OSPEDALE
SAN RAFFAELE

Advancing therapies in Cystic Fibrosis : the role of preclinical research

Alessandra Bragonzi,
PhD | Patient Advocate

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San Raffaele Scientific Institute, Milano, Italy

Università Bicocca, 21 maggio, 2026

ICF
Infections and Cystic Fibrosis

My professional journey

ALESSANDRA BRAGONZI

PROFILE OVERVIEW

As a scientist: 25+ years of experience in translational research, managing preclinical studies in Infection and Cystic Fibrosis.

As a patient advocate: I am passionate about bringing scientists, patients, and stakeholders together to create meaningful change. I focus on projects that promote Patient Engagement in Research.

EDUCATION

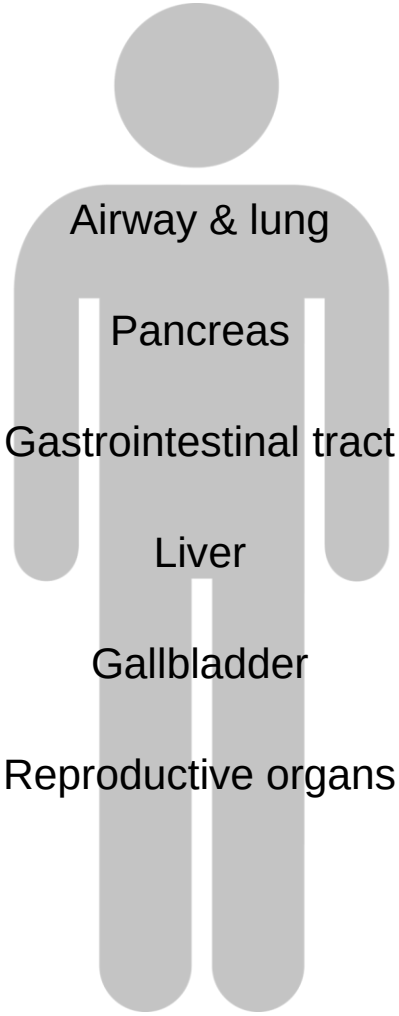
- Bachelor Biological Sciences, University of Milano, Milano, Italy
- Ph.D. in Natural Sciences**,** Eberhard Karls university of Tübingen, Germany
- Master in International Patient Advocacy, Catholic University, Italy
- European Patients' Academy on Therapeutic Innovation (EUPATI) patient expert

AREAS OF EXPERTISE

- Translational Research
- Preclinical Study Management
- Education and mentoring
- Patient Advocacy

I love to help others to share their voice and get involved...

Cystic Fibrosis: a lethal genetic disease



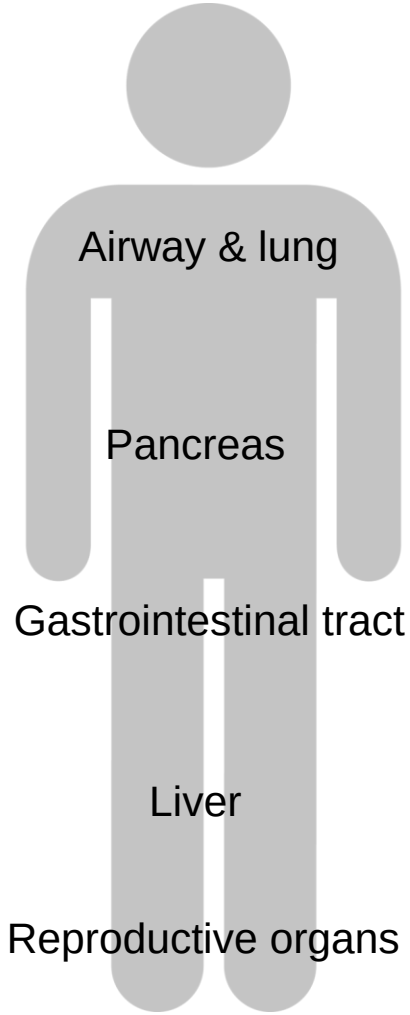
2,000 CFTR known mutations
ΔF508 most common mutation
1:25 carriers

1:2,500 live births

100,000 CF patients worldwide

Life expectancy about **46 years**

Cystic Fibrosis: a multi-organ disease



- Bronchiectasis
- Pneumonia
- Mucoid obstruction of bronchi
- Excessive inflammation
- Respiratory failure

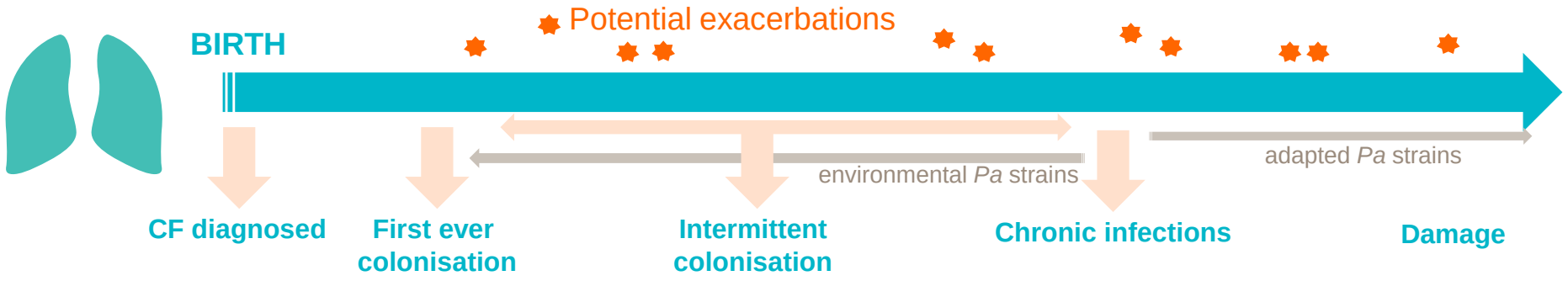
- Pancreatitis
- Insulin deficiency
- Symptomatic hyperglycemia
- Diabetes

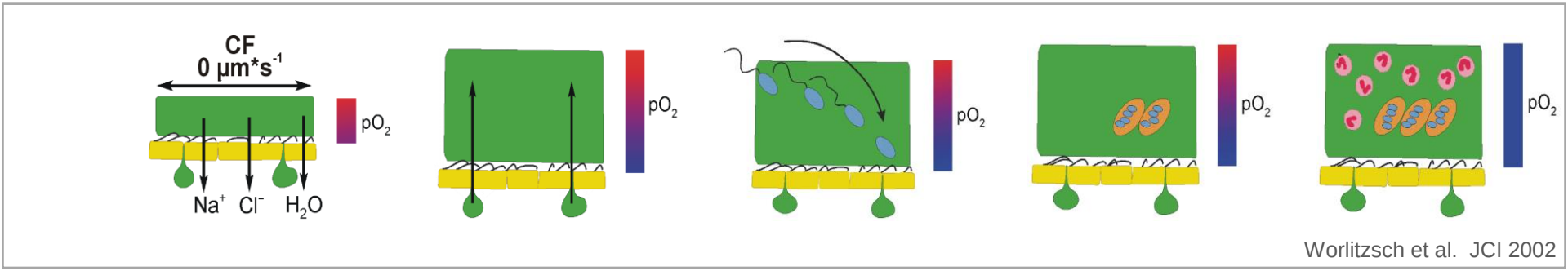
- Defect in gastric acid secretion.
- Significant lower net fluid secretion (gastric juice) and higher bilirubin reflux.
- Meconium ileus/peritonitis
- Distal intestinal obstruction syndrome

- Hepatic stenosis
- Portal hypertention

- Infertility
- Mucoid obstruction of vas deferens in newborn
- Absence of vas deferences in adult

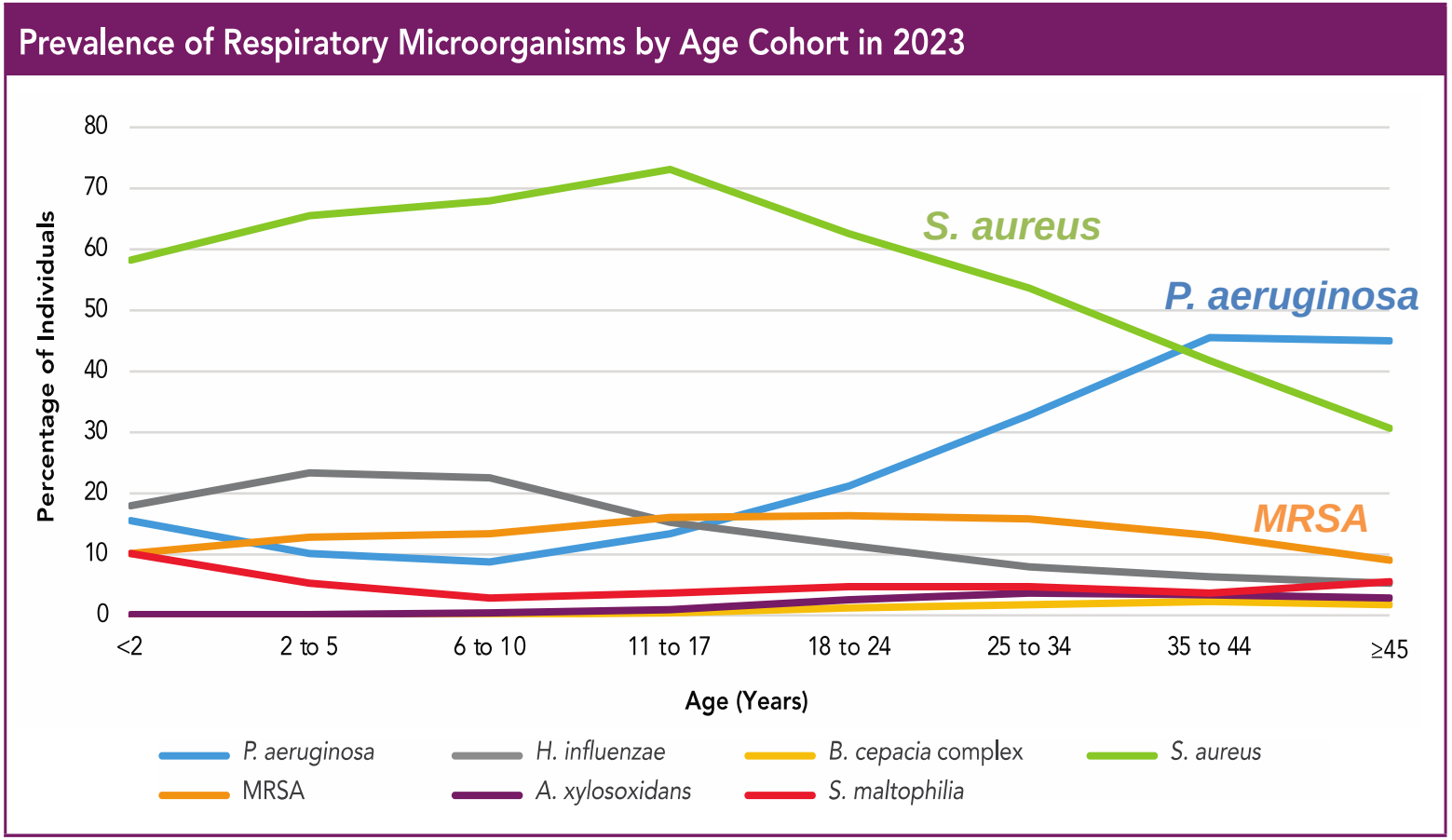
Timeline of pulmonary pathology in pwCF





Worlitzsch et al. JCI 2002

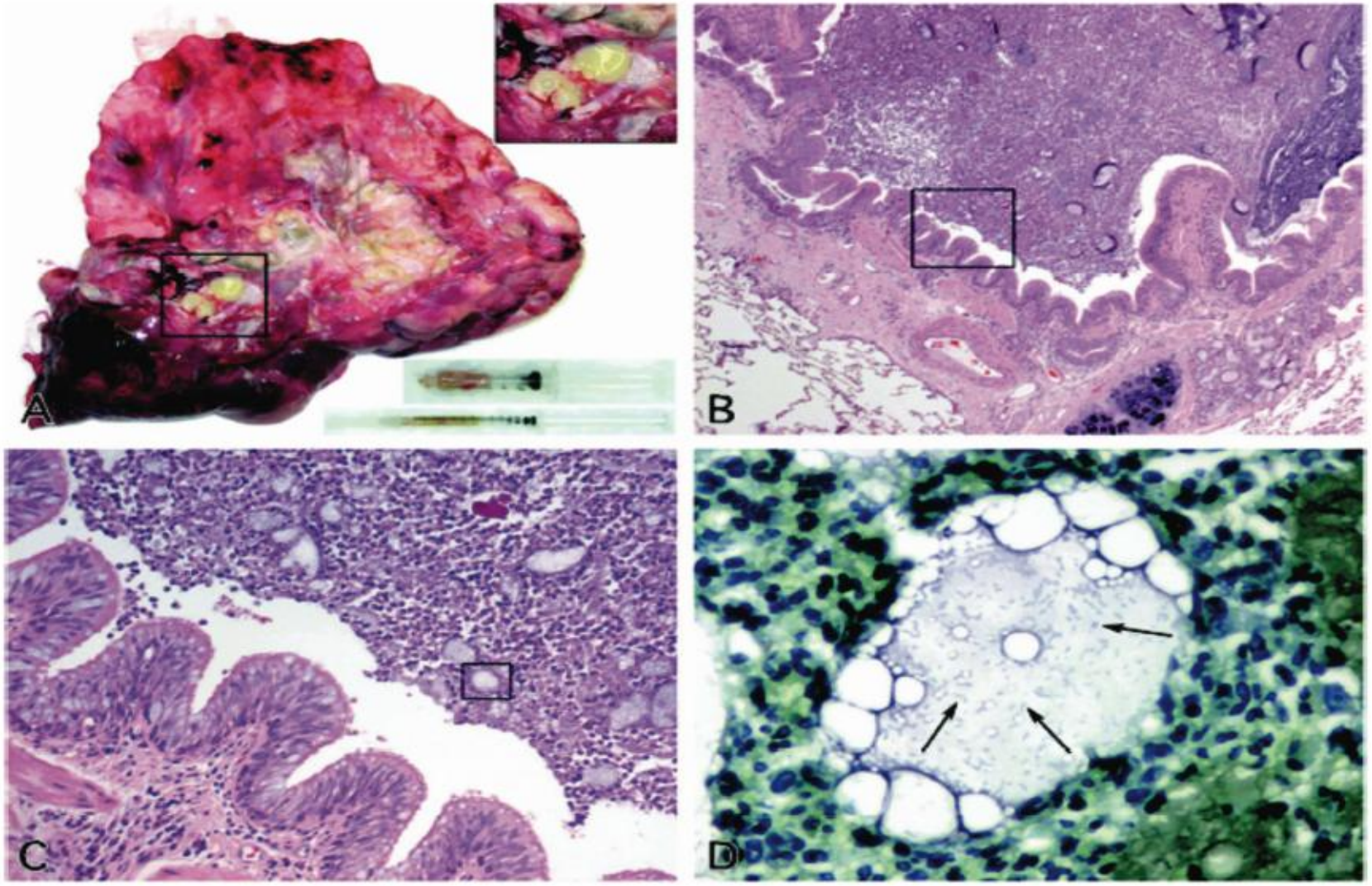
The Players:
the bacteria identified in the clinical lab



CF Foundation Patient Registry 2023

These are the most common “standard pathogens“,
we focus on the diagnosis and treatment

Pulmonary pathology in pwCF



Livraghi and Randell, Toxicology Pathology, 2007

The CF pathogenesis cascade & current therapies

Defective CF gene

Deficient CFTR Protein

Abnormal Chloride Permeability
Altered Ionic Transport

Act here to rescue the basic CF defect and block the cascade

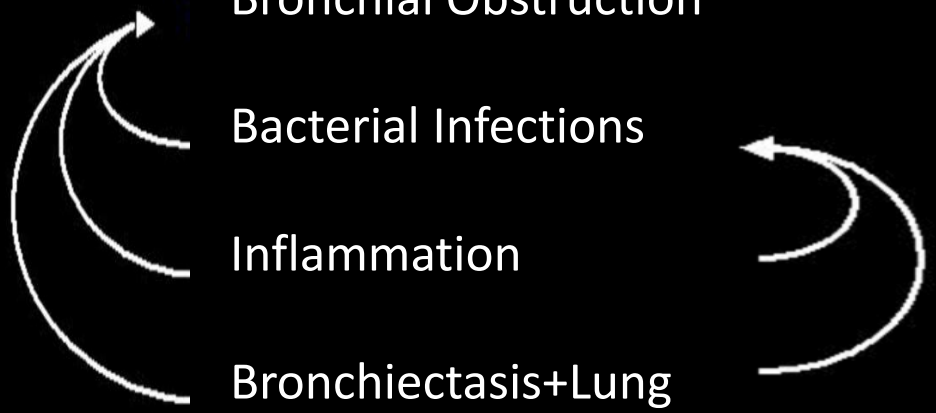
Decreased Water in ASL
Abnormal Mucus Composition

Bronchial Obstruction

Bacterial Infections

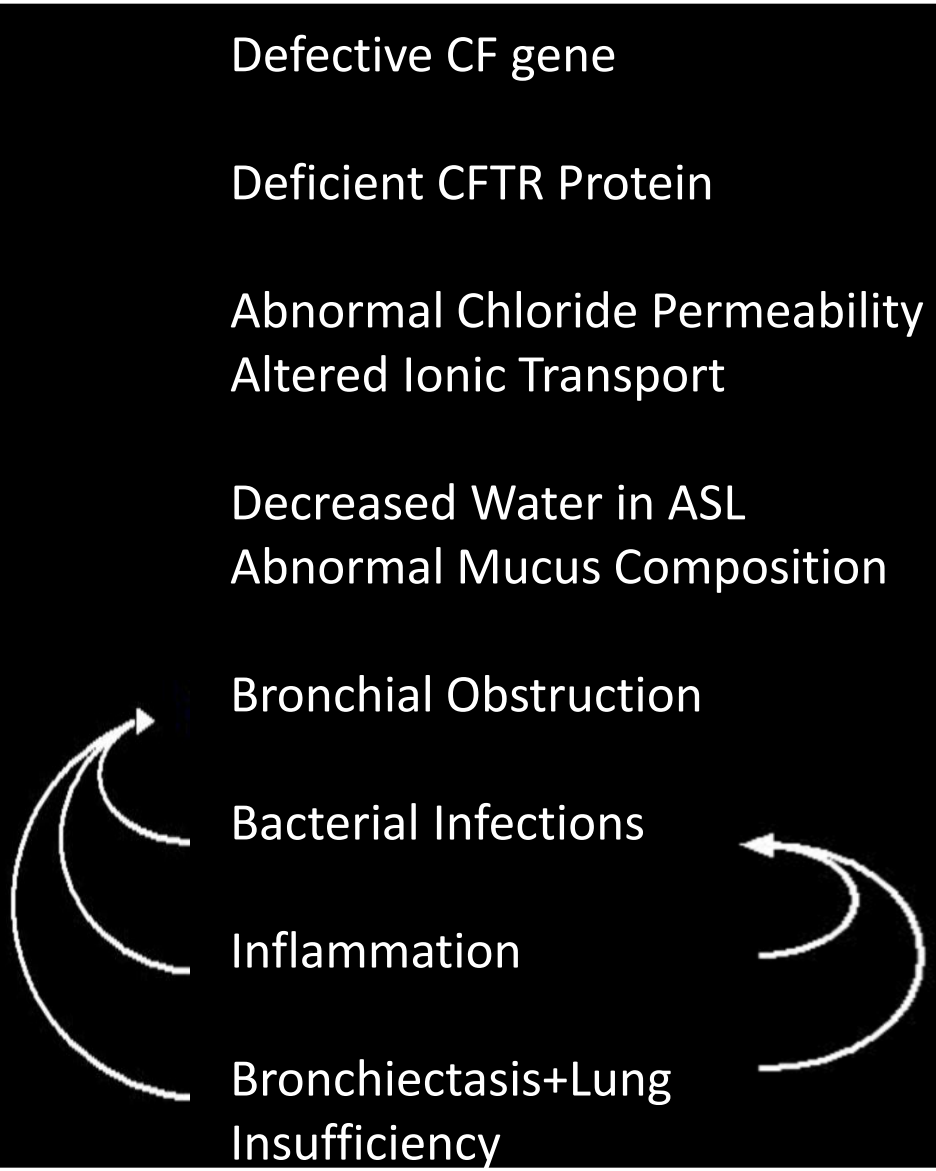
Inflammation

Bronchiectasis+Lung Insufficiency



Most current therapy in CF

The CF pathogenesis cascade & current therapies



Gene Therapy

↓

Activation of mutant CFTR

↓

Cl channel activators
Na channel blockers

↓

Airway clearance Rx
Bronchodilators, mucolytics

↓

Antibiotics

↓

Anti-inflammatory agents,
Anti-oxidants, anti-proteases

↓

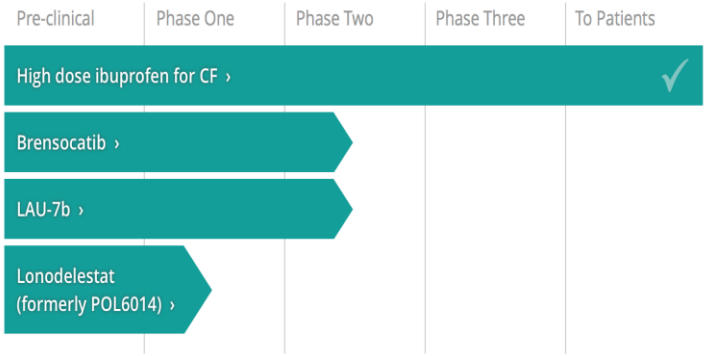
Lung transplant

Drug development pipeline

Attacking CF from every angle

Anti-infectives

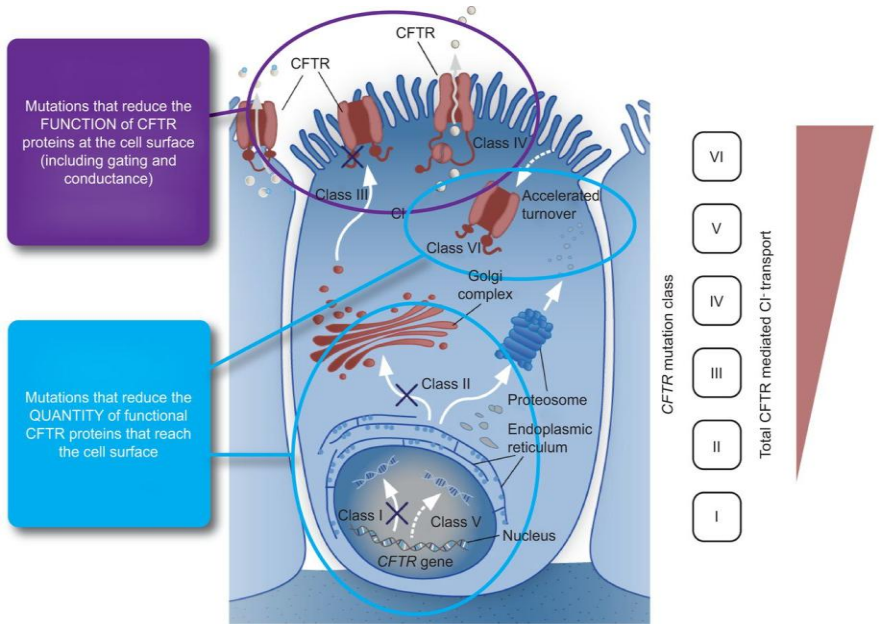
Anti-inflammatory



CYSTIC FIBROSIS FOUNDATION
ADDING TOMORROWS

CFTR-modulator therapy

A major breakthrough to address defect in CF

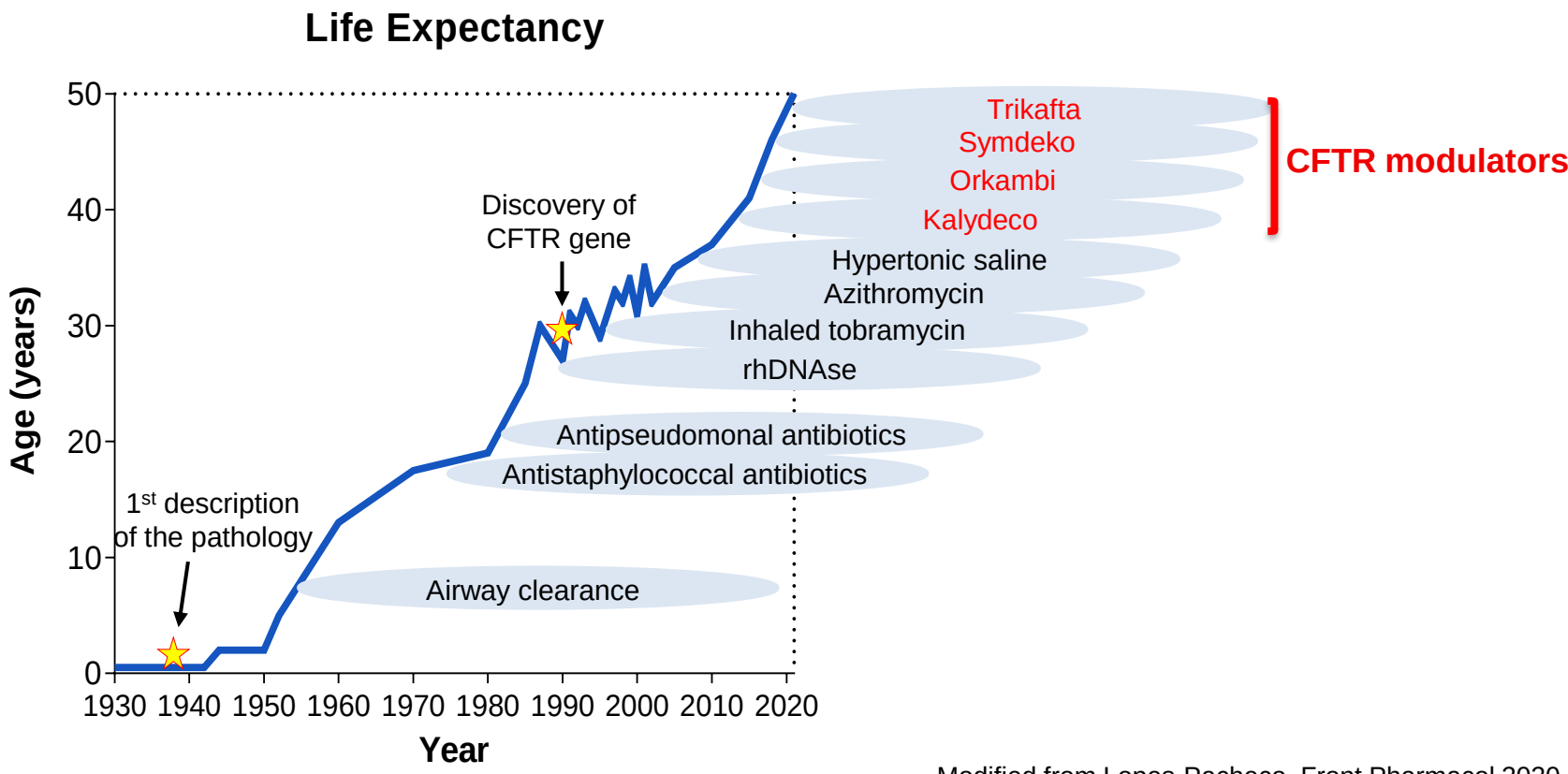


Restore CFTR function

Pre-clinical	Phase One	Phase Two	Phase Three	To Patients
Elexacaftor + tezacaftor + ivacaftor (Trikafta®) ›				✓
Ivacaftor (Kalydeco®) ›				✓
Lumacaftor + ivacaftor (Orkambi®) ›				✓
Tezacaftor + ivacaftor (Symdeko®) ›				✓
VX-121 + tezacaftor + VX-561 ›				
ABBV-2222 + ABBV-3067 ›				
ELX-02 ›				
VX-561 (deutivacaftor) ›				
4D-710 ›				
Arcturus Therapeutics ›				
Icagen, Inc. ›				
Pioneering Medicines ›				
Reata Pharmaceuticals ›				
ReCode Therapeutics ›				
SalioGen Therapeutics ›				
Sionna Therapeutics ›				
Southern Research Institutes ›				
Spirovant Sciences ›				
SpliSense ›				

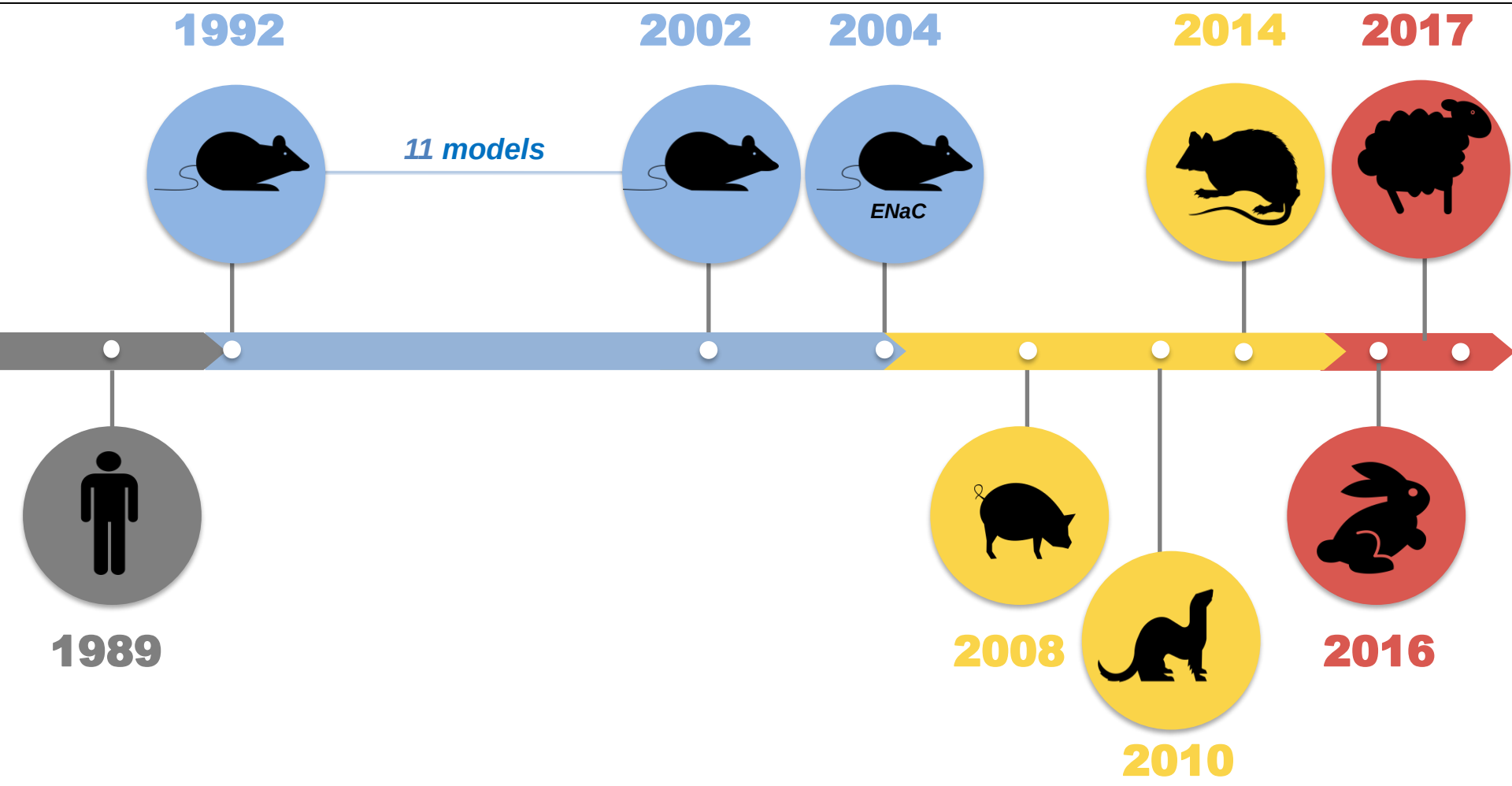
CYSTIC FIBROSIS FOUNDATION
ADDING TOMORROWS

Cystic Fibrosis: therapies



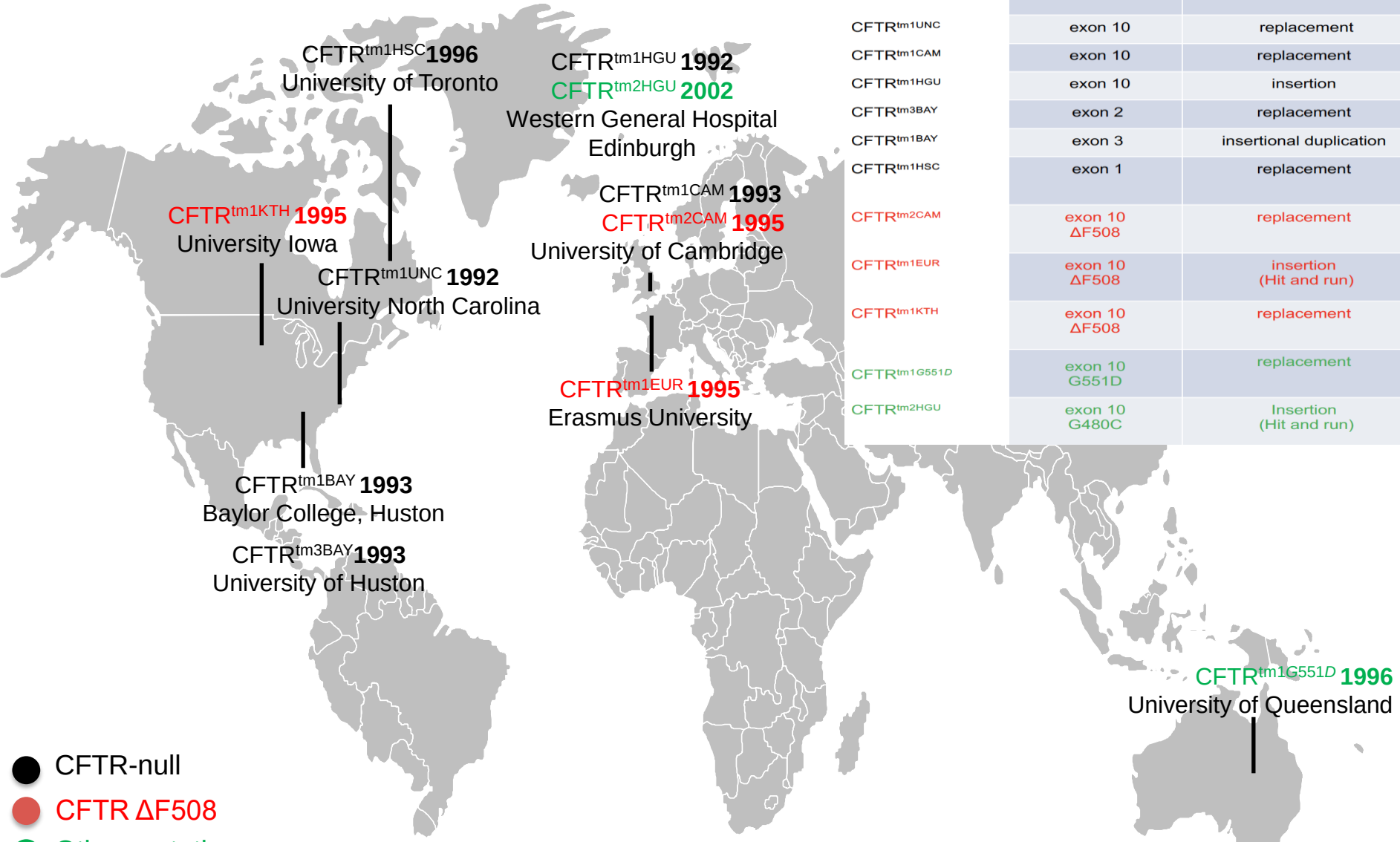
Modified from Lopes-Pacheco, Front Pharmacol 2020

Animal models of Cystic Fibrosis



CF benefits from the direct engineering of six different species

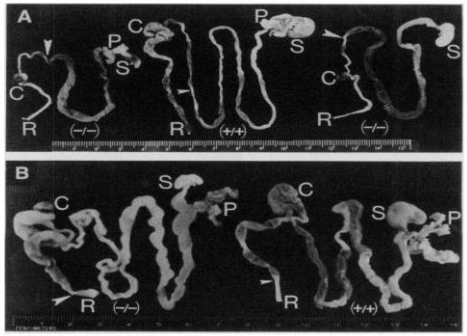
Cohorts of CF mice



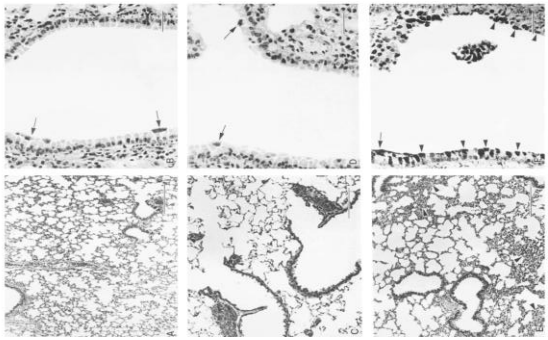
MOUSE	GENOMIC SITE	MUTATION
CFTR^{tm1UNC}	exon 10	replacement
CFTR^{tm1CAM}	exon 10	replacement
CFTR^{tm1HGU}	exon 10	insertion
CFTR^{tm3BAY}	exon 2	replacement
CFTR^{tm1BAY}	exon 3	insertional duplication
CFTR^{tm1HSC}	exon 1	replacement
CFTR^{tm2CAM}	exon 10 ΔF508	replacement
CFTR^{tm1EUR}	exon 10 ΔF508	insertion (Hit and run)
CFTR^{tm1KTH}	exon 10 ΔF508	replacement
CFTR^{tm1G551D}	exon 10 G551D	replacement
CFTR^{tm2HGU}	exon 10 G480C	Insertion (Hit and run)

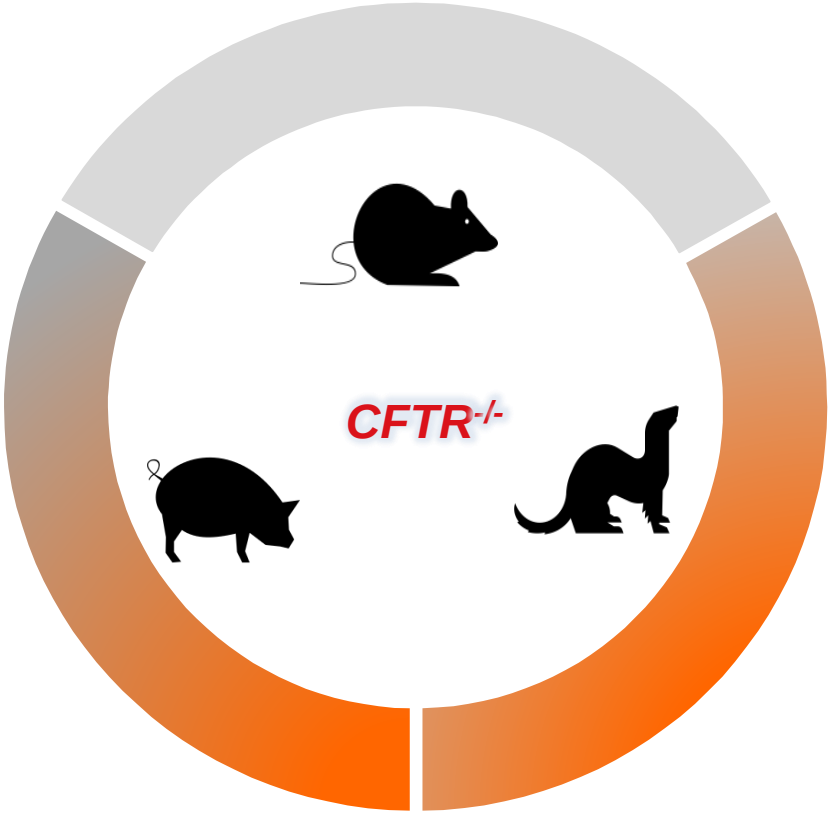
Major concerns with CF animal models

Absence of CF-like lung disease and other disease manifestations



Severe intestinal obstruction
Absence of lung phenotype





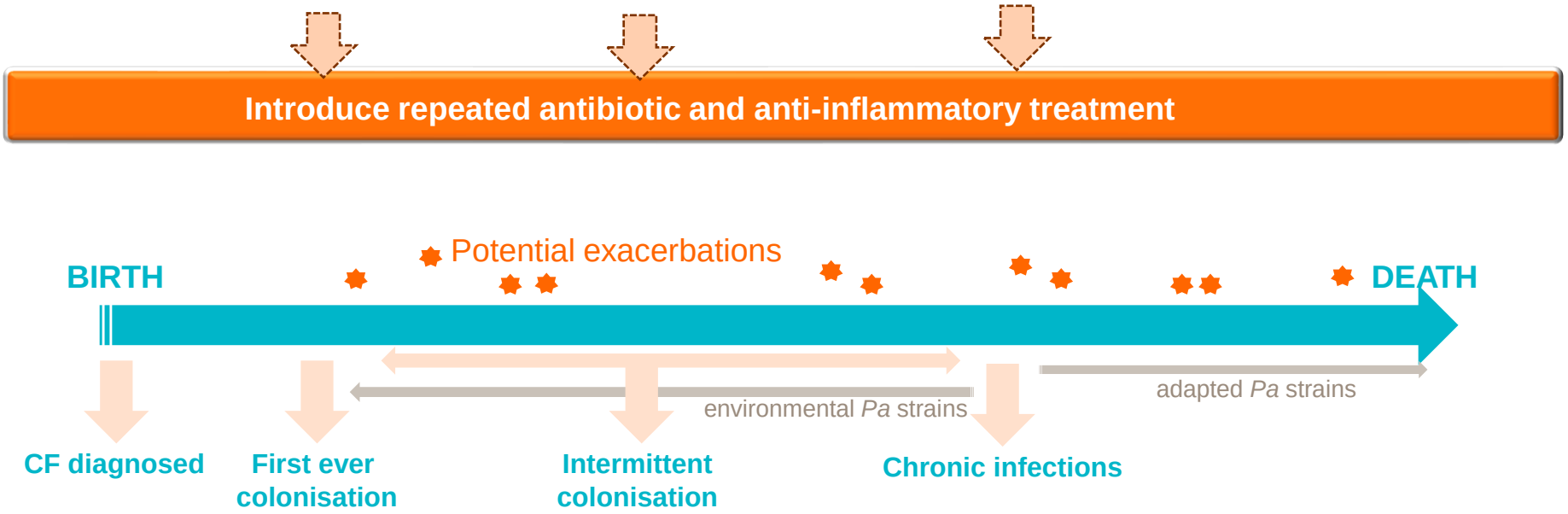
Severe meconium ileus and malabsorption
Moderate mucus-obstruction with high variability
Absence of *P. aeruginosa* infection

Unmet clinical needs in Cystic Fibrosis

- New and better antimicrobials and anti-inflammatory therapies
  - Identify better therapeutic targets

- Develop more reliable and pre-clinical informative animal models to test efficacy of new therapies
  - There are no clear guidelines for efficacy testing
  - There is failure of clinical phases

Natural history of *Pseudomonas aeruginosa* infection and response to antibiotic and anti-inflammatory treatment in cystic fibrosis lung



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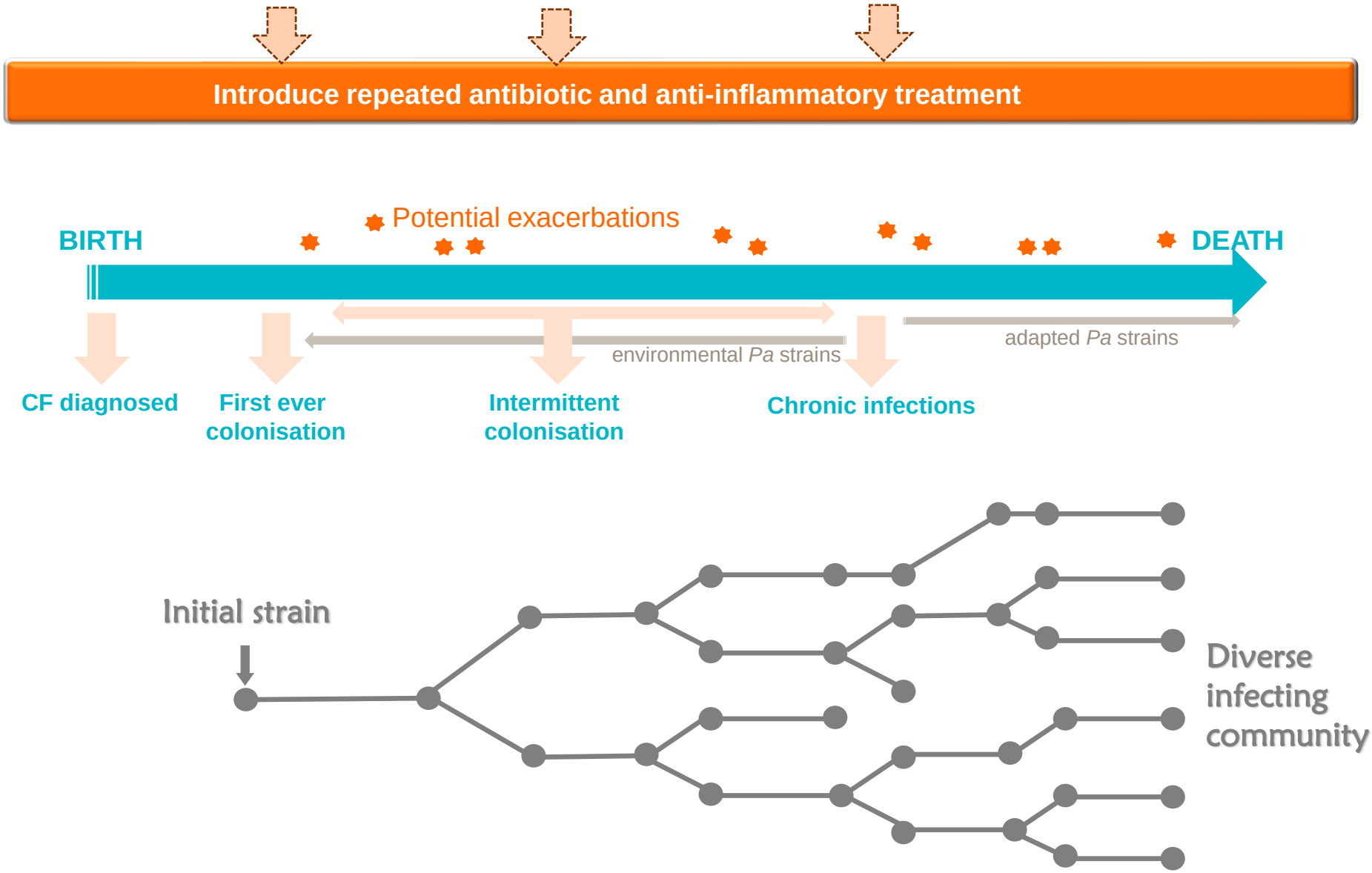


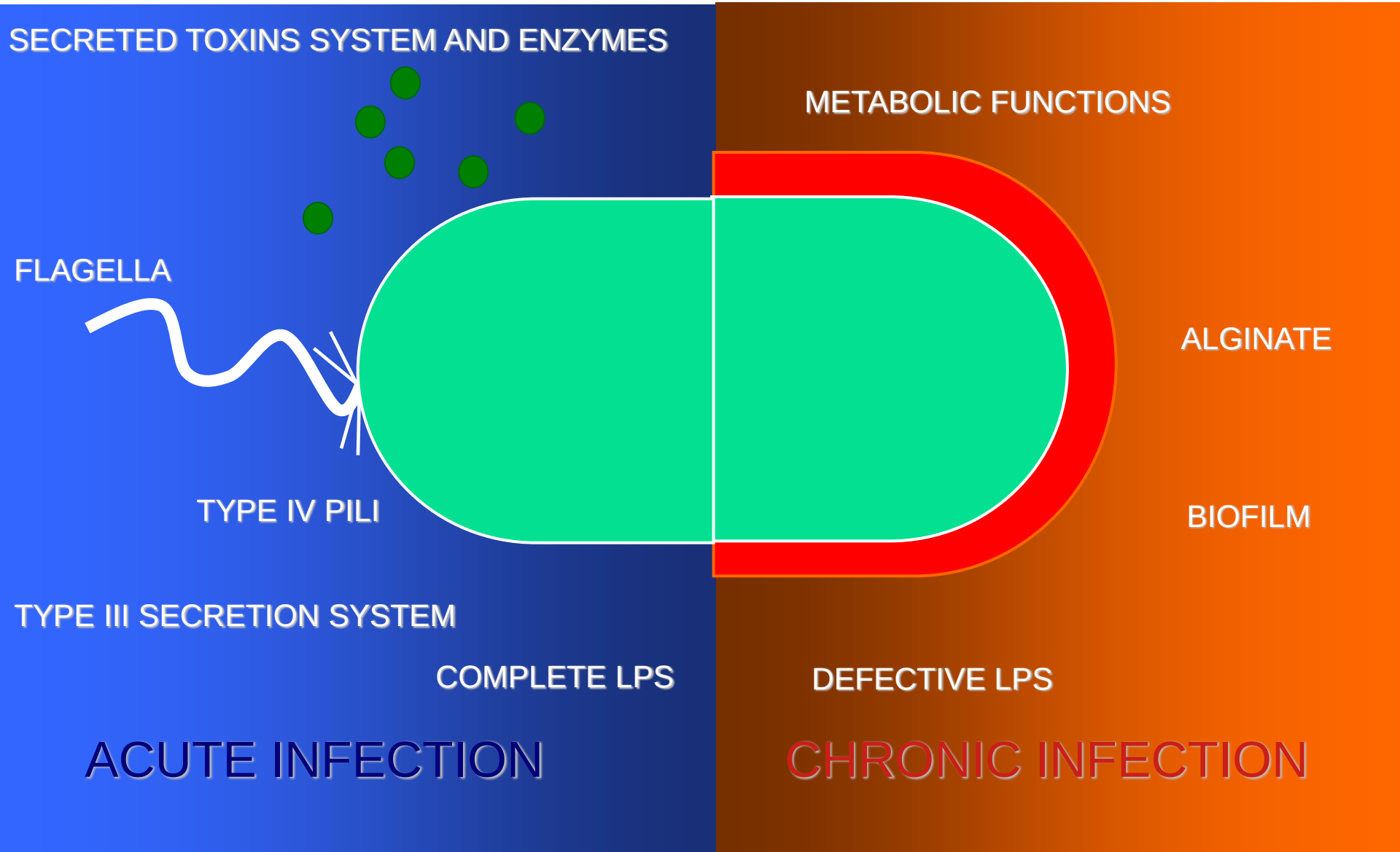
What have we learned about CF infections?

Nguyen & Singh, PNAS, 2006

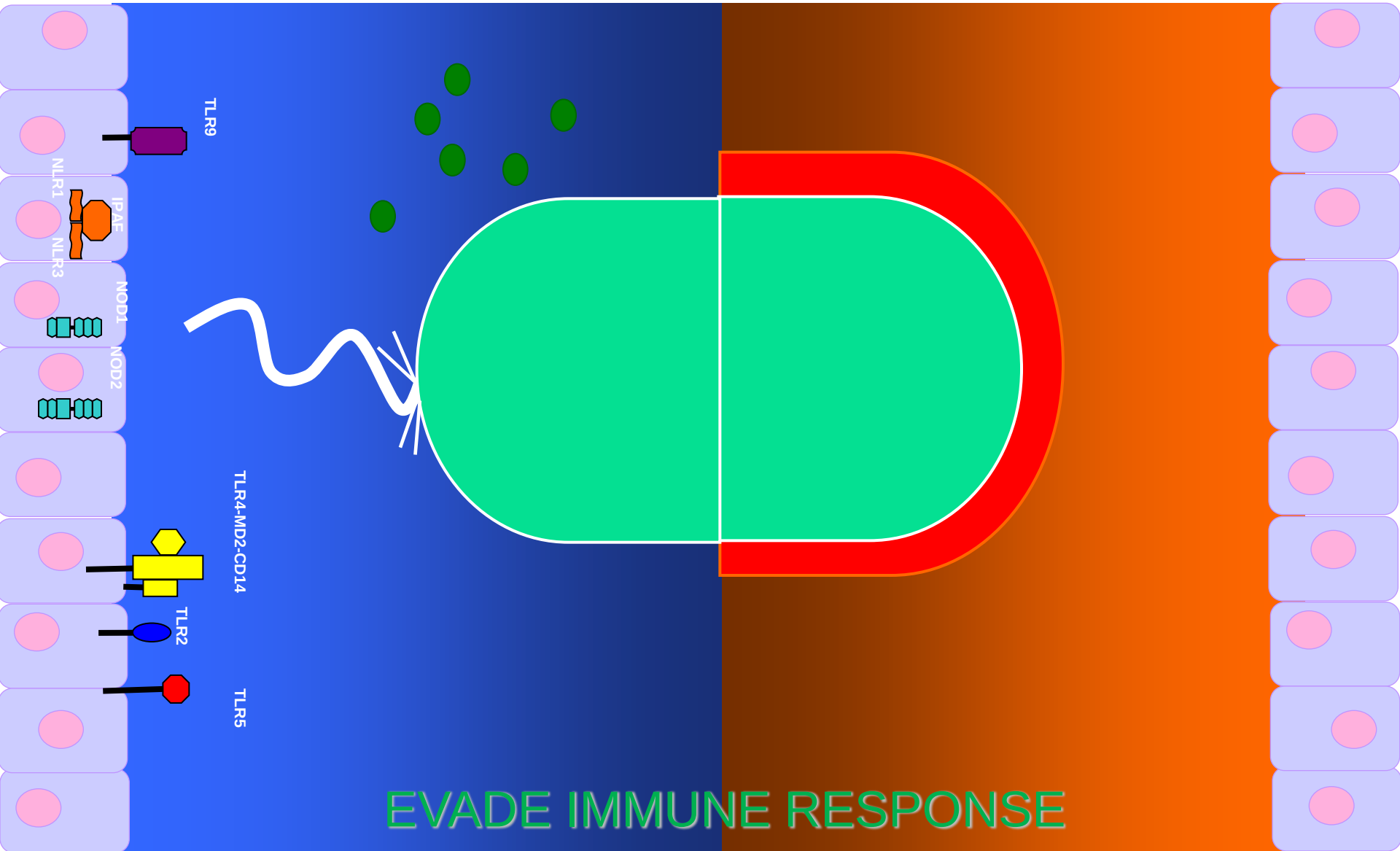
- Mucus
- The immune system
- Differences in oxygen and nutrients
- Antibiotics and other treatment
- Other microbes

Chronic infection is established through adaptation

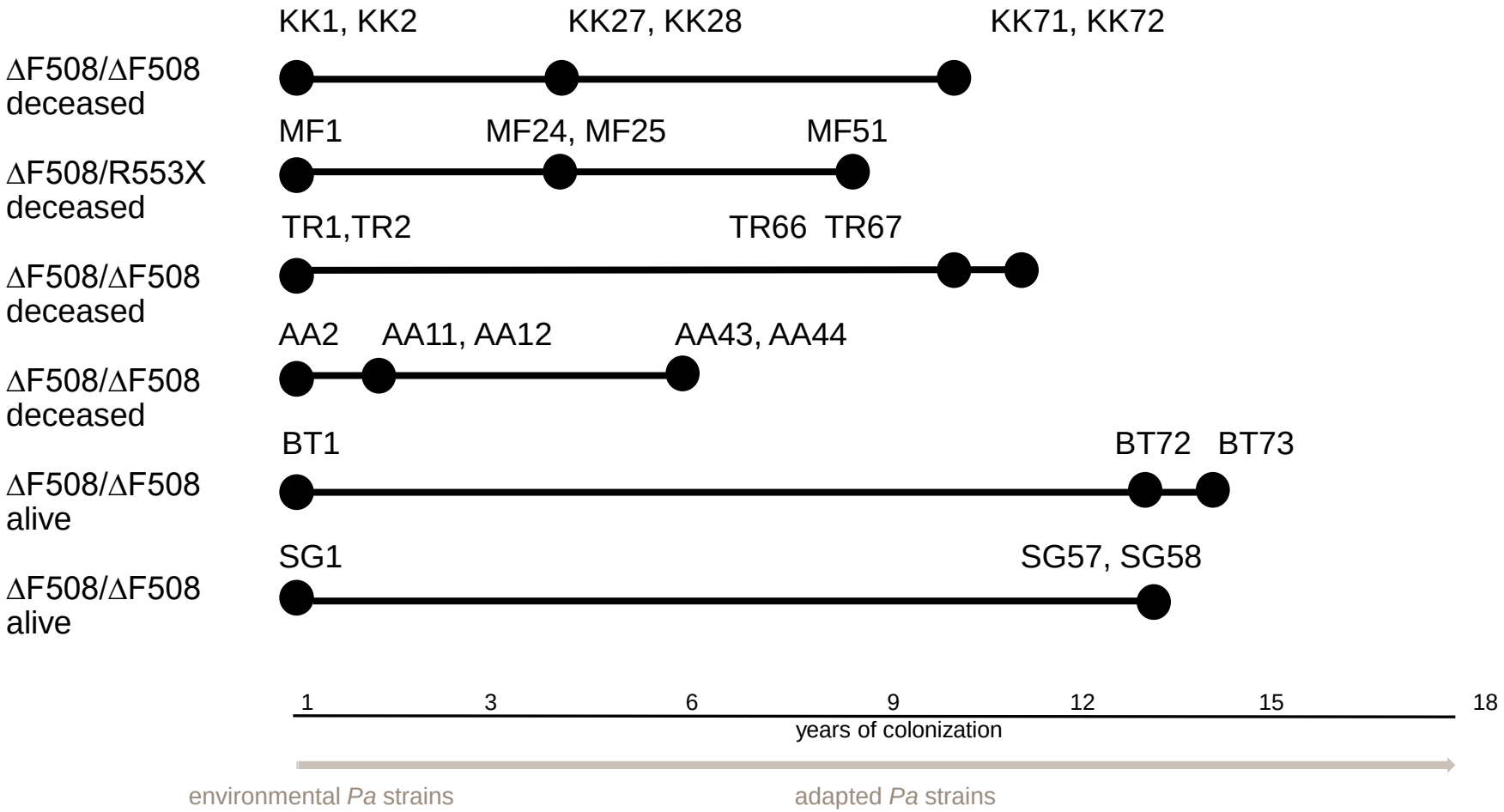
Pseudomonsa aeruginosa adaptation to the lungs of pwCF



Impact of the *Pseudomonsa aeruginosa* adaptation on the host response

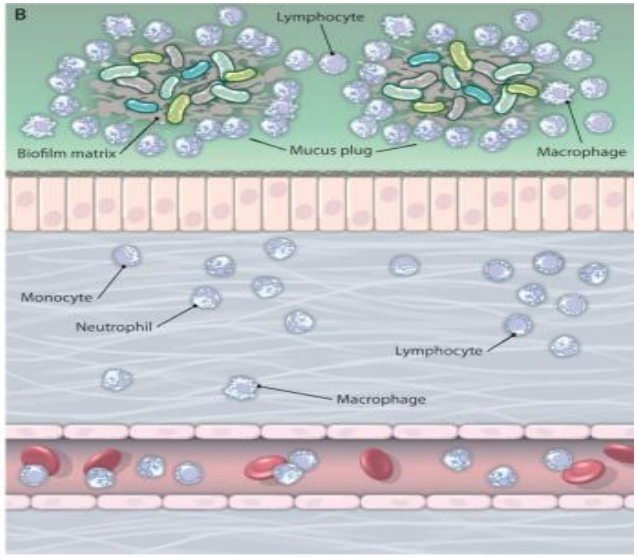


Selection of sequential *P. aeruginosa* strains from CF patients



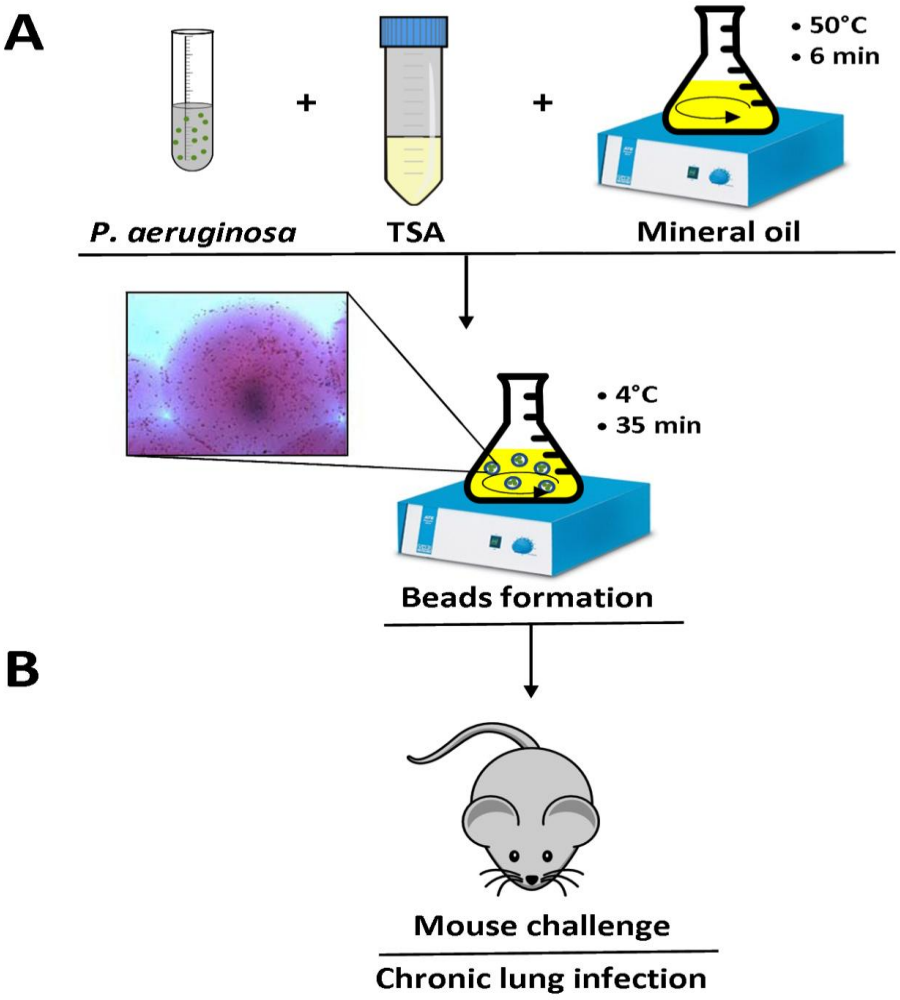
In collaboration with Burkhard Tummler

Chronic airways infection in pre-clinical mouse model



CHRONIC INFECTION

Biofilm like structure with bacterial genetic variants
Localized infection in single organ
Weeks or years

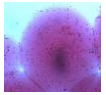


Bragonzi, Sci. Transl. Med. 2010

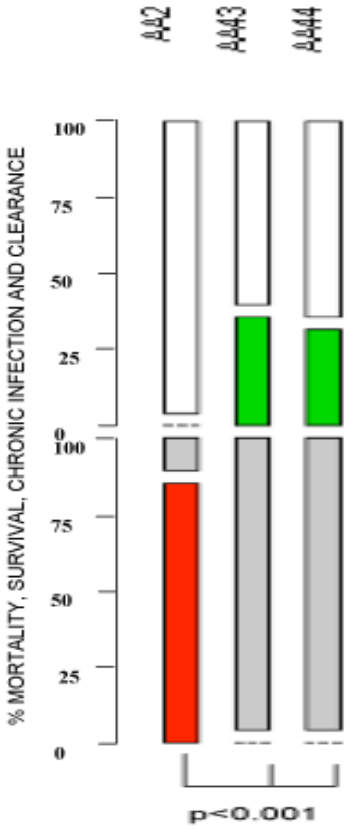
Chronic persistence is influenced by the *P. aeruginosa* strain and mouse model of infection

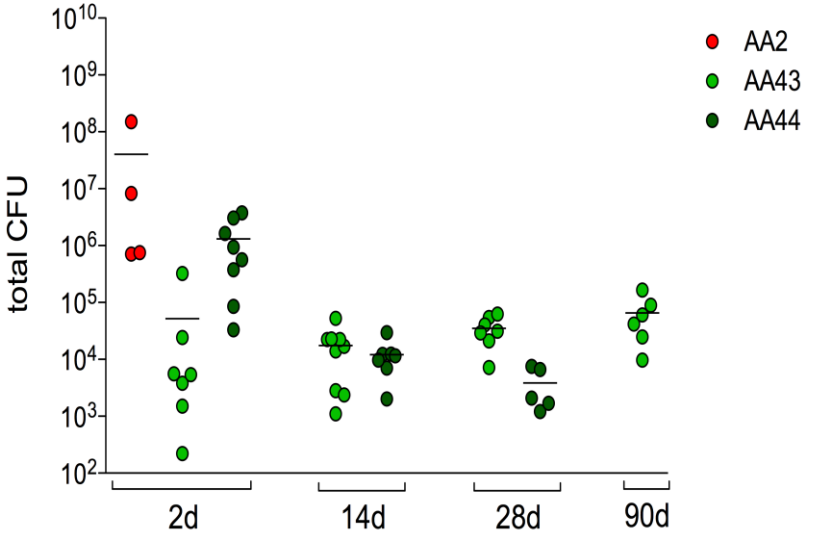
AA2 — AA43, AA44

Chronic infection

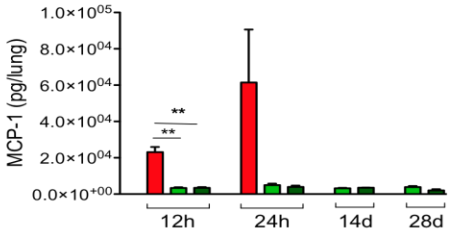


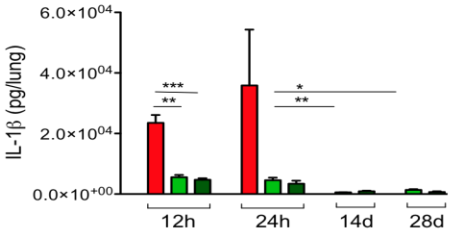
ΔF508/ΔF508
deceased

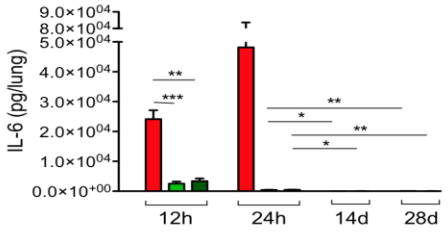


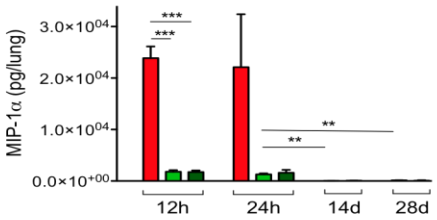


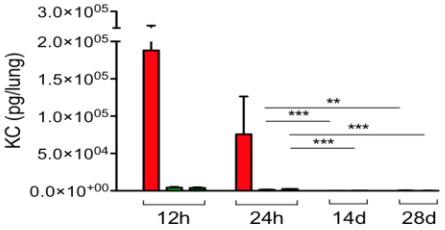
Host response is influenced by the *P. aeruginosa* strain and mouse model of infection

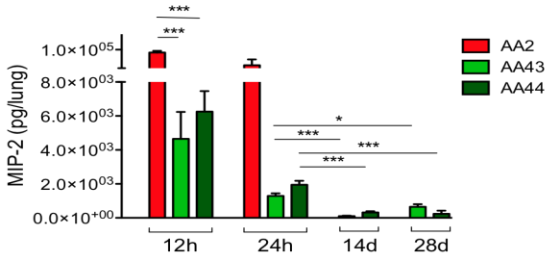


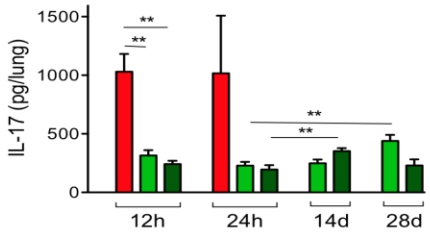


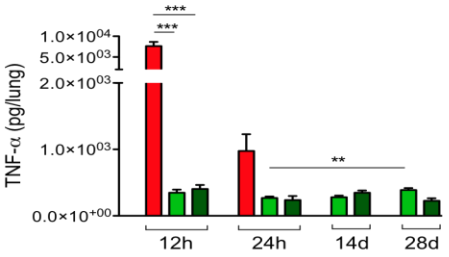


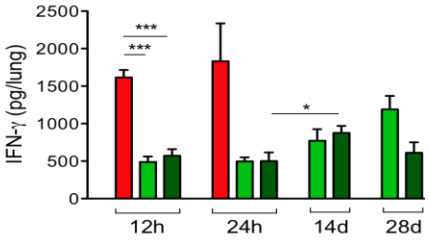




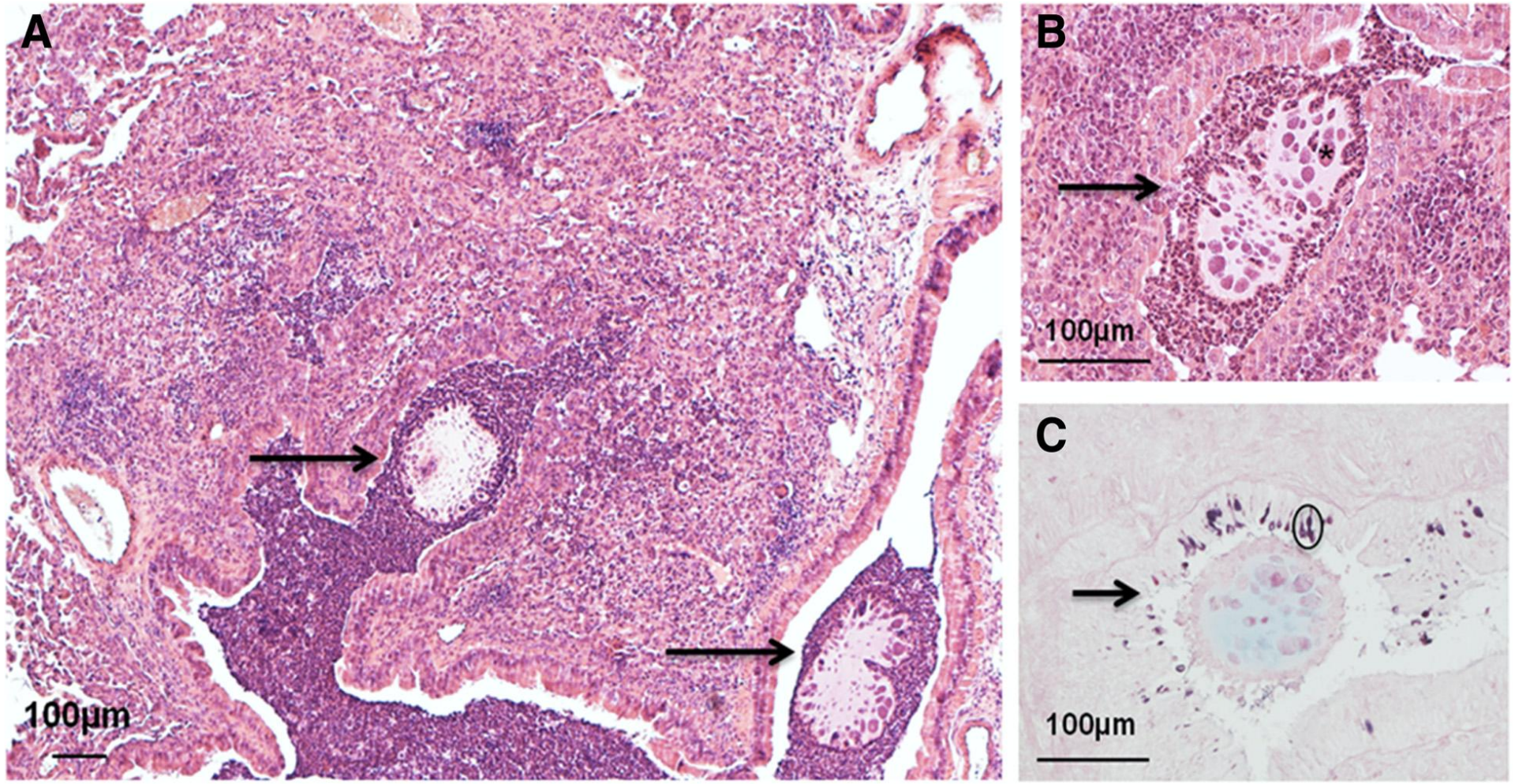








Lung pathology
after long-term chronic *P. aeruginosa* infection



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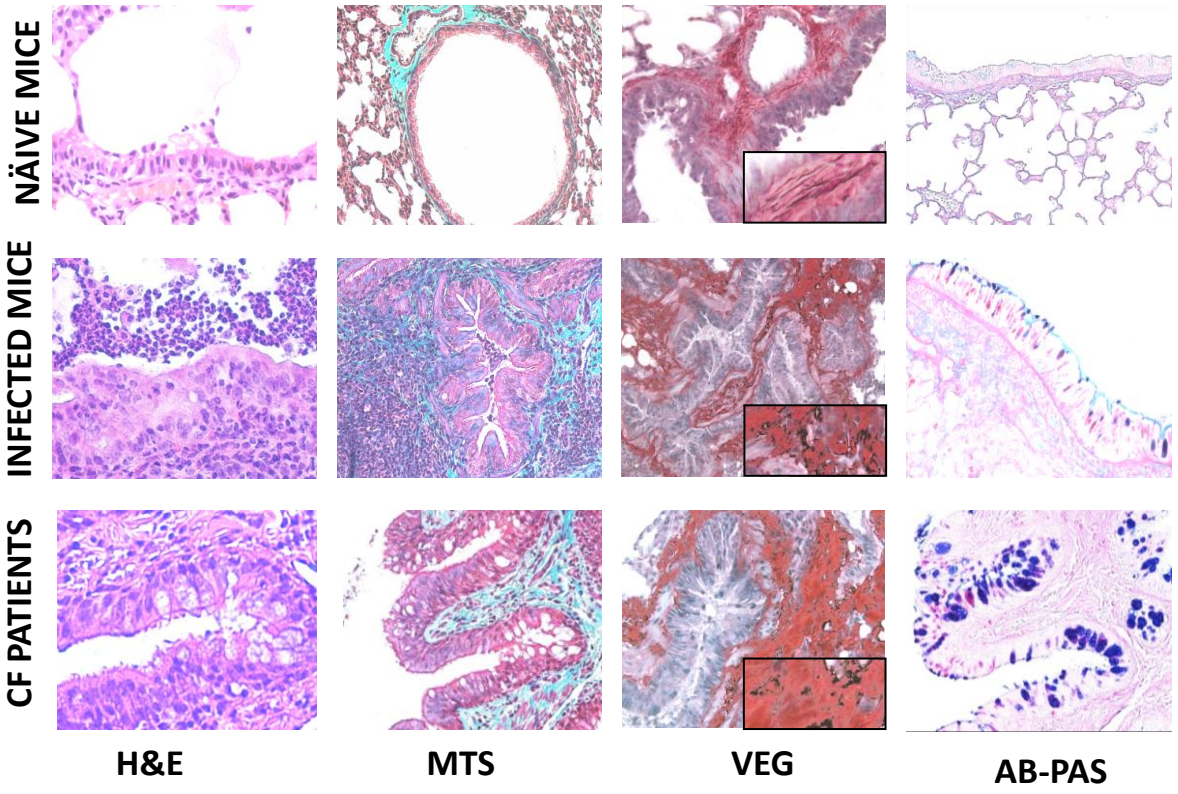
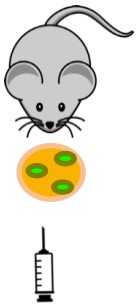
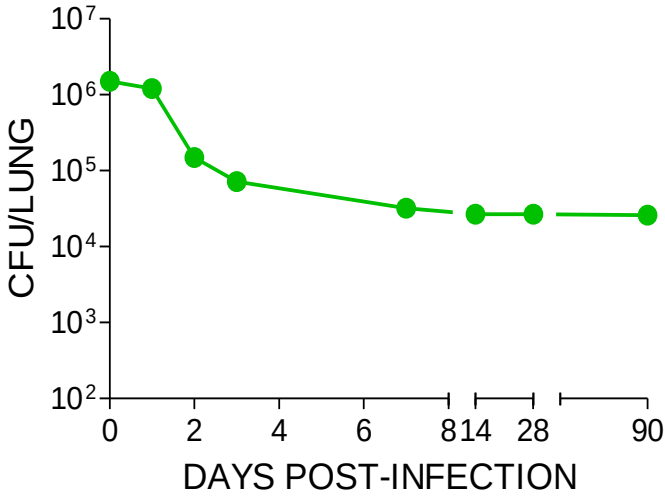




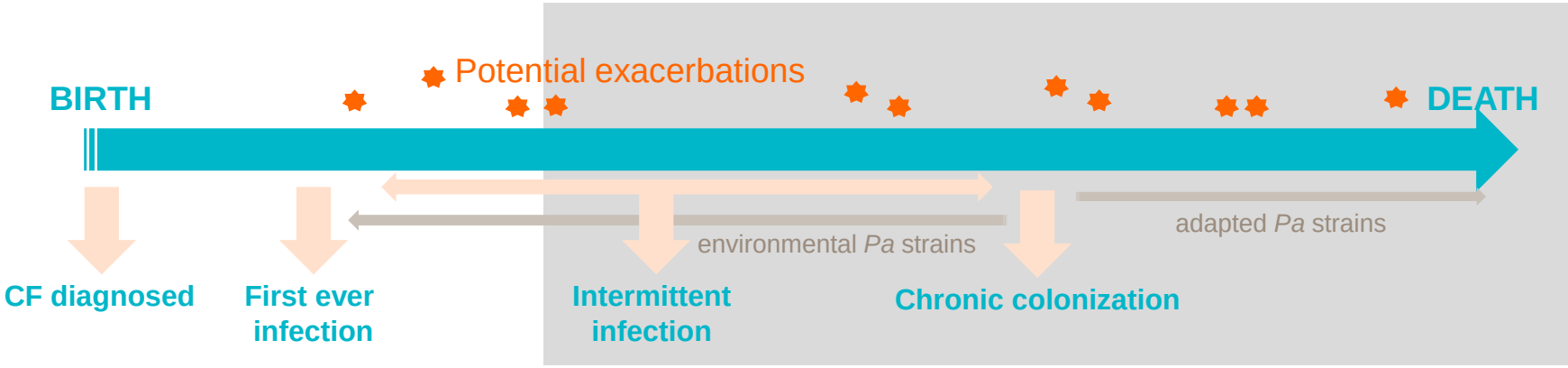


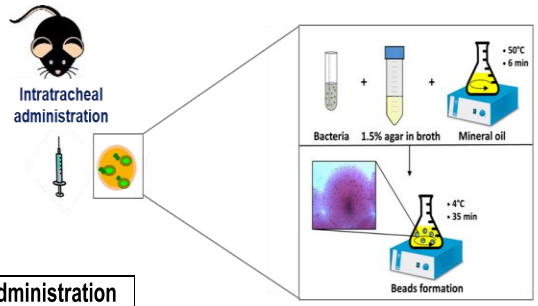
- stable infection and inflammation
- epithelial hyperplasia and structure degeneration
- goblet cells metaplasia

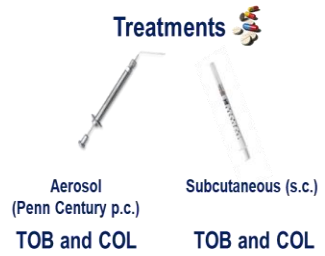
Can murine models of respiratory infection predict clinical efficacy in humans?

- How different administration routes – local vs parenteral - impact on host response and pathogen clearance
- How different schedules of treatment – soon after infection vs during chronic colonization – affect efficacy

Mouse model of chronic infection and schedule of early and late treatment

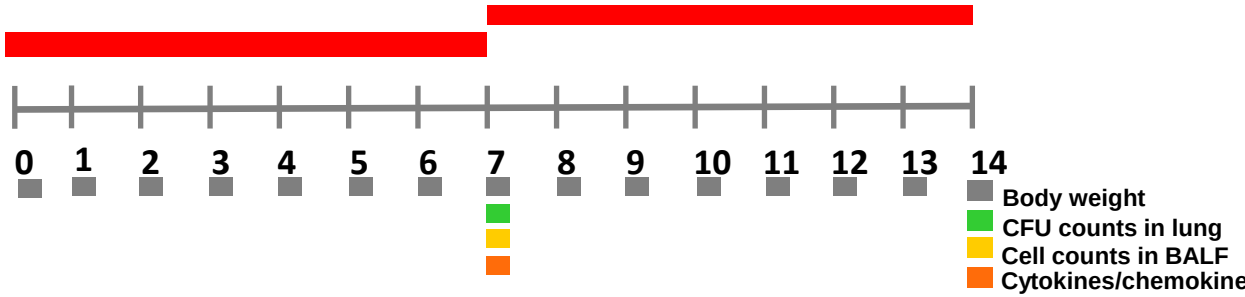




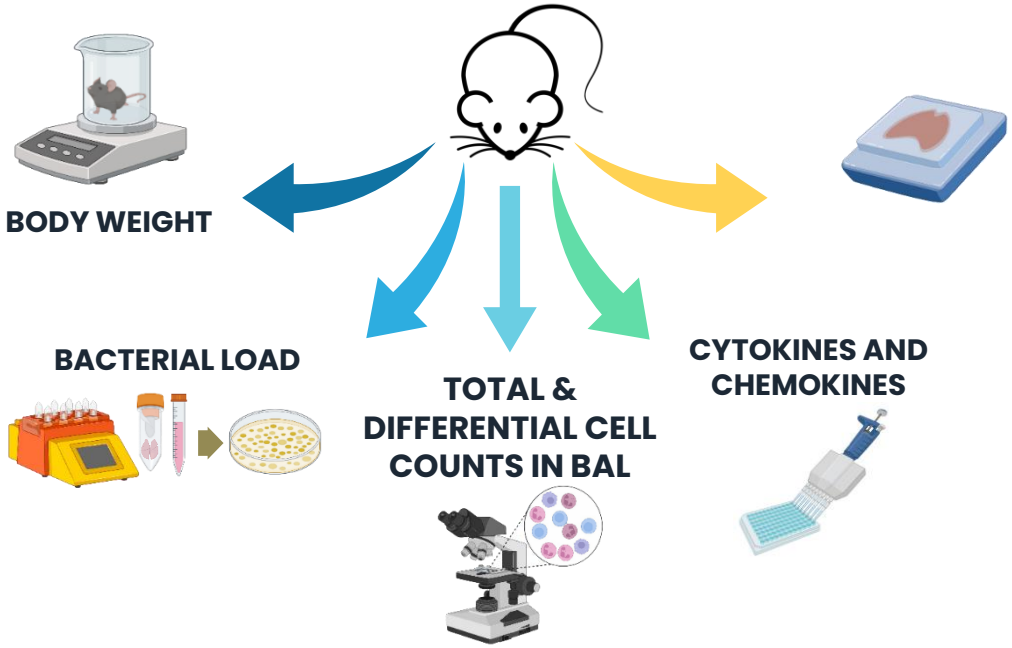


Strain	TOB MIC	Route of administration and dose		COL MIC	Route of administration and dose	
		Local	Systemic		Local	Systemic
PAO1	0.25	2 mg/kg	20 mg/kg	0.125	1 mg/kg	10 mg/kg
MDR-RP73	2	16 mg/kg	160 mg/kg	0.125	1 mg/kg	10 mg/kg

susceptible strains

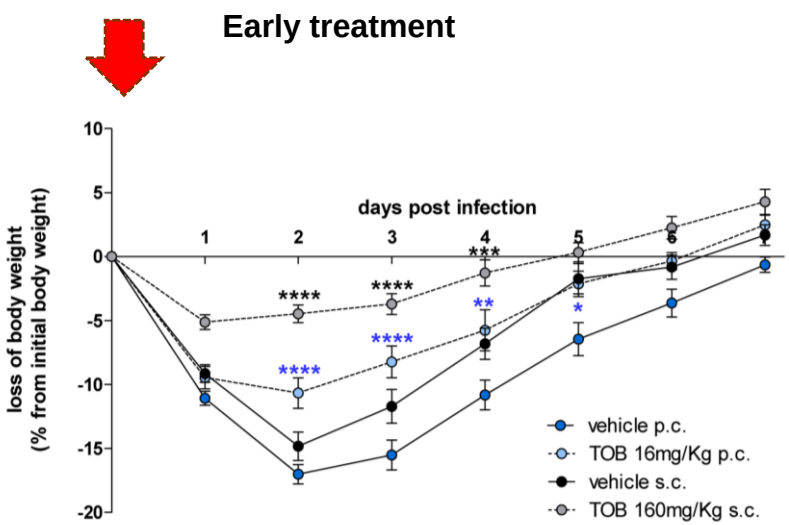


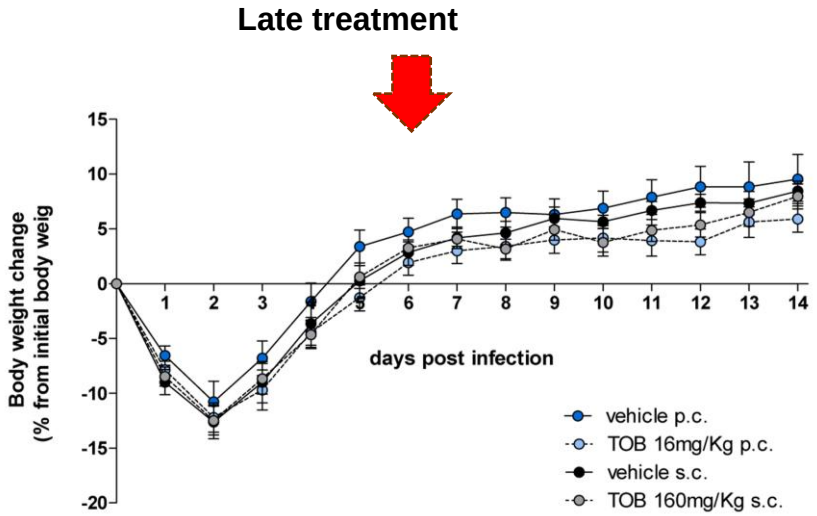
Read-out for efficacy studies in pre-clinical mouse model

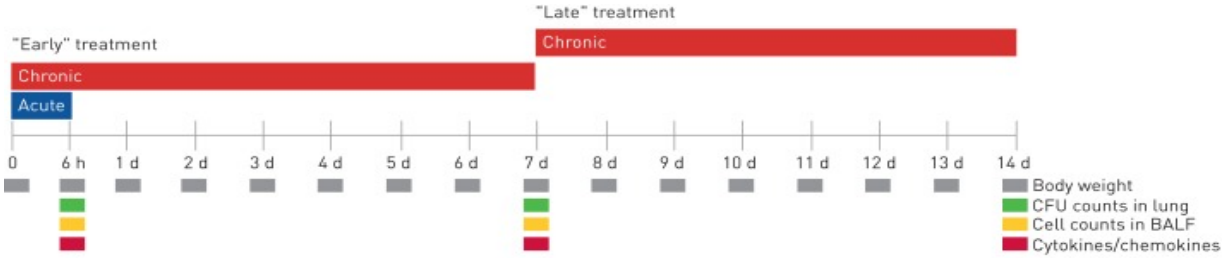


Early and late treatment in mouse model of chronic infection

Body weight



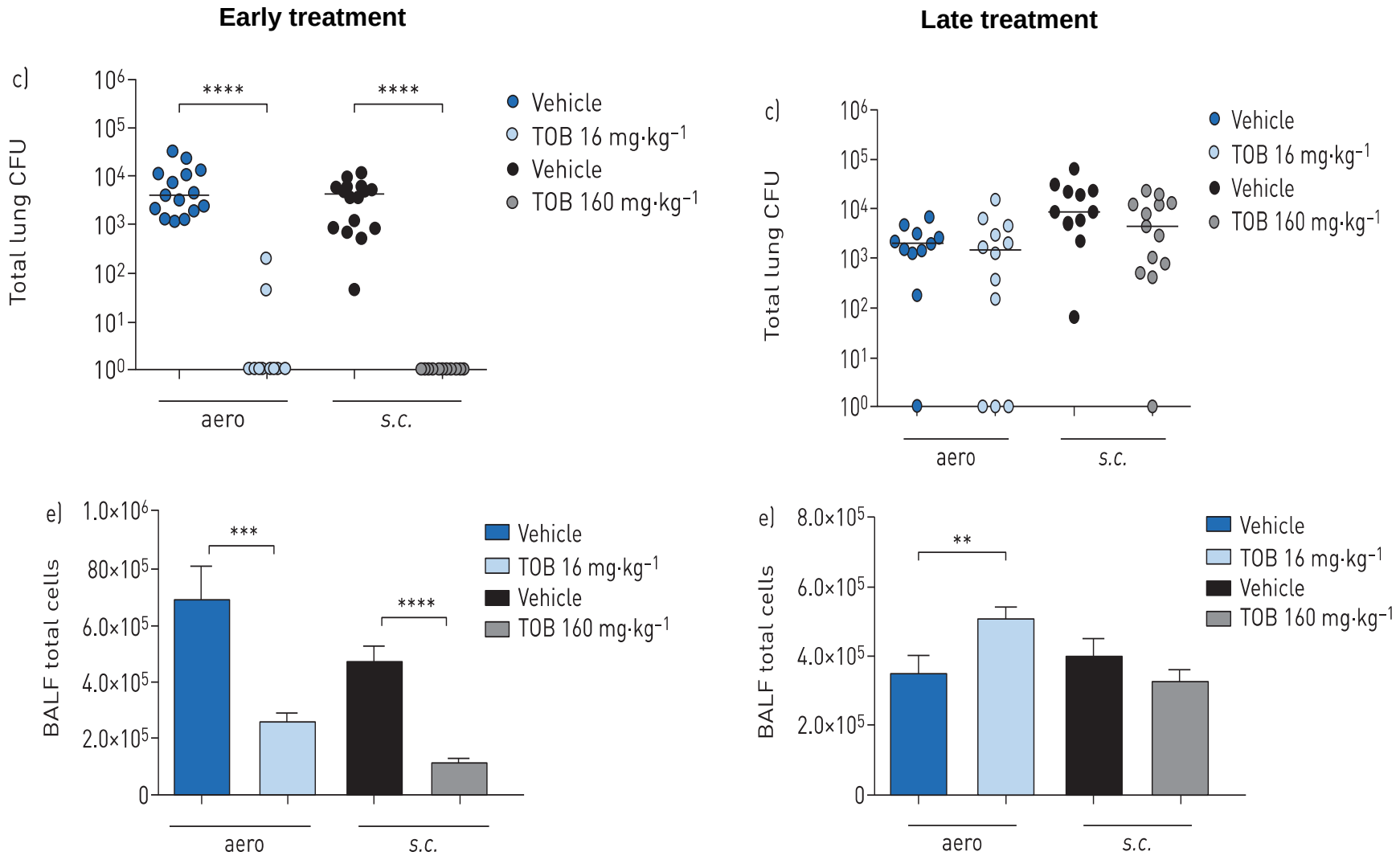




Cigana et al, Eur Respir J 2020

Early and late treatment in mouse model of chronic infection

Bacterial load and cells in broncho-alveolar lavage



Cigana et al, Eur Respir J 2020

Early and late treatment in mouse model of chronic infection

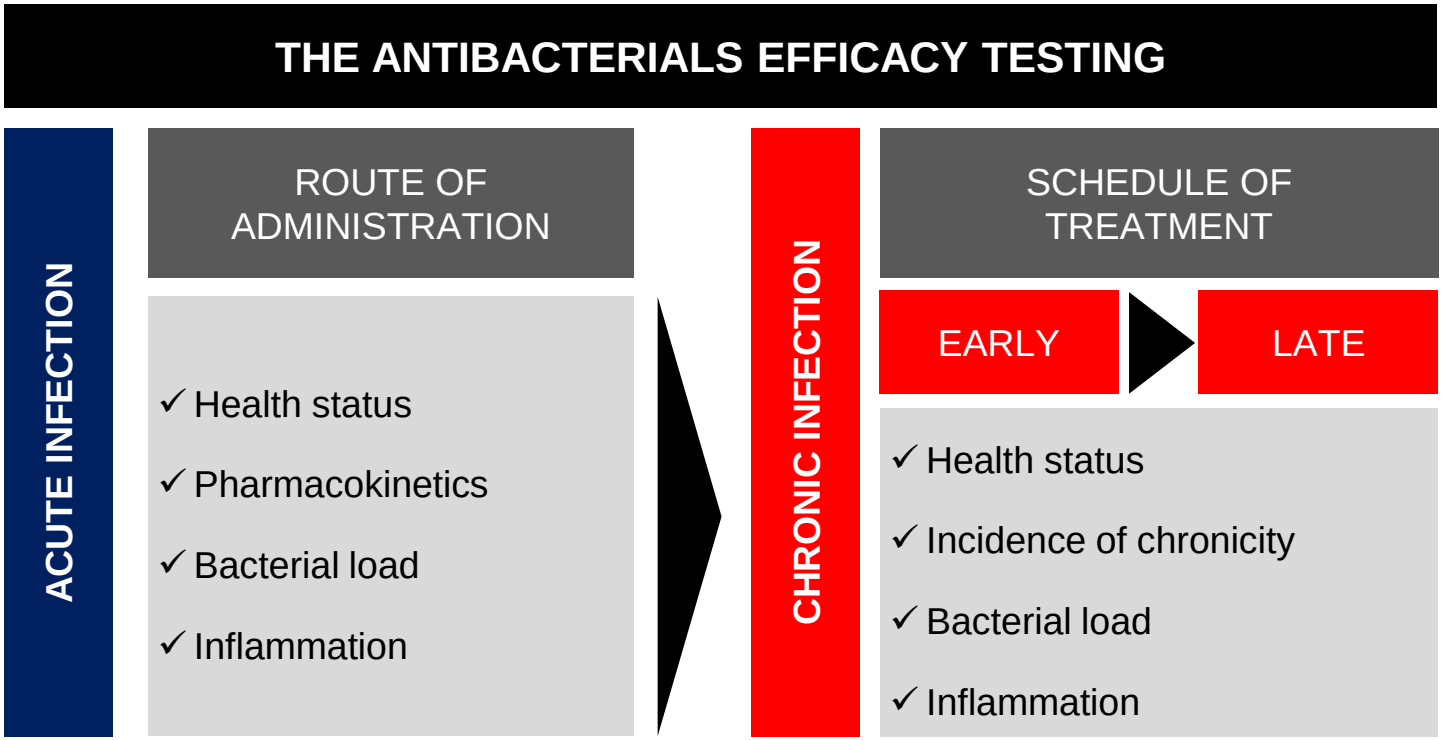
Cytokines and chemokines

Cytokine / Chemokine	Early treatment				Late treatment			
	Level (mean pg/500 µg ± SEM)							
	Vehicle p.c.	TOB p.c.	Vehicle s.c.	TOB s.c.	Vehicle p.c.	TOB p.c.	Vehicle s.c.	TOB s.c.
IL-1α	16.58±2.86	7.31±0.32**	12.48±1.95	6.34±0.47*	8.99±0.36	10.27±0.82	9.43±0.45	10.06±1.05
IL-1β	7.07±1.07	8.90±0.74	6.34±0.41	7.94±1.07	10.00±0.59	10.64±1.21	9.59±0.79	10.91±1.46
IL-2	4.33±0.09	7.49±0.89**	5.49±0.59	5.86±0.04	9.35±0.57	9.72±0.75	8.27±0.46	9.31±1.19
IL-3	2.24±0.14	2.52±0.18	2.44±0.17	2.89±0.07	3.49±0.16	3.55±0.24	3.54±0.31	3.71±0.30
IL-5	2.40±0.22	2.51±0.06	2.37±0.09	3.10±0.15**	3.14±0.13	3.03±0.18	2.80±0.48	3.11±0.39
IL-6	4.88±0.20	3.72±0.35*	4.45±0.34	3.15±0.08*	4.00±0.22	4.40±0.09	3.67±0.12	3.99±0.32
IL-9	16.49±0.48	19.44±1.21*	17.78±0.25	15.18±0.11	23.52±1.54	25.82±1.11	21.11±1.16	21.67±1.09
IL-10	9.44±0.53	9.65±0.83	10.65±0.60	10.41±0.47	8.38±0.29	8.88±0.88	7.92±1.14	8.74±1.11
IL-12p40	68.07±6.68	37.67±3.42***	59.37±4.66	32.50±2.47**	39.70±3.41	47.98±2.46	48.51±2.99	44.41±6.32
IL-12p70	26.77±2.45	20.46±1.77	26.56±2.86	22.80±1.42	24.39±2.27	28.76±2.99	21.45±3.02	23.51±3.02
IL-13	63.53±4.62	81.55±4.38*	74.97±3.83	83.52±3.25	71.57±2.65	80.26±4.35	72.43±4.10	73.37±6.23
IL-17A	15.08±2.24	6.01±0.17**	16.82±1.37	7.60±0.82**	10.31±0.52	9.25±0.66	12.59±0.51	11.31±1.10
eotaxin	637±67.26	489±59.16	634±31.45	448±47.34	451±20.08	482±48.88	397±20.91	394±28.88
G-CSF	8.50±0.95	7.26±0.00	8.26±0.51	7.26±0.00	7.73±0.37	7.36±0.00	7.36±0.00	7.36±0.00
GM-CSF	11.82±1.16	12.22±1.47	12.28±0.64	14.61±0.91	12.57±1.09	12.30±0.23	9.40±1.04	12.39±1.73
IFN-γ	24.84±1.72	28.83±1.84	26.24±0.94	23.24±0.36	34.24±1.36	38.43±1.71	30.13±1.81	32.32±2.49
KC	87.11±17.82	22.96±3.38***	49.11±7.62	23.85±3.43	33.99±4.66	31.75±5.09	26.09±2.53	23.63±2.23
MCP-1	150±15.00	108±12.14	123±10.31	121±9.36	139±7.78	152±19.78	129±11.02	128±6.85
MIP-1α	21.19±4.72	3.32±0.30**	17.06±4.22	2.56±0.27*	2.83±0.46	4.64±0.77	4.74±0.68	3.49±0.54
MIP-1β	27.40±1.99	26.89±3.08	30.13±2.78	20.10±0.89*	25.51±1.33	28.58±1.10	24.45±0.91	25.58±1.81
RANTES	324±67.69	69.49±6.66***	234±33.58	65.56±8.55*	76.33±6.34	75.65±3.13	75.11±4.74	77.92±7.76
TNF-α	44.31±4.31	34.57±1.71*	43.25±0.31	32.25±2.79*	54.38±4.27	59.62±2.66	50.12±2.79	51.15±4.00

significant differences compared to vehicle

Cigana et al., ERJ 2020

Guidelines of antibacterial efficacy testing in mouse model of respiratory infection



Cigana, ERJ 2020

Mice can explain the human response: the case of BIIL-284

- LTB4-receptor inhibition for reducing airways inflammation
- Phase II clinical trial with LTB4-receptor antagonist BIIL-284 (Boehringer)
- Oral BIIL-284 daily for 24 weeks
- Read-outs: change in FEV_1 and pulmonary exacerbation

- Terminated early for serious adverse events and increased pulmonary exacerbation

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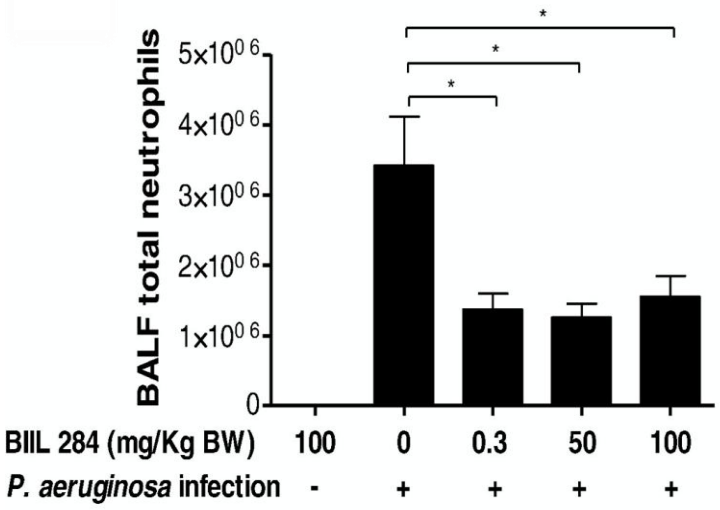
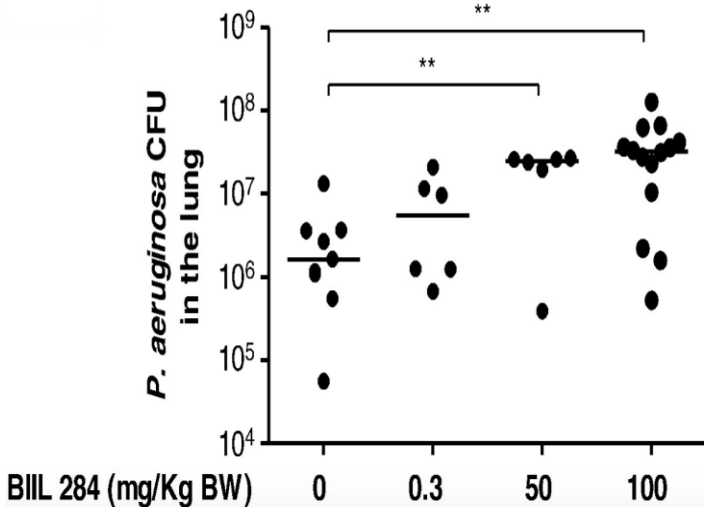
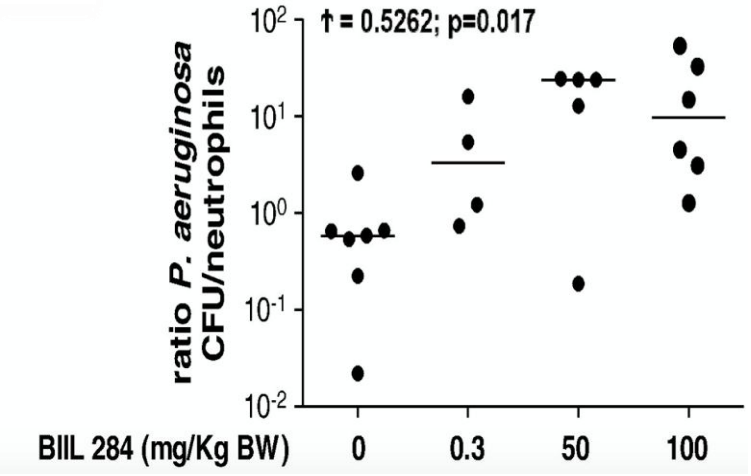
BIIL-284 –
oral administration

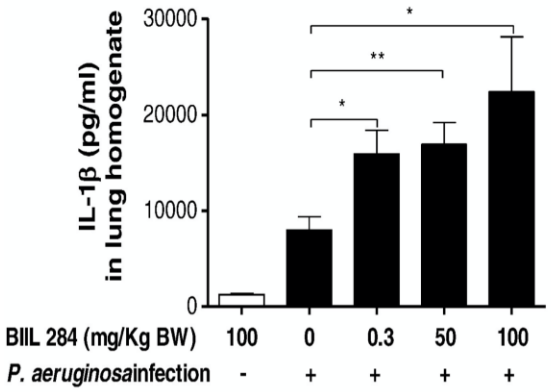


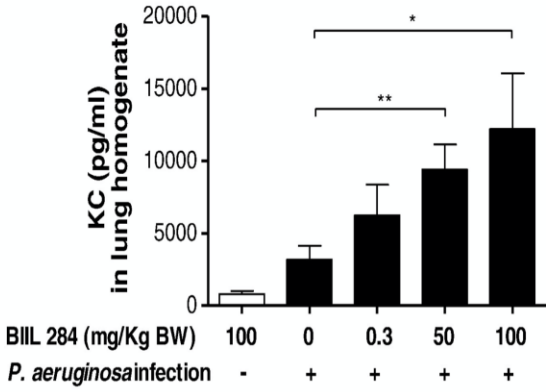


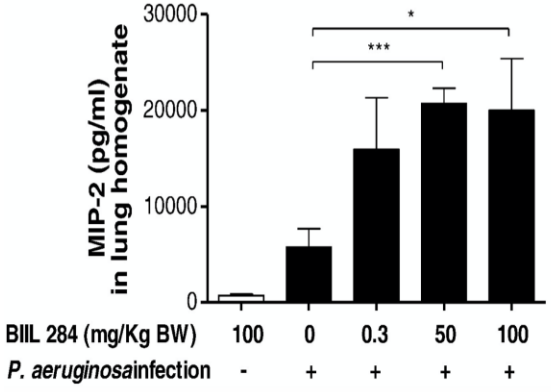
Konstan Doring, JCF 2014 ; Doring Bragonzi, JCF 2014

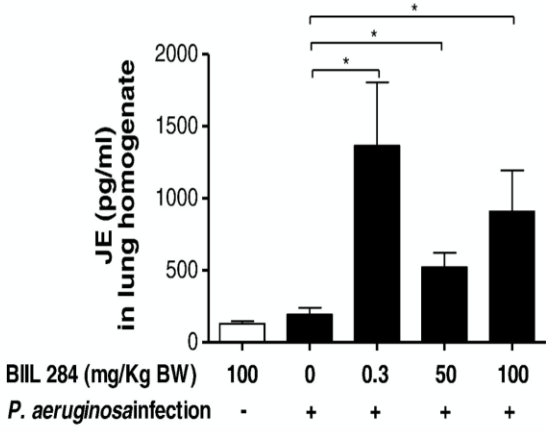
Mice can explain the human response: the case of BIIL-284







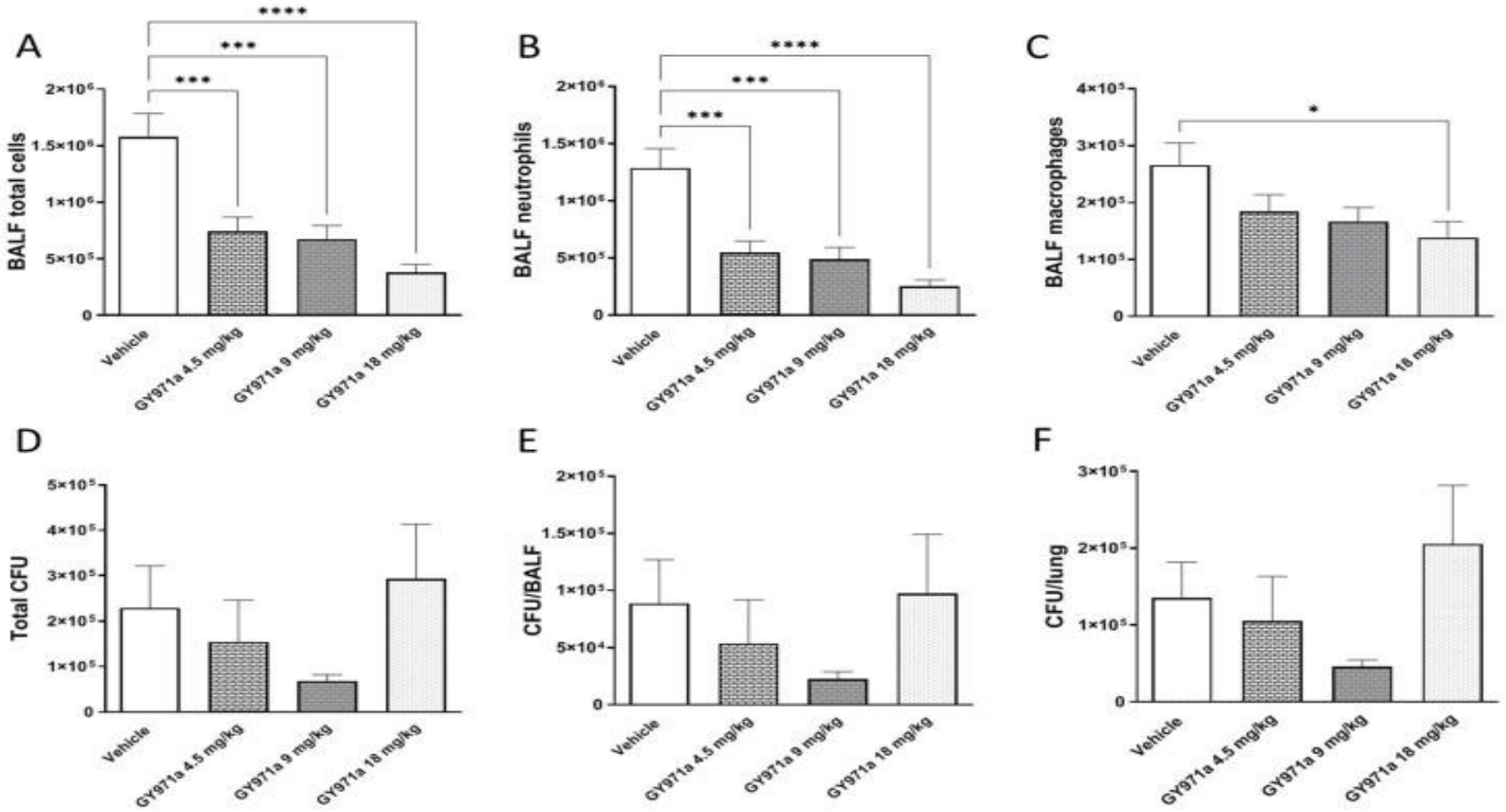




Robust pre-clinical safety and efficacy assessment in models of infection is paramount

Doring, Bragonzi, JCF 2014

GY971 as anti-inflammatory agents in the treatment of Cystic Fibrosis lung disease



Tupini, IJMS 2022

GY971 as anti-inflammatory agents in the treatment of Cystic Fibrosis lung disease

Cytokine/Chemokine	Concentration pg × 700 μg^{-1}			
	Vehicle (H_2O/DMSO 4%)	4.5 mg/Kg GY971a	9 mg/kg GY971a	18 mg/kg GY971a
IL-1α	88.45 ± 10.30	65.95 ± 7.91	54.31 ± 7.29 *	43.51 ± 3.03 **
IL-1β	9.21 ± 1.49	6.95 ± 1.05	4.91 ± 0.59 *	4.89 ± 0.75 *
IL-2	6.27 ± 0.28	5.58 ± 0.30	5.76 ± 0.24	5.28 ± 0.20 *
IL-3	5.11 ± 0.40	4.58 ± 0.24	4.36 ± 0.41	3.67 ± 0.25 *
IL-4	1.29 ± 0.09	1.23 ± 0.12	0.93 ± 0.14	0.87 ± 0.11 *
IL-5	8.46 ± 0.68	8.13 ± 0.37	7.89 ± 0.63	7.26 ± 0.44
IL-6	110.0 ± 27.68	64.30 ± 10.86	49.64 ± 9.34 *	69.79 ± 8.27
IL-9	38.58 ± 1.01	35.43 ± 1.74	32.17 ± 2.04 *	29.64 ± 0.69 ***
IL-10	20.76 ± 0.95	17.55 ± 1.23	16.41 ± 1.56 *	14.79 ± 1.05 **
IL-12p40	71.29 ± 4.32	61.72 ± 5.31	52.88 ± 4.67 *	45.67 ± 2.62 ***
IL-12p70	167.6 ± 16.08	123.7 ± 12.91	110.1 ± 13.58 **	94.45 ± 6.61 **
IL-13	88.02 ± 3.81	82.53 ± 2.63	80.58 ± 4.30	78.14 ± 3.36
IL-17A	5.49 ± 0.63	4.27 ± 0.26	3.98 ± 0.30 *	3.84 ± 0.17 *
Eotaxin	1627 ± 160.8	1638 ± 124.4	1618 ± 87.33	1414 ± 65.28
G-CSF	444.1 ± 86.41	259.1 ± 36.89	297.8 ± 58.72	181.7 ± 19.88 *
GM-CSF	48.66 ± 5.94	45.70 ± 1.52	33.60 ± 4.60 *	42.55 ± 3.18
IFN-γ	30.10 ± 1.43	27.02 ± 1.51	23.76 ± 1.65 **	22.51 ± 0.96 **
KC	2413 ± 456.1	2085 ± 263.6	1486 ± 150.8	2048 ± 248.3
MCP-1	1813 ± 322.6	1171 ± 136.4	979.0 ± 144.3 *	692.2 ± 78.41 **
MIP-1α	186.9 ± 36.52	131.7 ± 23.94	107.0 ± 12.10	92.11 ± 10.33 *
MIP-1β	291.2 ± 30.26	254.0 ± 28.16	219.8 ± 20.41	212.2 ± 15.66
RANTES	110.3 ± 10.35	123.0 ± 9.93	109.9 ± 9.72	101.9 ± 9.40
TNF-α	104.6 ± 9.05	93.45 ± 6.93	95.61 ± 11.04	84.06 ± 5.22

Tupini, IJMS 2022

The European Medicines Agency (EMA) Grants Orphan Drug Designation to GY971 for Cystic Fibrosis

- **Special status** granted to drugs intended to treat **rare diseases**

- Aims to **incentivize research** where commercial interest is limited.

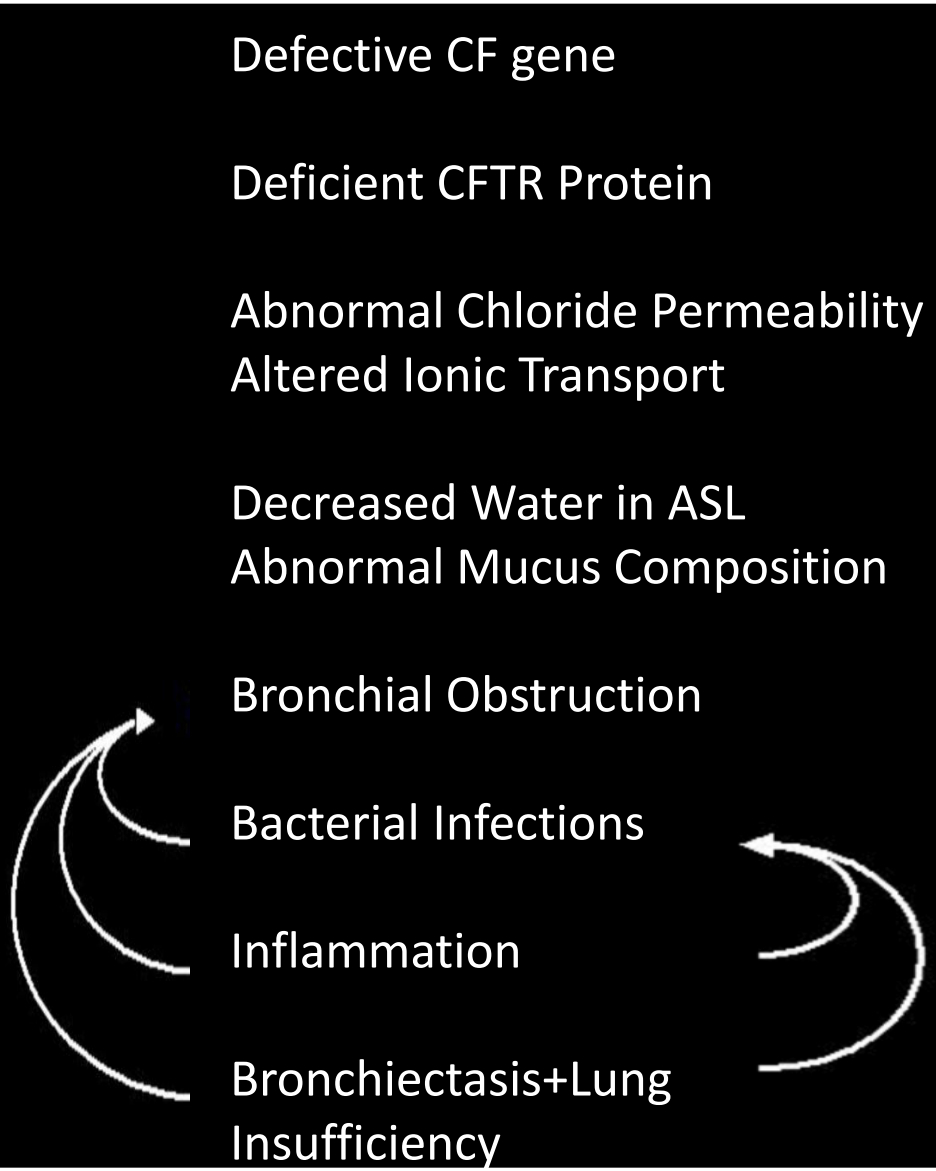
Key Benefits for Developers
- Market exclusivity (10 years in the EU, 7 in the US)
- Regulatory support (scientific advice, fee reductions)
- Access to incentives, grants, and facilitated approval pathways

Fondazione Ricerca Fibrosi Cistica - Onlus
fibrosicisticaricerca.it

21 Novembre 2024

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

The CF pathogenesis cascade & current therapies



Gene Therapy

↓

Activation of mutant CFTR

↓

Cl channel activators
Na channel blockers

↓

Airway clearance Rx
Bronchodilators, mucolytics

↓

Antibiotics

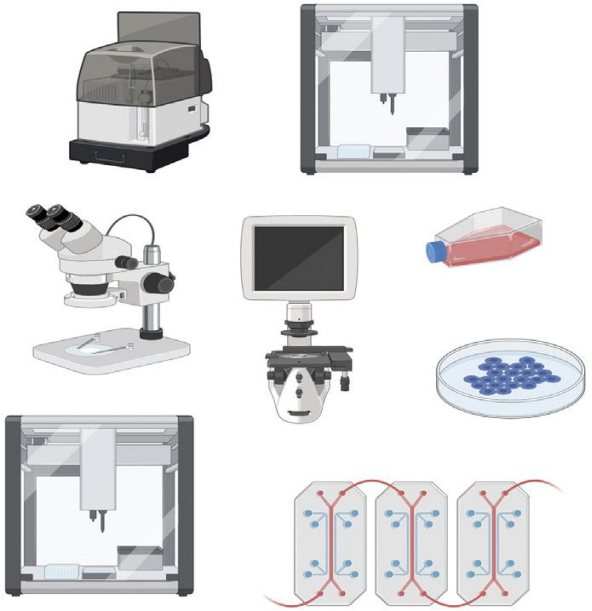
↓

Anti-inflammatory agents,
Anti-oxidants, anti-proteases

↓

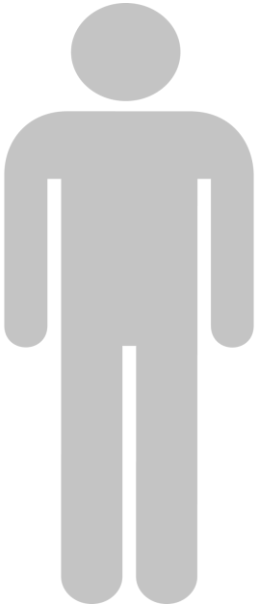
Lung transplant

The case of CFTR modulators



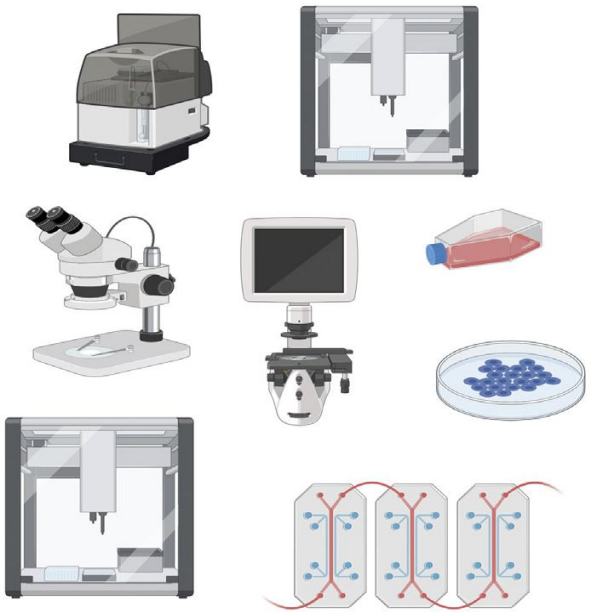
Efficacy testing

Replacement of *in vivo* studies



- target identification
- compound screening
- candidate selection

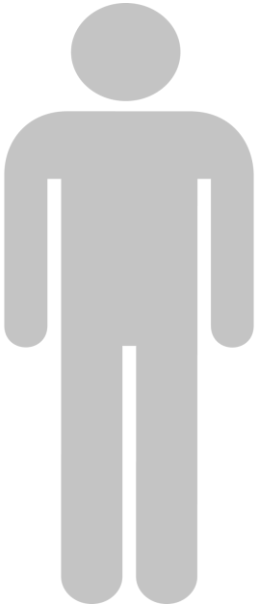
The case of CFTR modulators



- target identification
- compound screening
- candidate selection

Efficacy testing

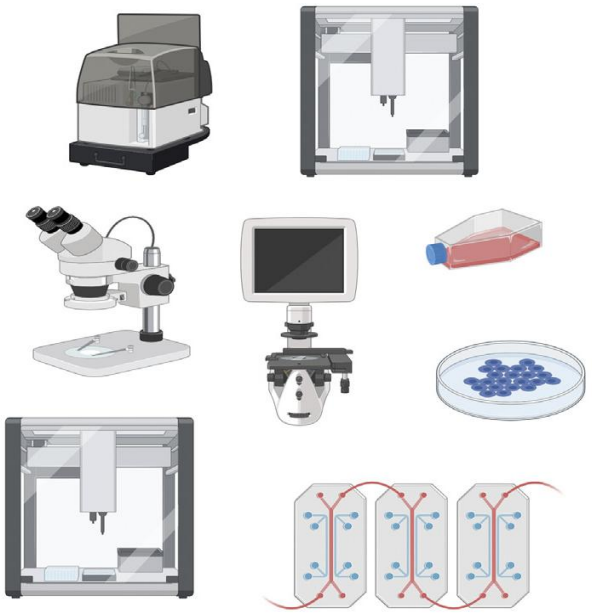
Replacement of *in vivo* studies



species	CFTR identity (%)
human	100
mouse	78

Human CFTR

The case of CFTR modulators

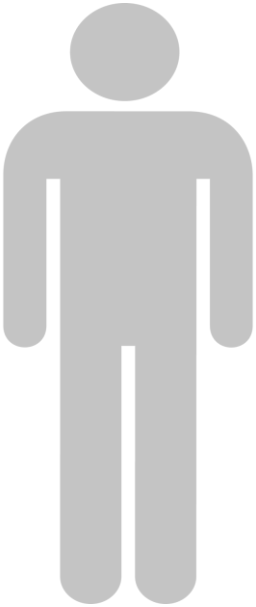


Efficacy testing

Replacement of *in vivo* studies

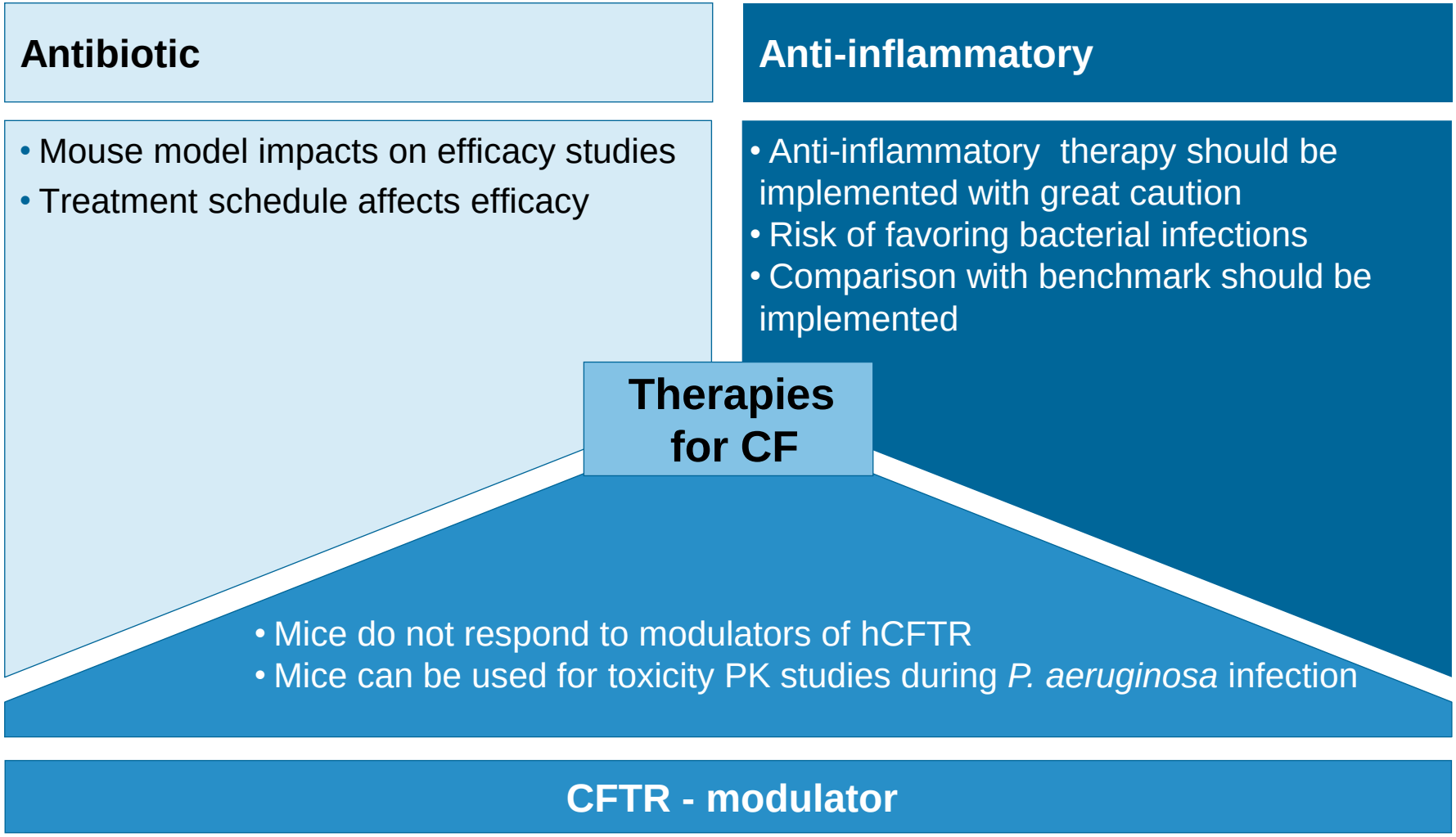
Toxicity

PK studies



- target identification
- compound screening
- candidate selection

- Phase I, II, III clinical trials
- dosage & safety monitoring
- post-approval surveillance


Antibiotic
Anti-inflammatory
• Mouse model impacts on efficacy studies
• Treatment schedule affects efficacy
• Anti-inflammatory therapy should be implemented with great caution
• Risk of favoring bacterial infections
• Comparison with benchmark should be implemented
Therapies for CF
• Mice do not respond to modulators of hCFTR
• Mice can be used for toxicity PK studies during *P. aeruginosa* infection
CFTR - modulator

Le fasi della ricerca

Studio del meccanismo
Identificazione del bersaglio
Scoperta di composti
Valutazione in vitro

Test in vivo
Produzione e formulazione

Fase I (Sicurezza)
Fase II (Efficacia)
Fase III (Conferma)
Fase IV (Sorveglianza post-marketing)

A cosa serve la sperimentazione animale?

1

Studio del meccanismo

Identificazione del difetto genetico e valutazione fisiopatologica. Le mutazioni genetiche vanno valutate in un organismo complesso, con tutti gli organi e sistemi che influenzano la malattia

2

Valutazione di un nuovo farmaco

L'efficacia di una terapia può manifestarsi pienamente solo in un organismo completo, dotato di tutti gli organi che possono ricevere e modificare la terapia stessa. L' effetto può non essere evidente nelle cellule isolate utilizzate nei primi esperimenti in vitro

E’ sostituibile?

1

Studio del meccanismo

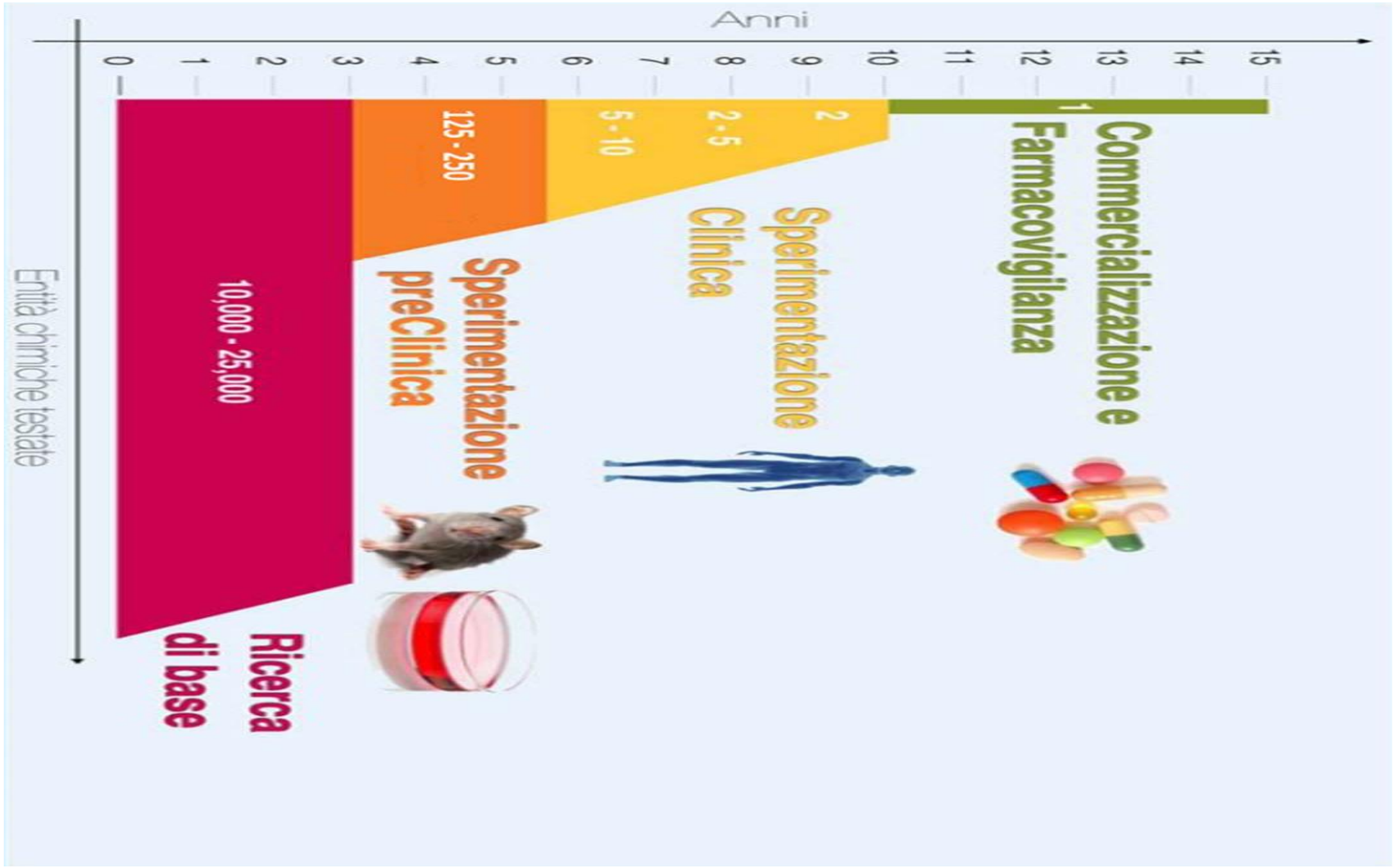
Le cellule sono isolate, ma nella natura interagiscono con altre cellule e organi che possono influenzarne la funzione. È essenziale verificare un meccanismo in un organismo completo e sufficientemente complesso.

2

Valutazione di un nuovo farmaco

Gli esperimenti negli animali sono richiesti per legge prima di passare alla sperimentazione clinica. La legge assicura che le terapie che arrivano al paziente siano efficaci e non siano dannose

Lo sviluppo di un farmaco



Obiettivi degli studi *pre-clinici*

- Il medicinale "**funziona**"? → la risposta verrà dalle valutazioni di **efficacia**.

- Come può essere **somministrato** il prodotto medicinale? E come **reagisce** il corpo ad esso? → queste risposte verranno dagli studi ADME (Assorbimento, Distribuzione, Metabolismo ed Escrezione).

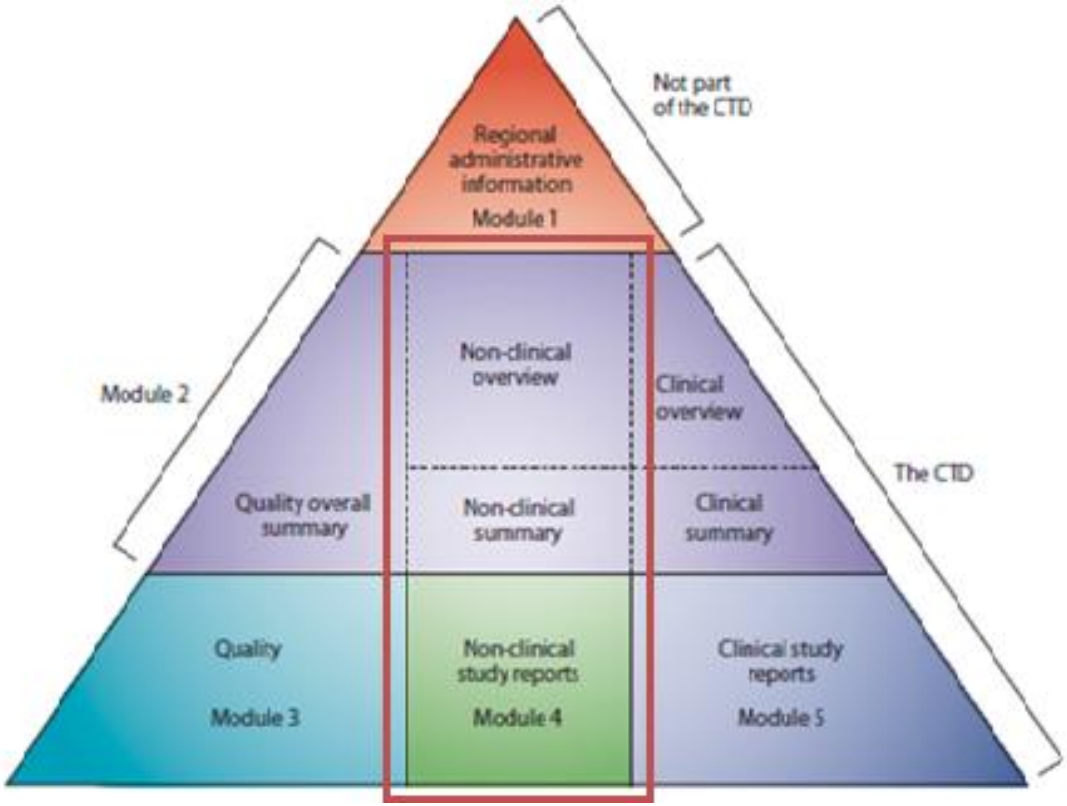
- Il medicinale è **sicuro**? → gli studi di **tossicologia/farmacologia** della sicurezza forniranno la risposta indicando la dose singola/ripetura, valutando genotossicità – alla cellula e mutazioni geniche-, cancerogenicità – possibilità di causare cancro.

- Stabilisce la **prima dose** da utilizzare negli **studi di Fase I** sugli esseri umani al fine di proteggere i partecipanti, senza effetti avversi osservabili.

https://learning.eupati.eu/local/coursecatalogue/index.php?categoryid=4

Linee guida regolatorie per un nuovo candidato medicinale

Prima che un nuovo **candidato medicinale** possa essere venduto sul mercato o prescritto ai pazienti, l'azienda farmaceutica deve presentare un documento che include dati sulla **Qualità**, **Sicurezza** ed **Efficacia** all'EMA. Deve dimostrare di avere un'efficacia superiore rispetto ai farmaci già presenti nel mercato.



Sperimentazione animale: la normativa

La sperimentazione è una pratica prevista dalla legge:

- legge europea, la Direttiva 2010/63/EU Protection of animals used for scientific porpoises

- normativa italiana con il decreto legislativo n. 26 del 2014 attuazione della direttiva 2010/63/EU sulla protezione degli animali utilizzati a fini scientifici.

Il progetto applica il principio delle 3R
Replacement, Reduction, Refiniment

The Principles of Humane Experimental Technique
W.M.S. Russell and R.L. Burch , 1959

Considerazioni etiche: il principio delle 3R

Replacement, Reduction, Refiniment
Sostituzione, Riduzione, Miglioramento

✓**EU Directive: Article 4** (Principle of replacement, reduction and refinement):

1. Gli stati membri devono assicurare che, quando possibile, siano utilizzati metodi scientifici o strategie di test che non implichino l'uso di animali vivi
2. Gli stati membri devono assicurare che il numero di animali coinvolti sia ridotto al minimo
3. Gli stati membri devono assicurare l'utilizzo di procedure che riducani al minimo il dolore, lo stress o il distress negli animali.

✓ **Dl.vo 26/2014: Articolo 1** (Oggetto e campo di applicazione)

1. Sostituzione, Riduzione e perfezionamento
2. E' consentito l'utilizzo degli animali a fini scientifici o educativi soltanto quando, per ottenere il risultato cercato, non sia possibile utilizzare altro metodo o una strategia di sperimentazione scientificamente valida, ragionevolmente e praticamente applicabile che non implichi l'impiego di animali vivi.