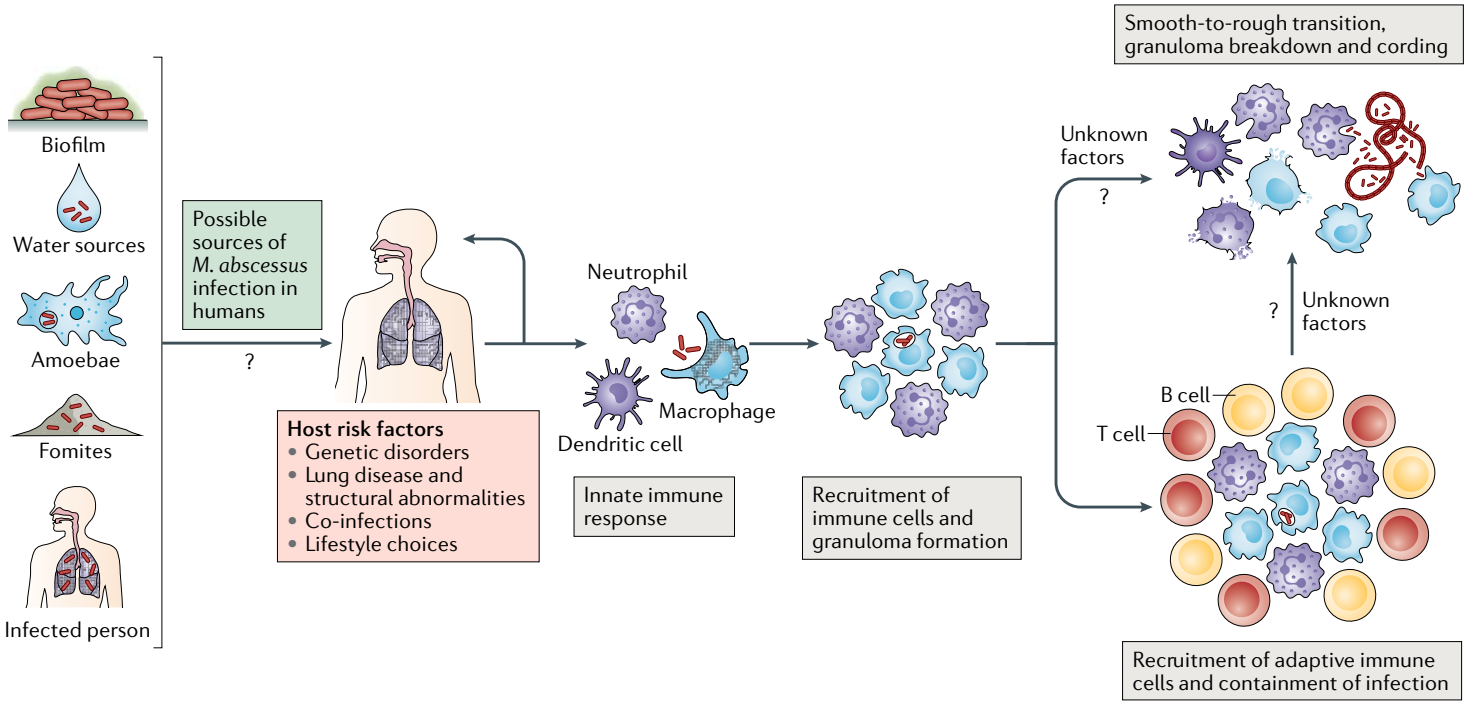


Rowe M.S. et al., N Engl J Med, 2005
Johansen MD et al, Nat. Rev., 2020
Daley C. et al ERJ 2020

Background

Nontuberculous mycobacteria (NTM) pulmonary disease (NTM-PD)



Johansen MD et al, Nat. Rev., 2020
Daley C. et al ERJ 2020

Background

NTM-PD treatment

Review > Chest. 2022 Jan;161(1):64-75. doi: 10.1016/j.chest.2021.07.035. Epub 2021 Jul 24.

Treatment of Mycobacterium abscessus Pulmonary Disease

David E Griffith [1], Charles L Daley [2]

Affiliations + expand

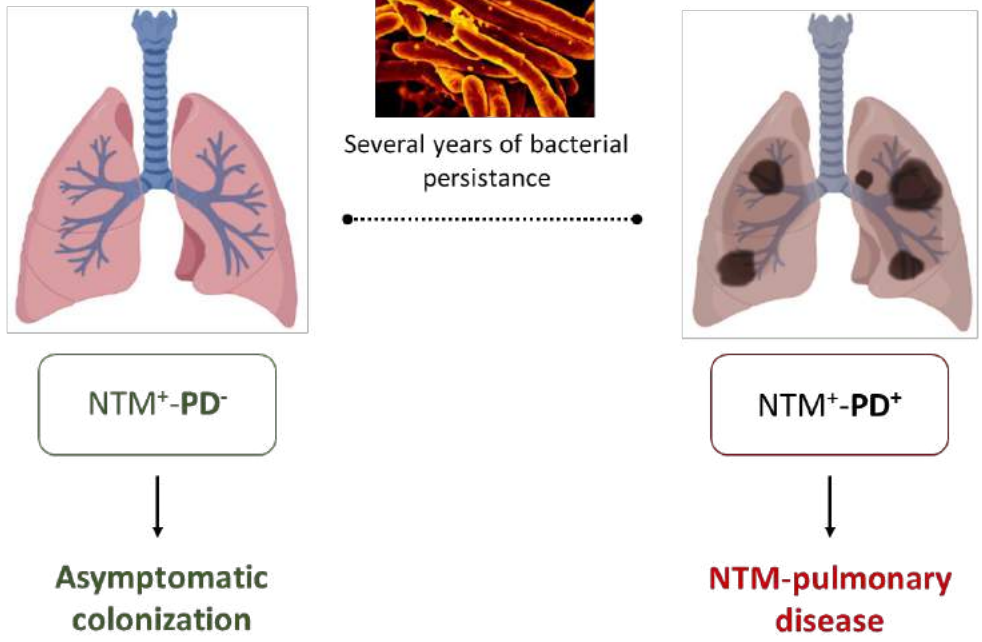
PMID: 34314673 DOI: 10.1016/j.chest.2021.07.035

TABLE 5] Recommended Treatment Regimens for *Mycobacterium abscessus*

Mutational	Inducible	No. of Drugs	Preferred Drugs		Frequency of Dosing
Susceptible	Susceptible	Initial Phase ≥ 3	Parenteral (choose 1-2)[a] Amikacin[b] Imipenem (or cefoxitin) Tigecycline	Oral (choose 2)[c] Azithromycin[d] Clofazimine Omadacycline Linezolid or tedizolid Bedaquiline	Daily (3 times weekly may be used for parenteral aminoglycosides)
		Continuation phase ≥ 2	Oral/inhaled (choose 2-3)[a] Azithromycin[d] Clofazimine Omadacycline Linezolid or tedizolid Inhaled amikacin Bedaquiline		
Susceptible	Resistant	Initial phase ≥ 4	Parenteral (choose 1-2)[a] Amikacin Imipenem (or cefoxitin) Tigecycline	Oral (choose 2)[c] Azithromycin[e] Clofazimine Omadacycline Linezolid or tedizolid Bedaquiline	Daily (3 times weekly may be used for parenteral aminoglycosides)
		Continuation phase ≥ 2	Oral/inhaled (choose 2-3)[a] Azithromycin Clofazimine Omadacycline Linezolid or tedizolid Inhaled amikacin Bedaquiline		
Resistant	Susceptible or resistant		As above: treatment recommendations for macrolide-resistant *M abscesses* are the same regardless of the mechanism of macrolide resistance		
Resistant	Susceptible or resistant	Salvage therapy	Parenteral imipenem with ceftaroline or ceftaroline or ceftazidime; combine with best available oral/inhaled agents		Daily

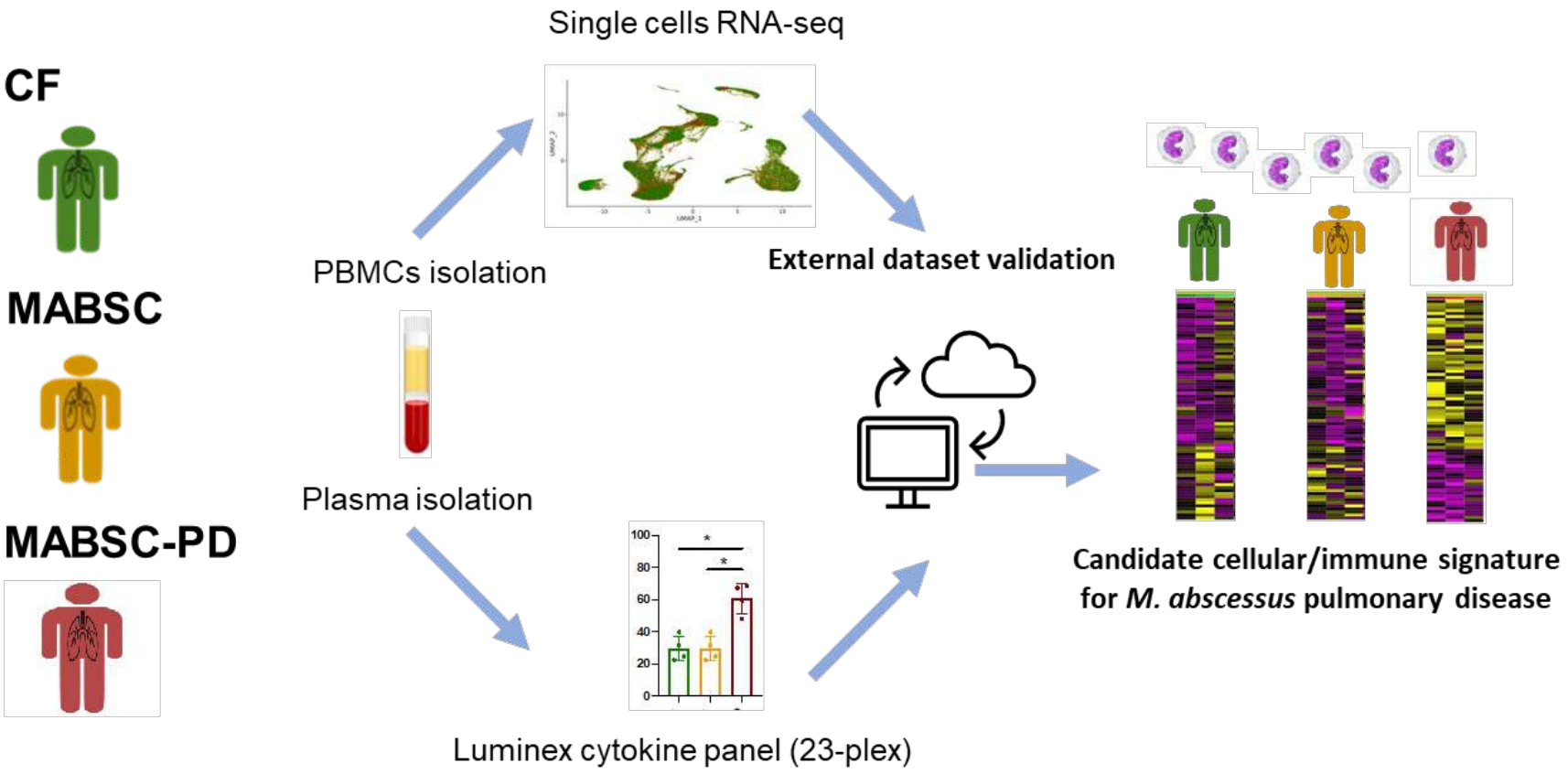
Recommended antibiotic doses are consistent with the 2020 nontuberculous mycobacteria guidelines[22] unless specifically noted.
[a]Preferred order of choice for parenteral drugs for amikacin-susceptible *M abscessus*: amikacin, imipenem, cefoxitin, and tigecycline.
[b]Optimal amikacin dosing has not been established. Details are provided in the 2020 Guidelines.[22] We recommend expert consultation for amikacin dosing guidance.
[c]Preferred order of choice for oral medications for macrolide-susceptible *M abscessus*: clofazimine, omadacycline, tedizolid, linezolid, and bedaquiline.
[d]Azithromycin is active and can be counted as one of the active drugs in the treatment regimen.
[e]Azithromycin is unlikely to be active and cannot be counted as one of the active drugs in the treatment regimen but can be given as an immune modulator.

NTM-PD Diagnosis Challenges

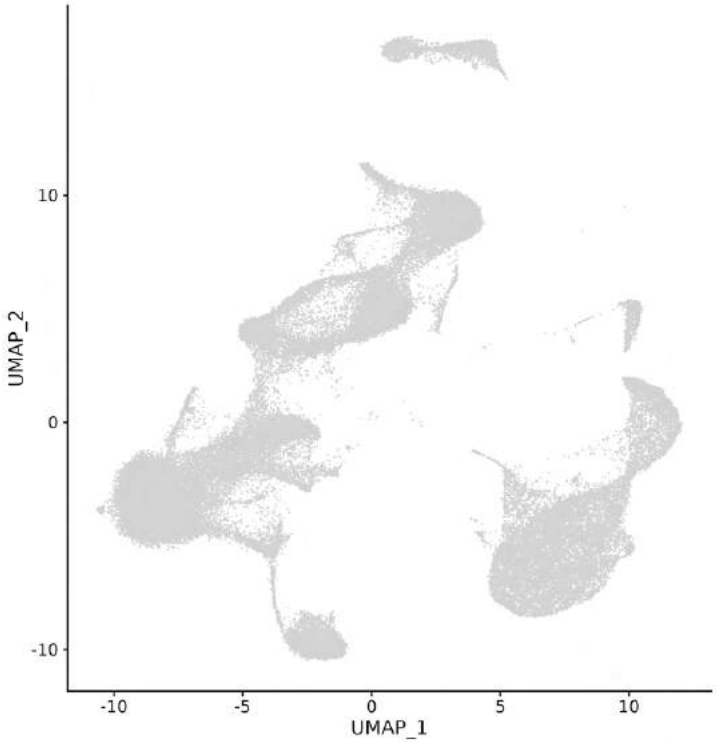


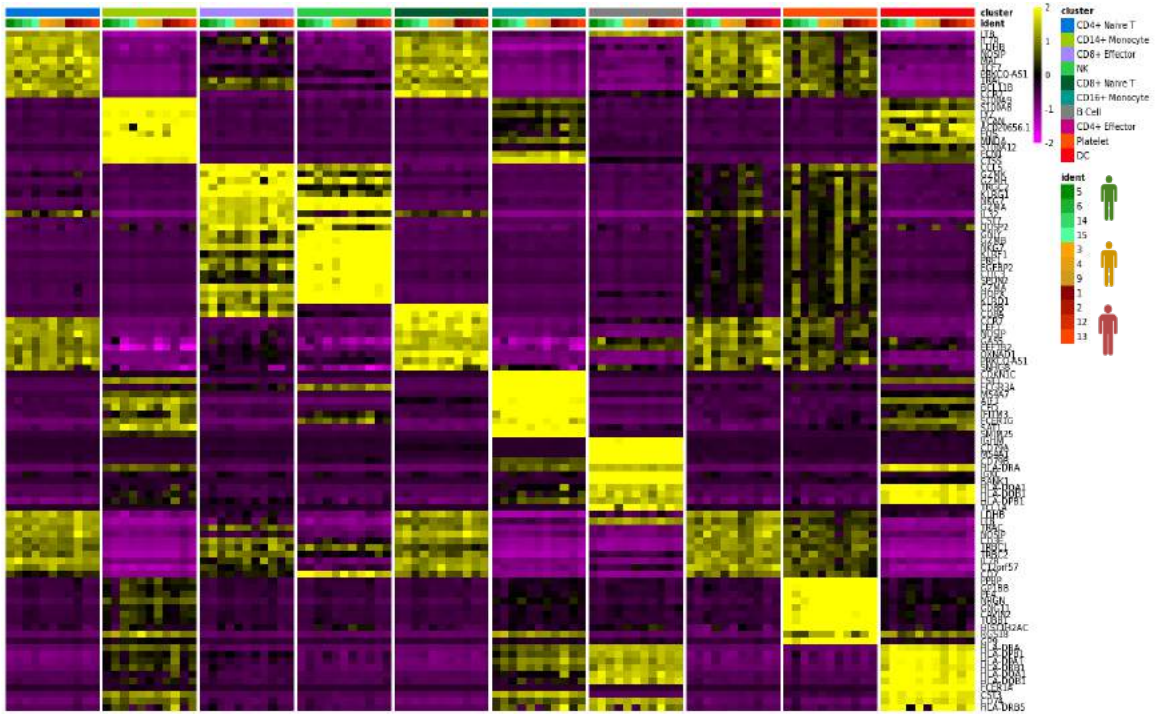
Rowe M.S. et al., N Engl J Med, 2005
Johansen MD et al, Nat. Rev., 2020
Daley C. et al ERJ 2020

Work flow

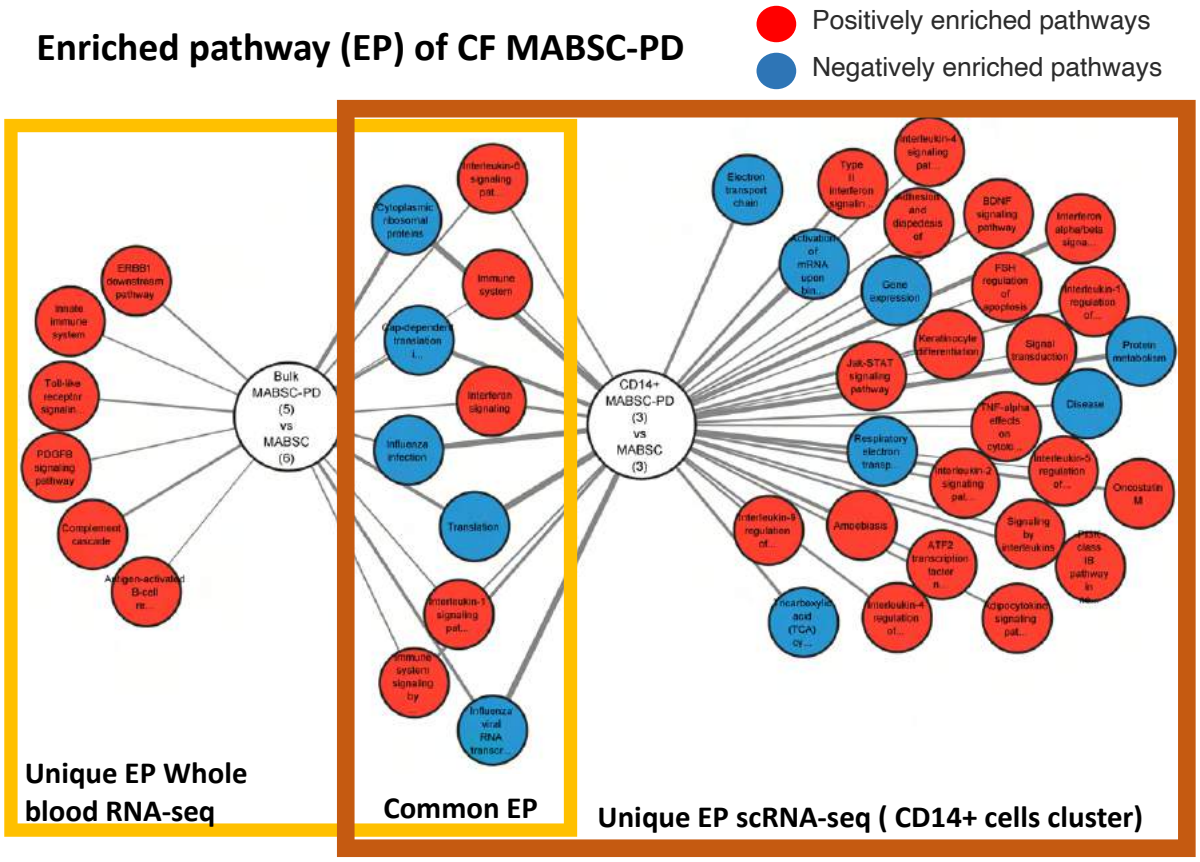


PBMC subpopulations





Validation of enriched pathways with bulk RNA-seq



Unpublished data - confidential

Conclusion

Cystic fibrosis and NTM polmonary disease

- CFTR loss-of-function alters the mucociliary clearance and predisposes patients to be colonized by opportunistic pathogens
- CFTR modulators are improving the lung health status in cystic fibrosis patients, although this cure is not available for al the patients
- Among CF pathogens, NTMs can cause asymptomatic colonization or promote sustained lung disease called NTM-PD
- Define biomarker to define better the NTM-PD is an unmet clinical and research to improve therapy and limits the progression NTM-PD
- scRNA seq allow us to determined the relevance of hyperinflammatory Monocytes (CD14+ Monocytic cells) in NTM-PD
- Through bioinformatic approaches and publicly available datasets we validated the relevance of hyperinflammatory Monocytes

Acknowledge

- **Emerging Bacterial Pathogens Unit** DITID- IRCCS Ospedale San Raffaele, Milan, Italy

Daniela Cirillo
Fabio Saliu
Francesca Nicola
Federico di Marco
Federica Vacca
Enrico Tortoli

THANK YOU!

Fondazione Ricerca Fibrosi Cistica - Onlus
italian cystic fibrosis research foundation

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FFC #7/2022
FFC #23/2020

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Milano, Monday April 15, 2024

Unimib

Corso laurea magistrale

Malattia Genetiche: dalla diagnosi alla malattia

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Università Vita-Salute San Raffaele, Milan, Italy

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