Fondazione per la Ricerca
sulla Fibrosi Cistica - Onlus
italian cystic fibrosis research foundation

CFaCore –
Cystic Fibrosis animal Core Facility

Alessandra Bragonzi, PhD

Ospedale San Raffaele, Milano
bragonzi.alessandra@hsr.it

Agenda webinar CFaCore

- Servizi offerti da CFaCore
- Scelta, gestione e costi topi FC
- Modelli murini di infezione e valutazione di efficacia
- Accesso al servizio

- Validazione del modello CFaCore in studi di efficacia terapeutica per antibiotici noti

fondazione per la ricerca sulla fibrosi cistica - onlus
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CFaCore
Cystic Fibrosis animal Core Facility

OSPEDALE SAN RAFFAELE

WHAT

Pre-clinical research to accelerate new strategies for the treatment of CF.

WHY

Infrastructure (stabulary and BSL2) and expertise in animal models for CF are not available in single research centre.
High costs for maintaining CF colony in individual research center.

WHO

Myself and three collaborators (Ida De Fino, Alice Rossi and Davide Gugliandolo) with unique expertise in maintaining CF colonies and modeling infectious disease.

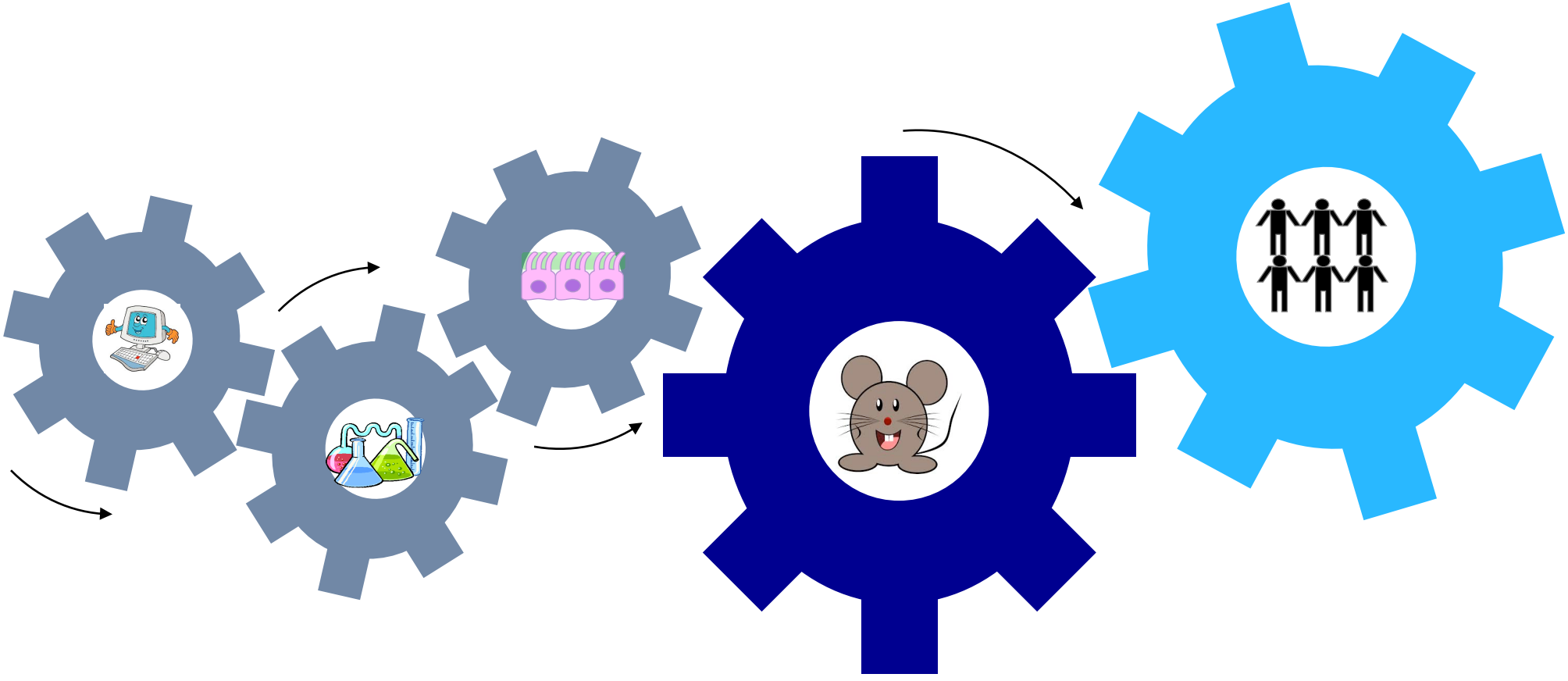
WHERE

In Milan at OSR, with financial support of FFC

WHEN

Running since 2009

CFaCore
Cystic Fibrosis animal Core Facility



Phase 1
BASIC RESEARCH

Phase 2
PRE-CLINICAL RESEARCH

Phase 3
CLINICAL RESEARCH

MODELS OF CF DISEASE
SPECIAL INFRASTRUCTURES
KNOW-HOW FOR TESTING NOVEL DRUGS

Servizi offerti ai ricercatori FFC

RICERCATORE

Livello I

DISTRIBUZIONE DI TOPI FC

Topo FC

Livello II

FORNITURA DI TRATTAMENTI SPECIALIZZATI IN ANIMALI

- Attività descritta al Livello I
- Richiesta al comitato etico
- Modello di infezione acuta o cronica/ infiammazione
- Trattamenti farmacologici
- Monitoraggio del benessere animale
- Sacrificio e raccolta campioni biologici

Campione biologico

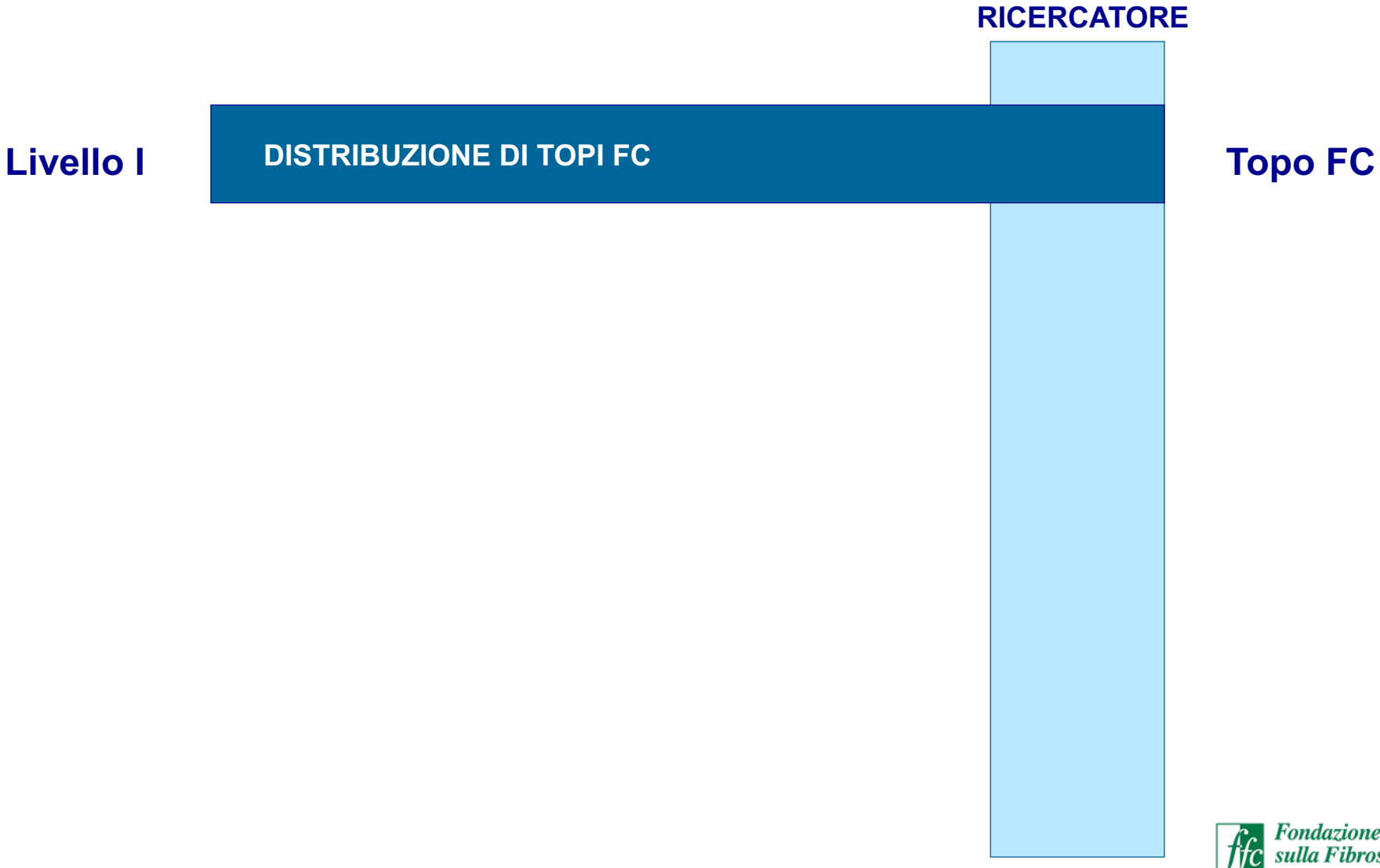
Livello III

ESECUZIONE DEL PROGETTO DI RICERCA

- Attività descritte al Livello II
- Processamento ed analisi di campioni biologici
- Analisi statistica

Risultati

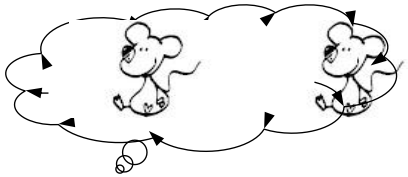
Servizi offerti ai ricercatori FFC



Staff

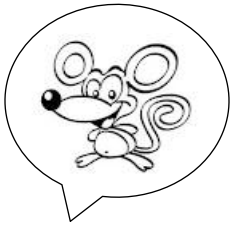
CF mice/Level I

Ida De Fino

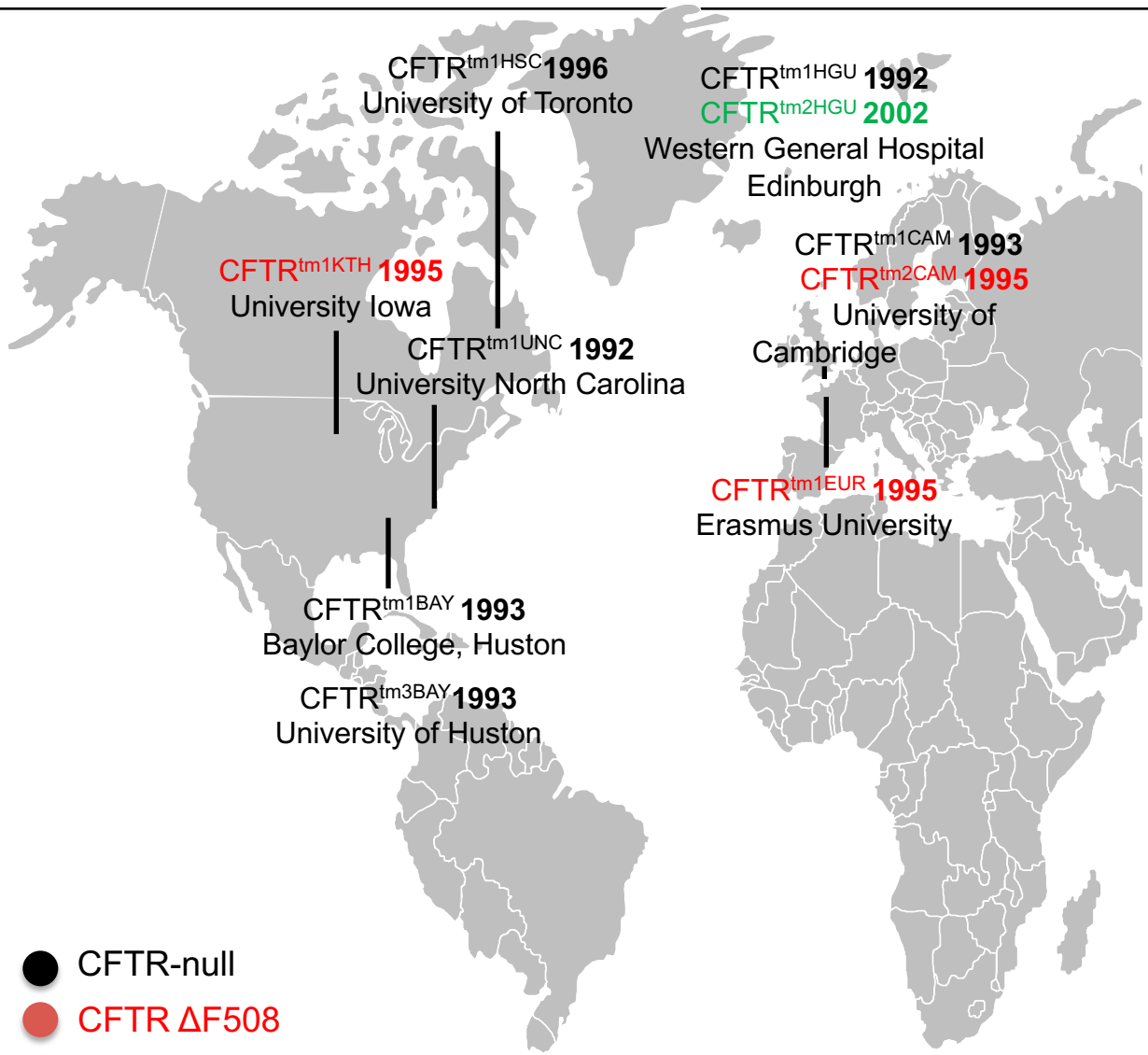


Services/Level II

Alice Rossi **Davide Gugliandolo**

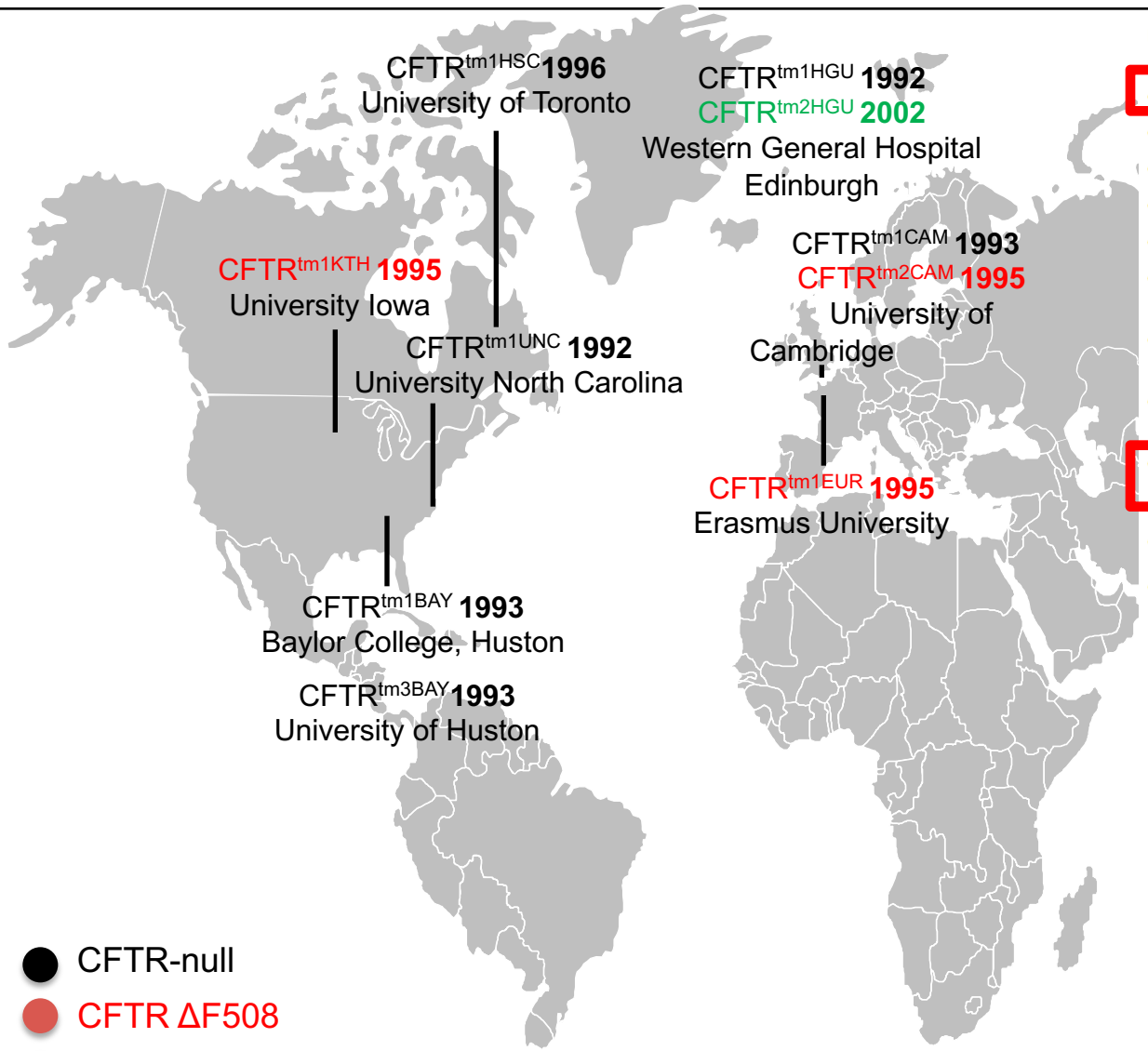


Cohorts of CF mice



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)

Cohorts of CF mice



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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)

Modelli animali di fibrosi cistica /Livello 1 - Topi CF

- Topo knock-out: **C57BL/6 Cftr tm^{1Unc} TgN(FABPCFTR)** (Snouwaert, 1992; Zhou, 1994)

Cftr exon 10 replacement (S489X)/ FABPhCFTR insertion

- Background strain: C57BL/6J inbred
- Respiratory system phenotype: abnormal nasal potential difference / inflammatory response / infection clearance
- Gut phenotype: normal
- Alimentary phenotype: normal
- Lethality-postnatal: normal

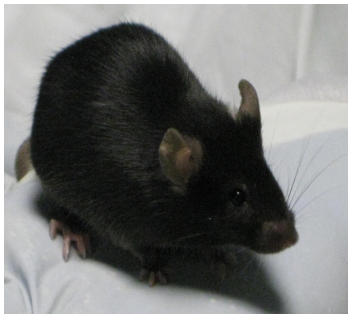
Action on the colony:

- Genotyping by PCR
- Sanitary control
- Rederivation in the year 2009 for SPF profile
- Backcross over the years 2014, 2017, 2019 to avoid genetic drift

SCHOOL OF MEDICINE
CASE WESTERN RESERVE UNIVERSITY

Collaborators: Tracey Bonfield / Craig Hodges/ Mitchell Drumm
Case Western Reserve University / CF Core Center Animal Core, Cleveland, OH

Modelli animali di fibrosi cistica /Livello 1 - Topi CF



Topo deltaF508: **C57Bl/6 *Cftr^tm1Kth*** (Zheier et al, 1995)

Cftr exon 10 replacement deltaF508

- Background strain: C57BL/6J inbred
- Gut phenotype: obstruction
- Respiratory system phenotype: abnormal nasal potential difference / inflammatory response / infection clearance
- Alimentary phenotype: normal + laxative
- Lethality-postnatal: high mortality pre and post-wearing

Action on the colony:

- Genotyping by PCR
- Sanitary control
- Rederivation in the year 2016 for SPF profile

SCHOOL OF MEDICINE
CASE WESTERN RESERVE UNIVERSITY

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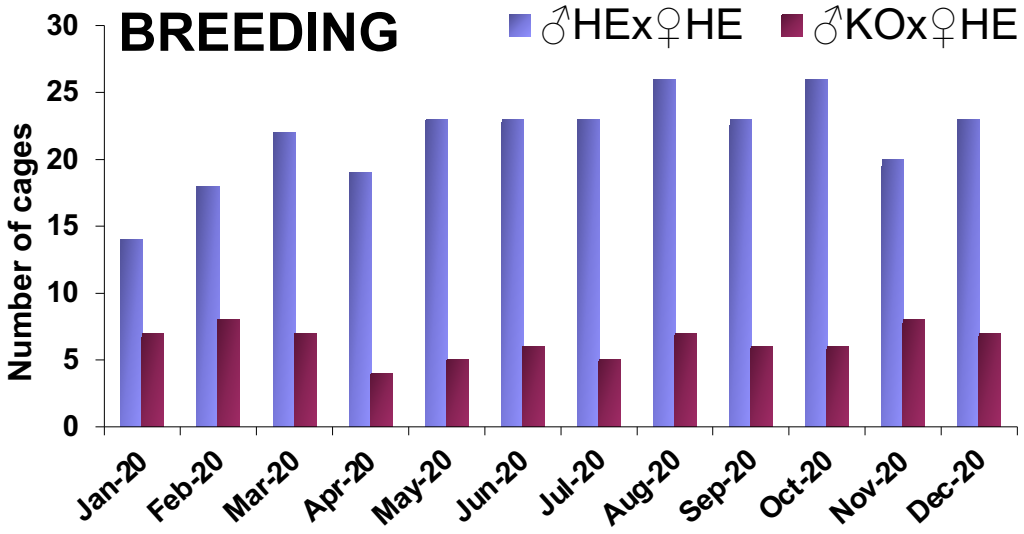
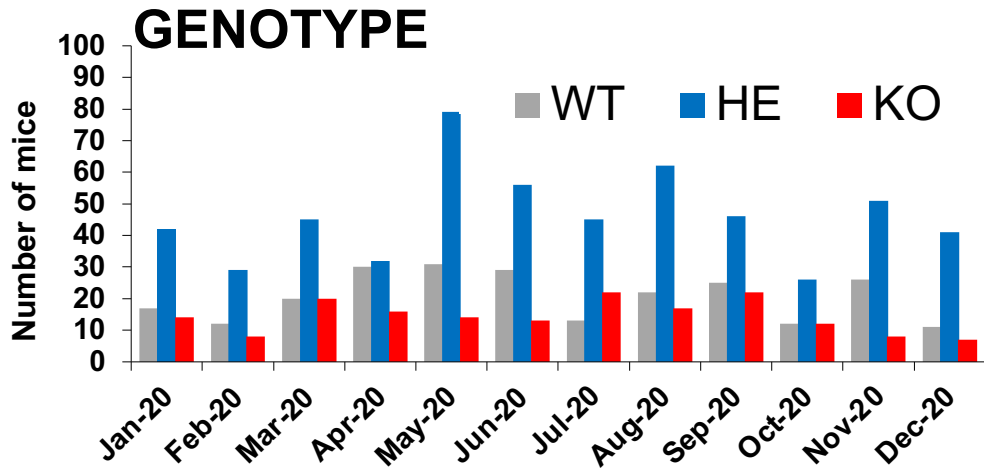
Colonia topi C57BL/6 Cftr tm^{1Unc} TgN(FABPCFTR)

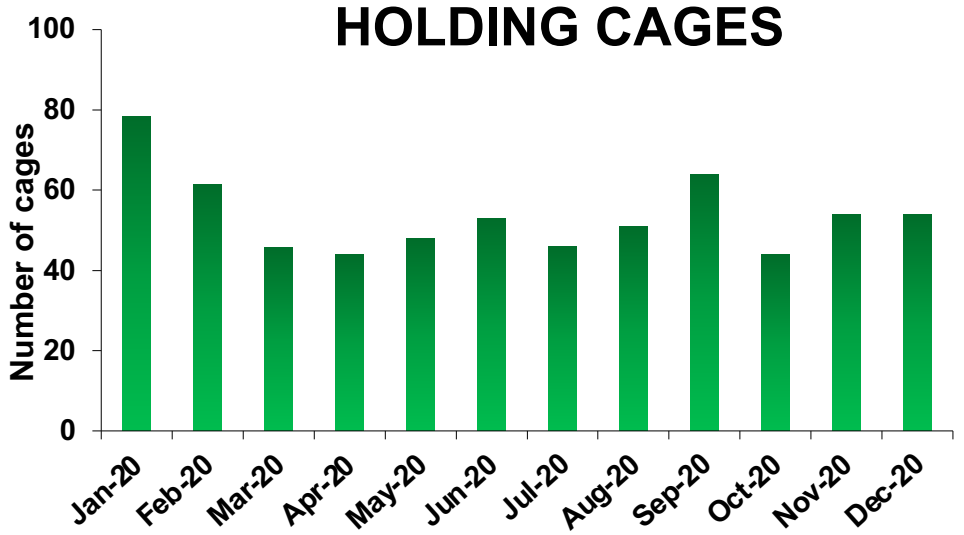




Breeding cages /month: 28

WT mice /month: 20
HE/month: 46
KO/month: 14

Holding cages: 53



Vantaggi colonia topi CF centralizzata

- Disponibilità di una colonia CF sul territorio nazionale per uso preferenziale dei ricercatori FC
- Esperienza sviluppata nel mantenimento della colonia CF
- Servizio centralizzato con contenimento dei costi

Costi

CF mice

- **Wild type o hetero (20€)**: 100% charged on the project
- **CF KO mice (207€)**: 50% charged on the project

(**103 €** / mouse), 50 % charged on CFaCore

Payment to FFC

Servizi offerti ai ricercatori FFC

RICERCATORE

Livello II

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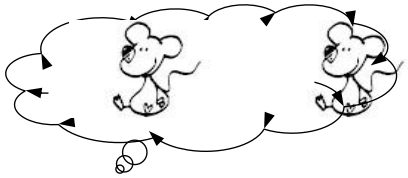
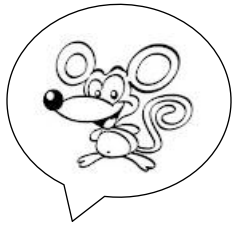
Ida De Fino

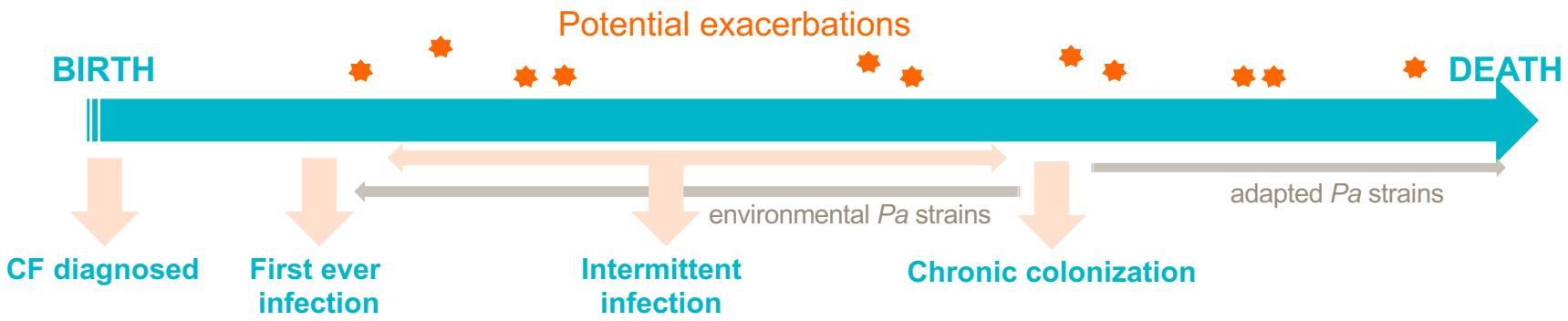


Services/Level II

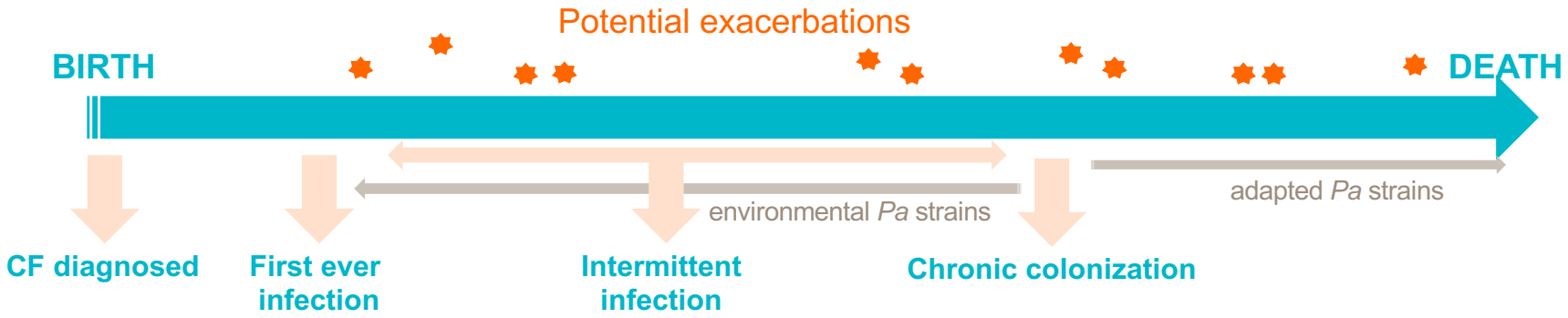
Alice Rossi **Davide Gugliandolo**

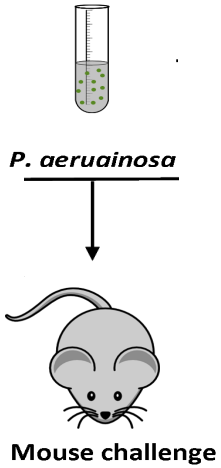


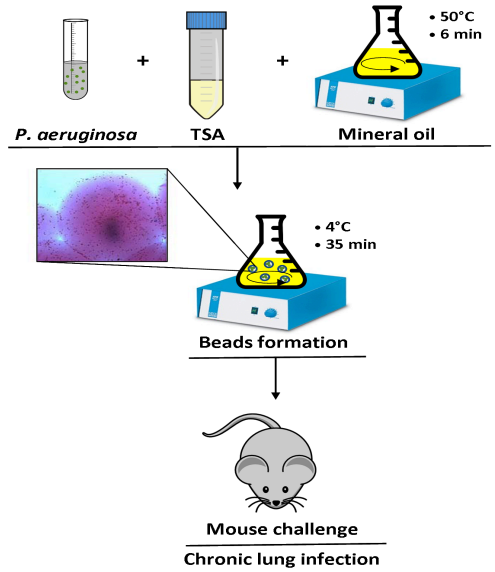
Modelli murini di infezione acuta e cronica per mimare diverse fasi di infezione in pazienti FC



Modelli murini di infezione acuta e cronica per mimare diverse fasi di infezione in pazienti FC

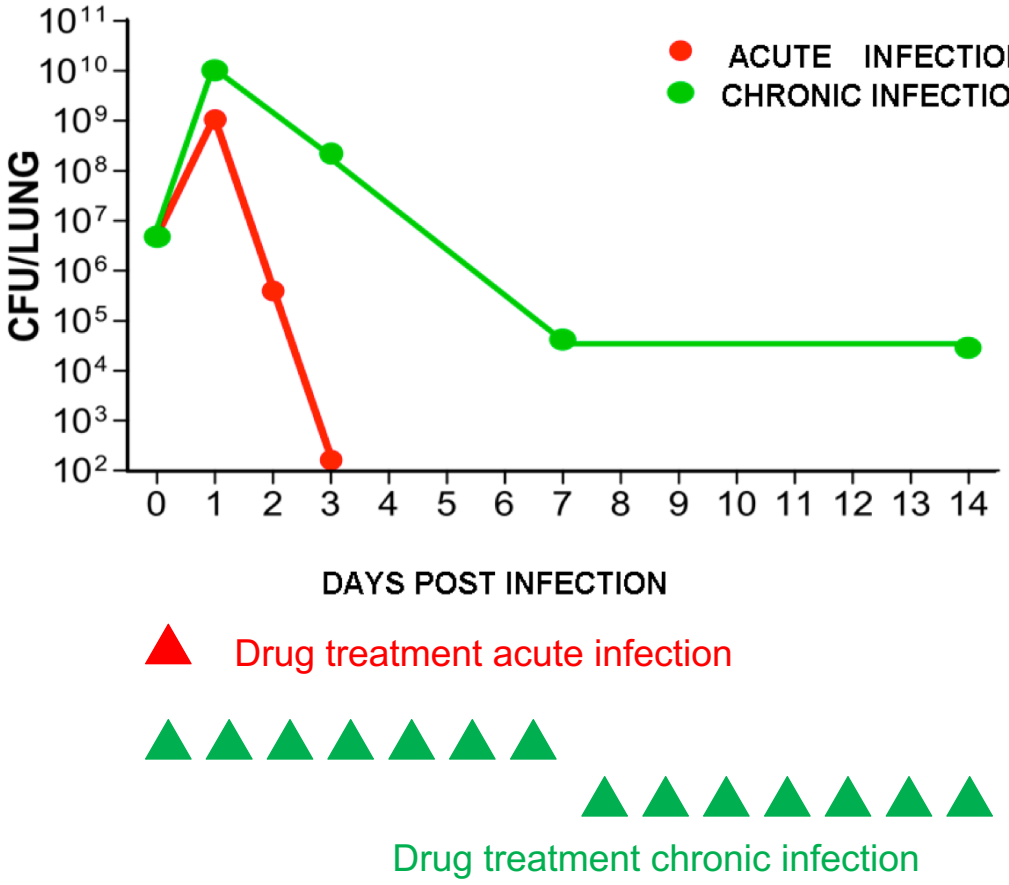




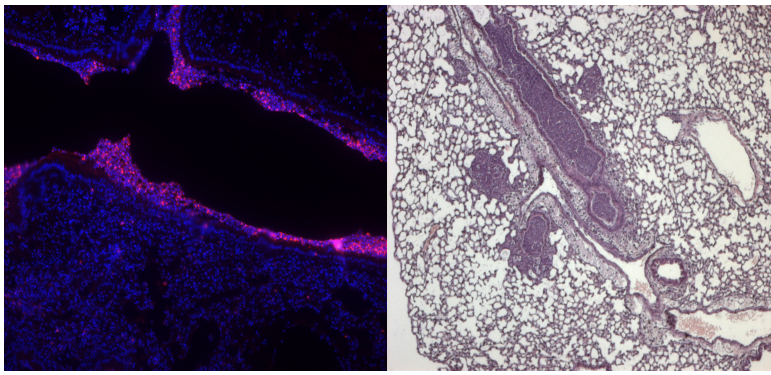


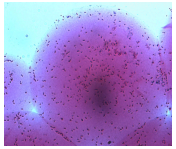
Bragonzi, JID 2014 and additional 72 pubblications

Modelli murini di infezione acuta e cronica per mimare diverse fasi di infezione in pazienti FC

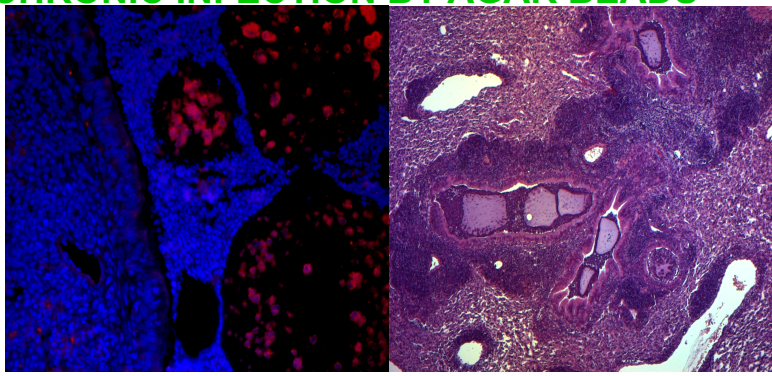


ACUTE INFECTION BY PLANKTONIC CELLS



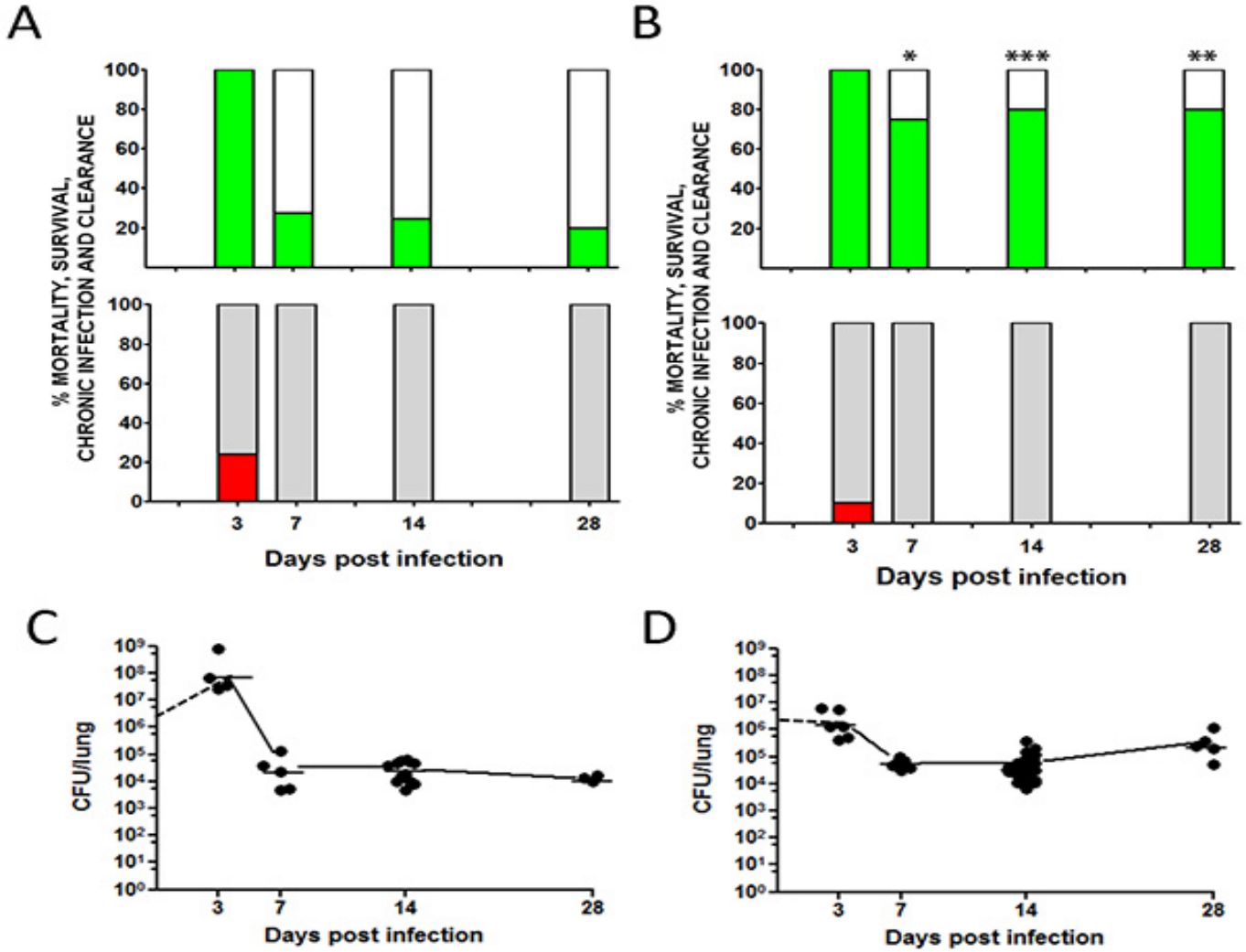


CHRONIC INFECTION BY AGAR BEADS



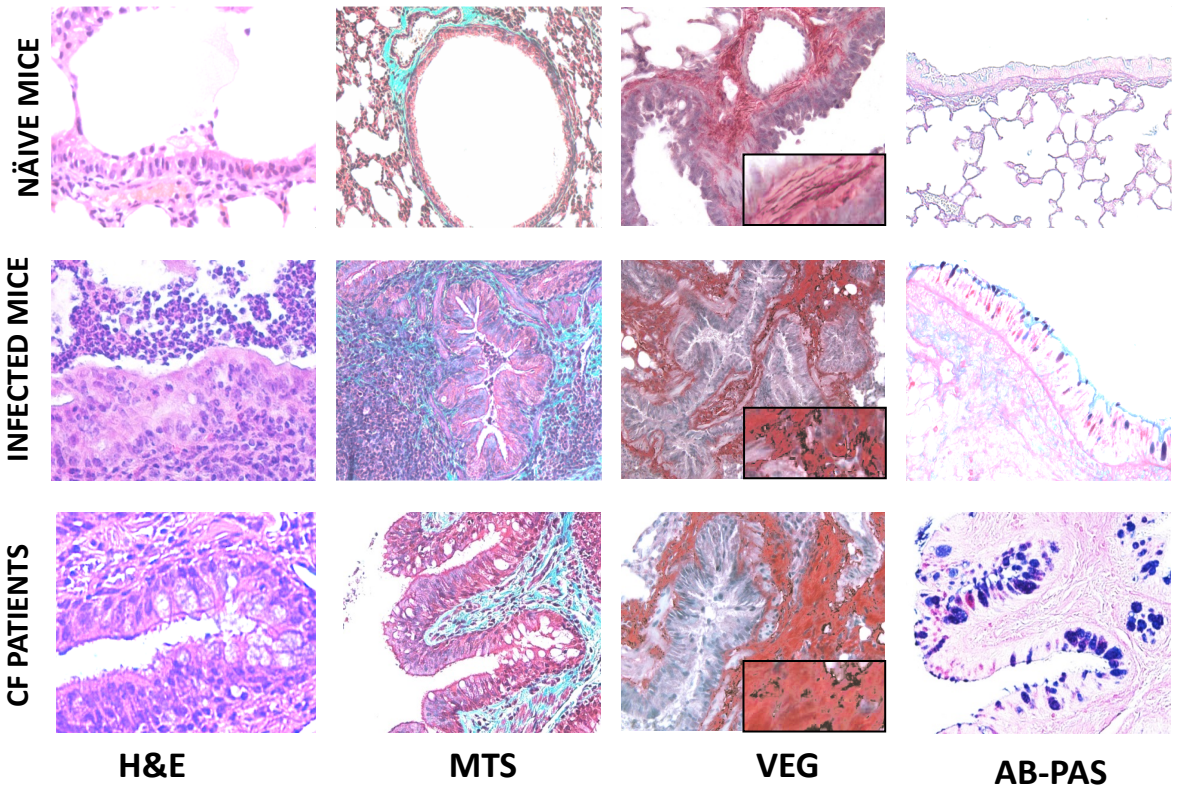
Facchini, Jove 2014

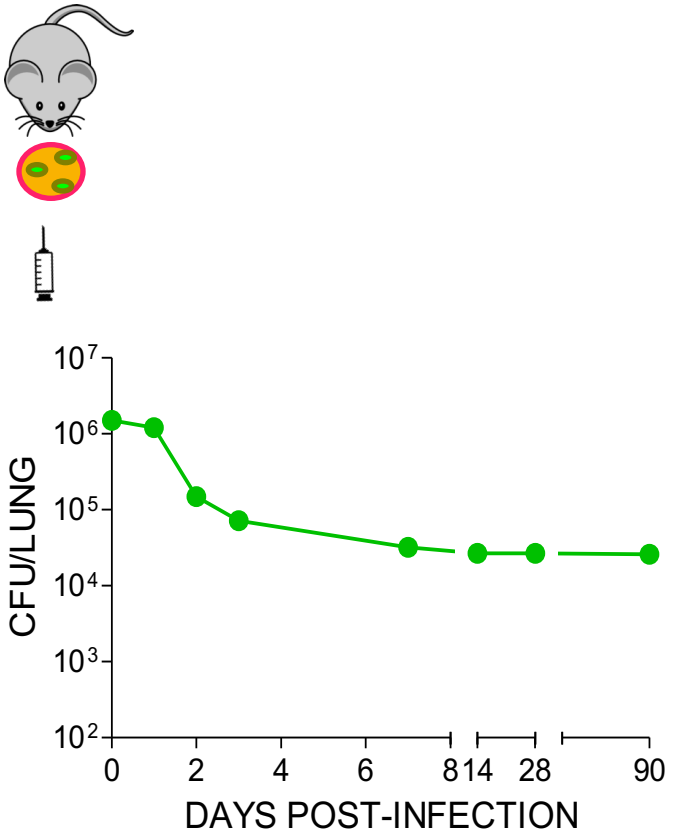
Modelli murini di infezione cronica con ceppi batterici di riferimento e clinici (*P. aeruginosa*)



Facchini, Jove 2005

Patologia polmonare dopo infezione da *P. aeruginosa* a lungo termine

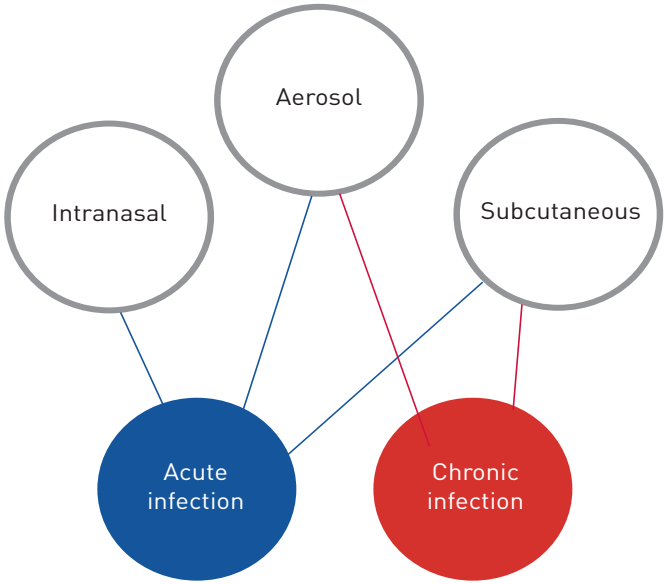




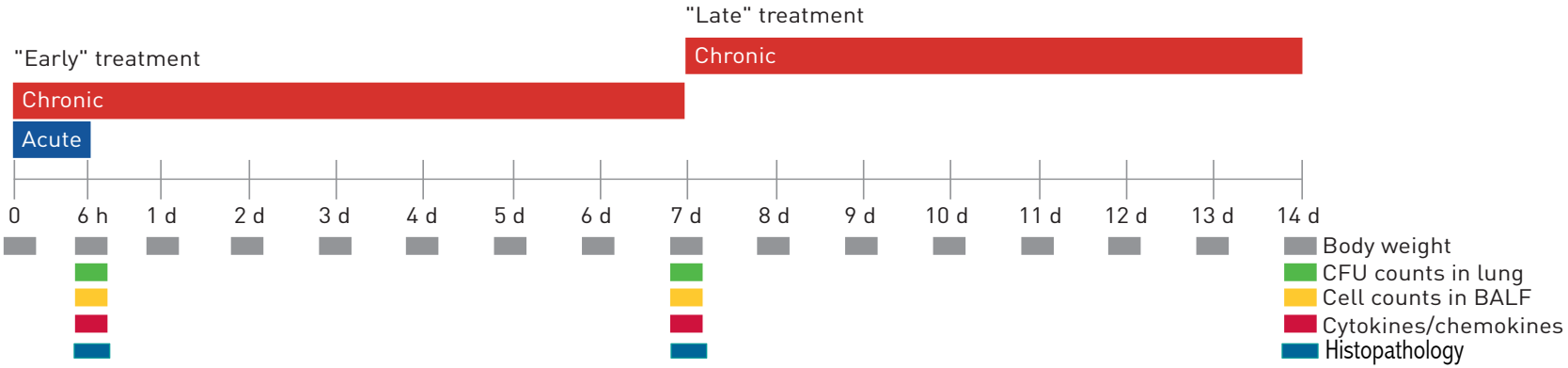
- stable infection and inflammation
- epithelial hyperplasia and structure degeneration
- goblet cells metaplasia

Cigana, Scientific Report 2016

Modelli murini di infezione, vie di somministrazione, e analisi



Aerosol delivery by MicroSprayer® Aerosolizer and the Dry Powder Insufflator™



Servizi addizionali di supporto a CFaCore in OSR

- **TOXICITY STUDIES**
  - *Toxicology*
  - *Necropsy and tissues collection managed by computerized system (Ascentos)*

- **PATHOLOGICAL STUDIES**
  - *Histological preparation,*
  - *Special staining,*
  - *Immuno histochemistry.*
  - *Histopathological evaluation*
  - *Reporting managed by computerized system (Ascentos)*

- **HEMATOLOGY**
  - *Complete Blood count (WBC, WBC count, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, and reticulocytes by Idexx Procyte analyzers)*

- **CLINICAL BIOCHEMISTRY**
  - *21 parameters of serum biochemical tests for liver, kidney, heart, pancreas and other*

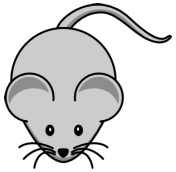
Francesca Sanvito
Pathological Anatomy Unit,
Ospedale San Raffaele

Fondazione per la Ricerca sulla Fibrosi Cistica - Onlus
fibrosicisticaricerca.it

Michele Raso
Animal Biochemistry
Ospedale San Raffaele

Risorse topi e utilizzo

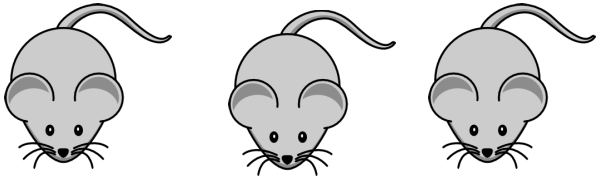
charles river
accelerating drug development. exactly.



C57Bl/6 Charles River

24 €

fondazione per la ricerca sulla fibrosi cistica - onlus



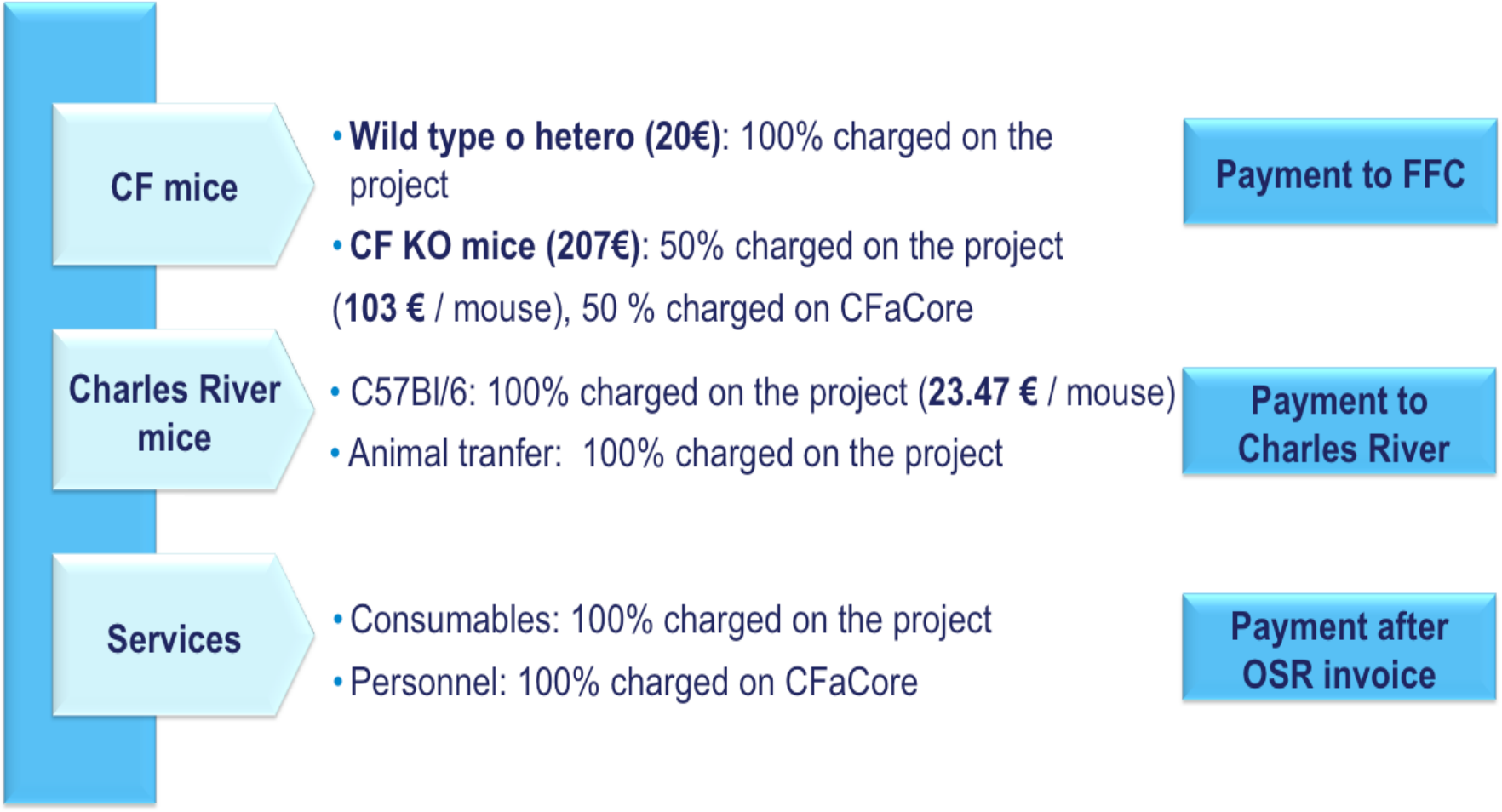
CFTR $^{-/-}$ **wt** **he**

103 € **20 €**

Toxicity studies
First efficacy studies of novel drugs
Set-up experiments

Differences between wild-type and CF mice
Final experiments for pubblication

Costi


CF mice
• **Wild type o hetero (20€)**: 100% charged on the project
• **CF KO mice (207€)**: 50% charged on the project
(**103 €** / mouse), 50 % charged on CFaCore
Payment to FFC
Charles River mice
• C57Bl/6: 100% charged on the project (**23.47 €** / mouse)
• Animal tranfer: 100% charged on the project
Payment to Charles River
Services
• Consumables: 100% charged on the project
• Personnel: 100% charged on CFaCore
Payment after OSR invoice

Come accedere al servizio

PROPOSTA BANDO FFC

- Contatto CFaCore-ricercatore
- Proposta del piano sperimentale
- Collaborazione CFaCore nel progetto
- Analisi statistica numero di animali da utilizzare
- Proposta budget

META' FEBBRAIO 2022

NEGOZIAZIONE PROGETTI FFC

- Revisione piano sperimentale in accordo ai referees e budget
- Preventivo di spesa
- Richiesta approvazione sperimentazione al comitato etico

LUGLIO 2022

ESECUZIONE PROGETTI FFC

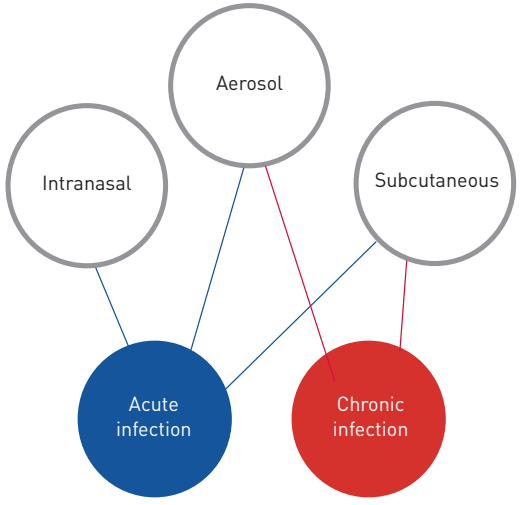
- Richiesta di risultati solidi in vitro
- Esecuzione di un esperimento pilota
- Report al ricercatore
- Esecuzione esperimento completo / modifica del protocollo

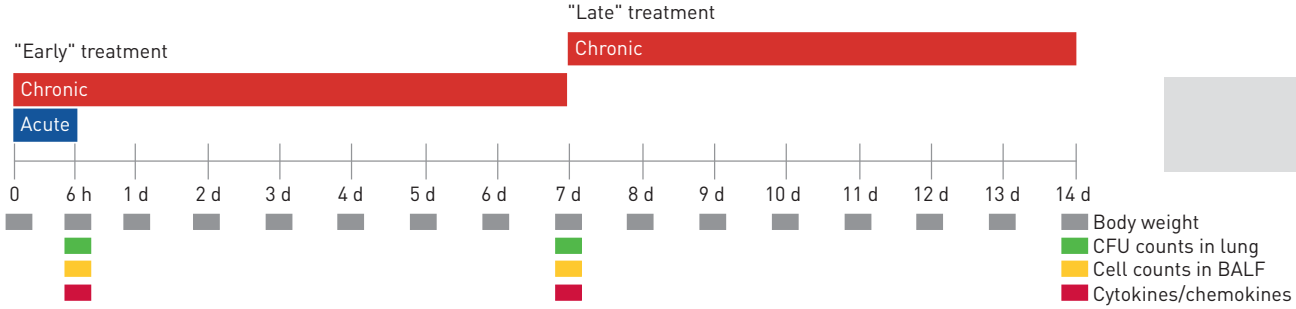
DA CONCORDARE

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

- Whether and how efficacy testing differs in murine models of acute vs chronic respiratory infection
- 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

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





ORIGINAL ARTICLE
LUNG INFECTION

Antibiotic efficacy varies based on the infection model and treatment regimen for *Pseudomonas aeruginosa*

Cristina Cigana[1,2], Serena Ranucci[1], Alice Rossi[1], Ida De Fino[1], Medede Melessike[1] and Alessandra Bragonzi[1,2]

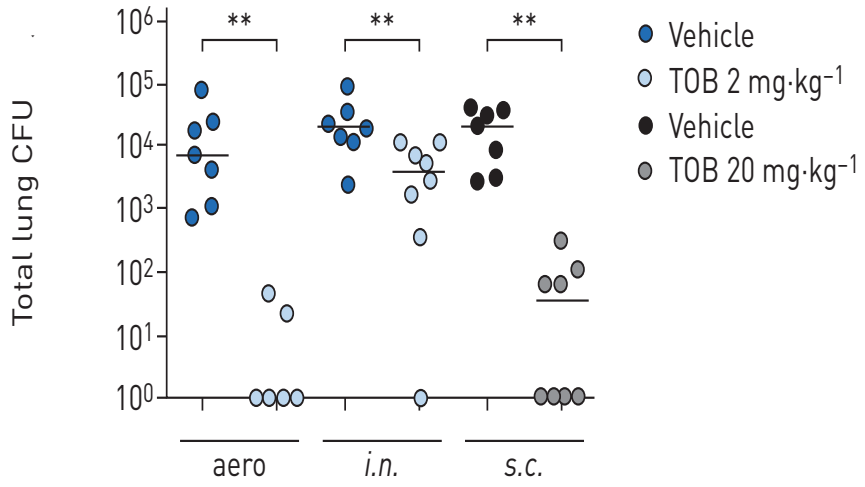
Affiliations: [1]Infections and Cystic Fibrosis Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milano, Italy. [2]Both authors contributed equally and share senior authorship.

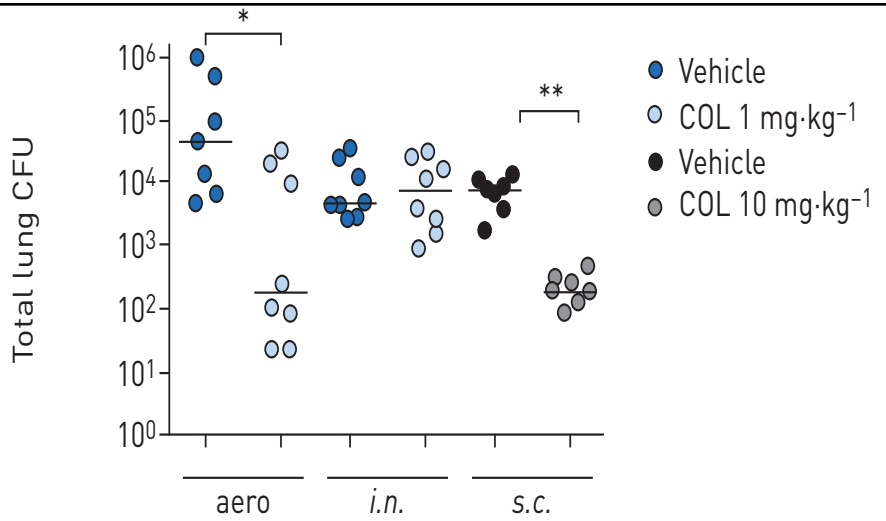
Correspondence: Alessandra Bragonzi, Infections and Cystic Fibrosis Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milano, Italy.
E-mail: bragonzi.alessandra@hsr.it

@ERSpublications
Disease-specific animal models and treatment regimens are essential in order to optimise anti-*Pseudomonas* drug testing http://bit.ly/2ISfBiB

TOB and COL efficacy in mouse model of acute infection

Bacterial load and pharmacokinetics analysis

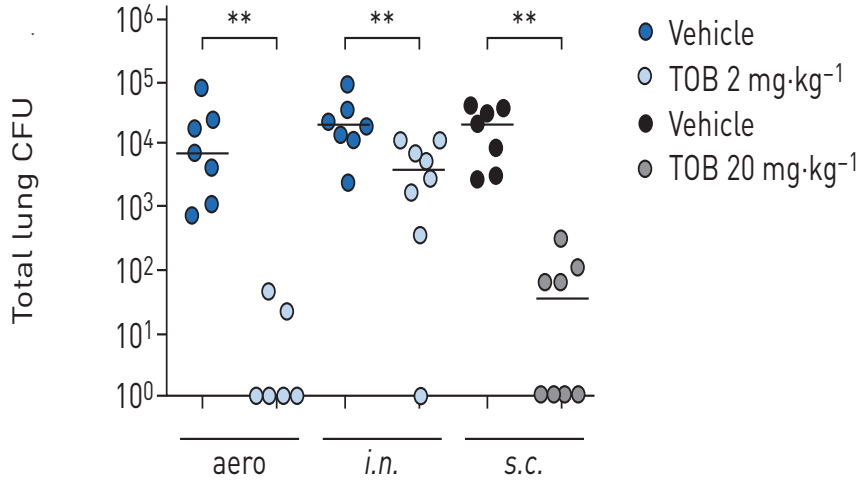


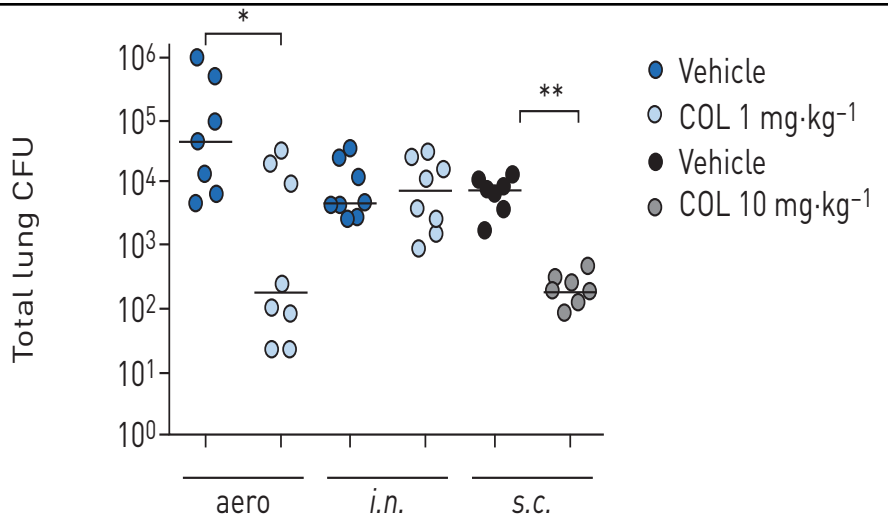


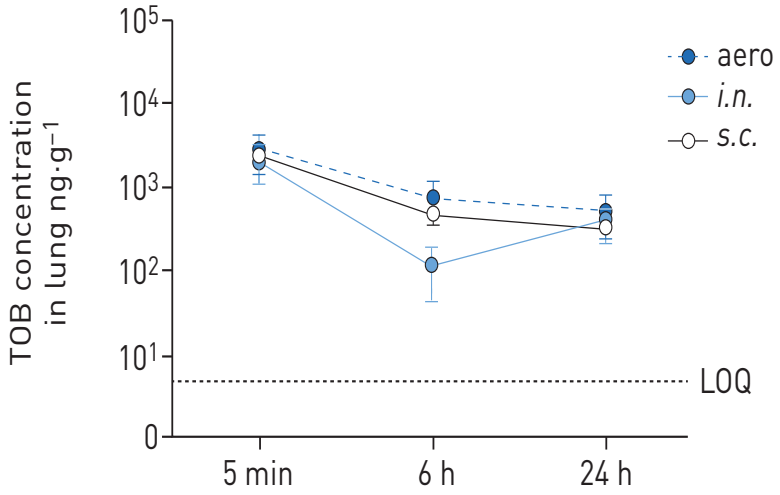
Cigana et al., ERJ 2020

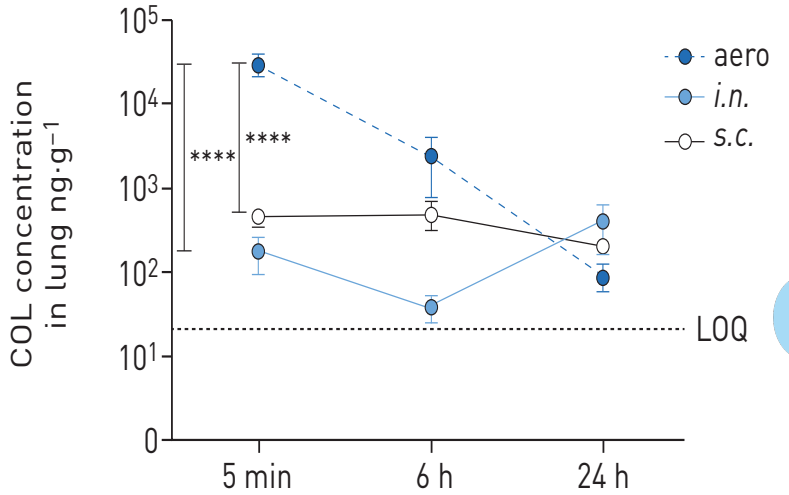
TOB and COL efficacy in mouse model of acute infection

Bacterial load and pharmacokinetics analysis









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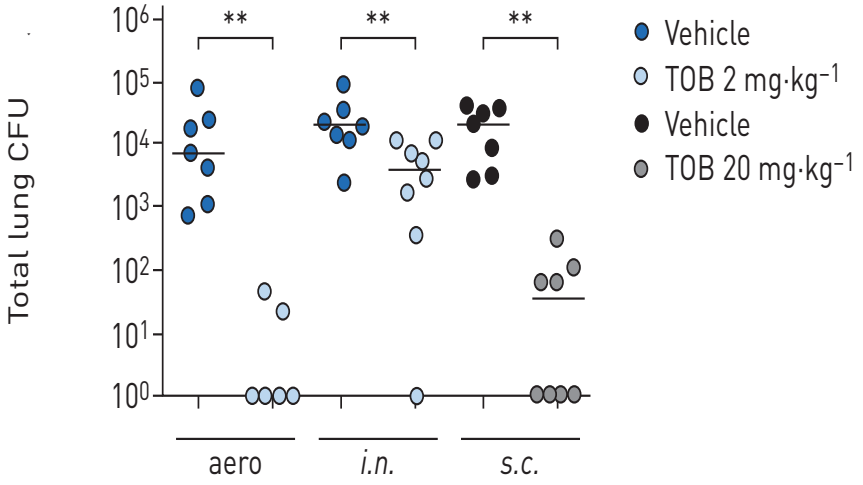
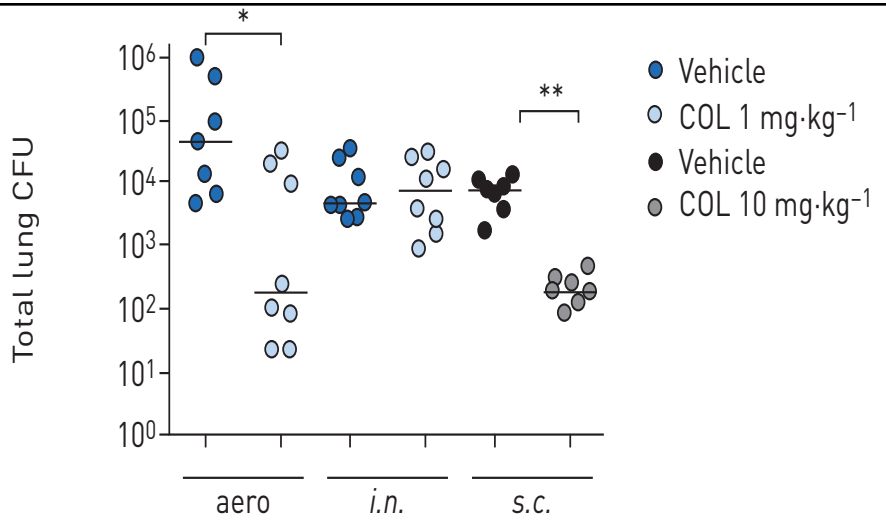
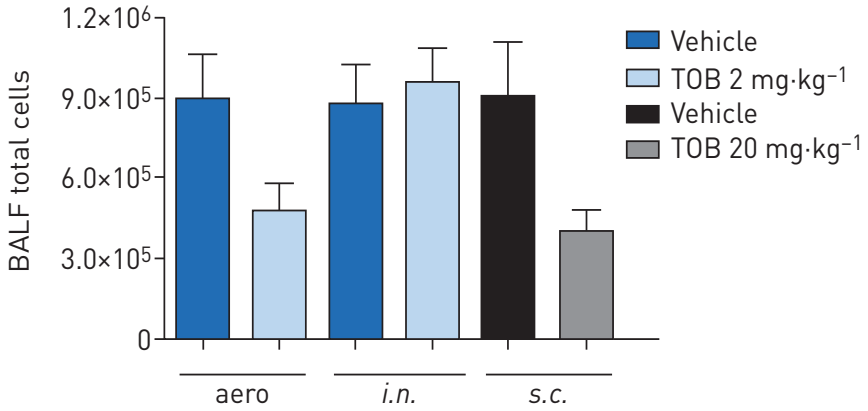
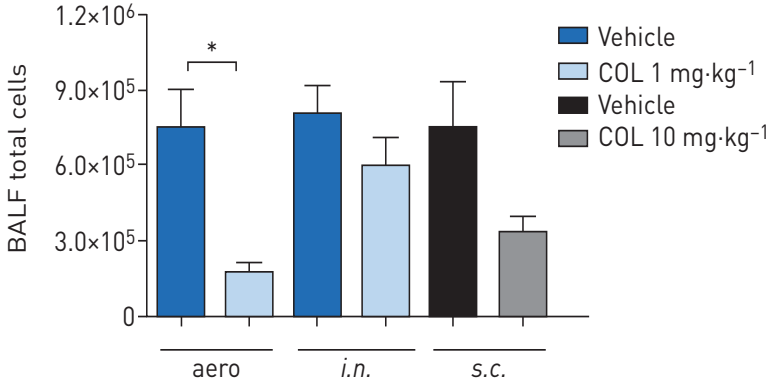
TOB and COL efficacy in mouse model of acute infection

Bacterial load and cells in broncho-alveolar lavage









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Treatment in mouse model of acute infection

Cytokines and chemokines

Cytokine / Chemokine	Level (mean pg/500 µg ± SEM)							
	Vehicle p.c.	TOB p.c.	Vehicle s.c.	TOB s.c.	Vehicle p.c.	COL p.c.	Vehicle s.c.	COL s.c.
IL-1α	76.65±0.40	79.00±8.43	87.51±10.76	60.14±5.34*	89.22±3.69	32.12±0.80***	71.86±10.94	39.26±4.19**
IL-2	6.23±4.01	6.62±0.59	5.96±0.45	5.99±0.16	6.18±0.42	6.43±0.15	6.18±0.24	6.01±0.19
IL-3	1.49±0.17	1.20±0.06	1.33±0.14	1.23±0.07	1.30±0.05	1.27±0.09	1.19±0.07	1.09±0.07
IL-4	3.37±0.23	2.76±0.19	3.10±0.34	2.62±0.22	2.84±0.23	2.21±0.21	2.33±0.22	2.09±0.15
IL-5	5.76±0.62	6.77±0.67	5.53±0.42	4.46±0.17	5.30±0.18	9.69±1.43**	4.49±0.51	3.91±0.35
IL-6	51.96±10.57	10.75±1.27*	80.74±18.29	6.30±0.80****	95.96±19.19	8.90±0.78****	37.55±9.90	9.70±1.02
IL-10	11.33±1.52	10.25±1.14	9.58±0.97	9.00±0.82	9.33±0.52	9.17±0.79	8.04±0.53	7.67±0.52
IL-12p40	31.46±1.55	30.80±5.78	36.57±3.54	27.28±2.68	27.85±2.55	19.97±0.30	28.26±2.45	22.96±2.37
IL-12p70	25.33±2.34	18.09±1.76	28.70±3.09	18.24±1.29**	26.86±2.00	16.65±1.00***	22.83±1.67	17.73±1.18*
IL-17A	5.54±1.20	4.40±0.66	6.30±1.08	4.88±0.32	4.59±0.55	6.17±0.45	4.63±0.66	4.95±0.42
Eotaxin	2036±365	1112±180*	2353±192	778±42.52***	2197±293	1307±276	1842±297	1011±82.36*
G-CSF	366±62.42	77.16±11.46**	516±92.23	43.44±8.56****	586±100	38.87±1.83****	279±49.68	52.66±8.57*
GM-CSF	36.32±5.24	20.27±0.86*	34.34±3.62	20.10±0.64*	36.27±3.73	18.45±0.73**	25.85±3.67	18.36±1.21
IFN-γ	26.45±0.74	28.33±2.96	25.70±1.09	26.42±0.70	25.71±2.00	24.20±0.86	23.94±1.48	26.53±3.42
KC	1136±229	282±33.36**	1402±236	276±41.22***	1722±201	226±82.49****	853±145	365±34.11*
MCP-1	942±206	368±62.05	1224±289	339±50.89**	1259±194	201±13.15****	615±105	320±27.06
MIP-1α	118±16.58	49.68±10.51*	153±28.71	49.52±11.41**	146±32.71	5.88±0.12***	69.84±5.65	23.51±3.39
MIP-1β	59.13±7.72	36.31±4.90*	67.52±6.45	36.21±3.00**	66.20±5.23	30.88±0.37***	57.28±7.09	37.61±2.16*
RANTES	127±12.29	85.93±13.03	129±14.42	81.00±7.62*	127±12.48	46.20±1.21****	117±12.21	68.67±5.54**
TNF-α	165±24.18	97.01±14.73*	180±22.34	83.42±4.56**	159±12.40	71.83±2.25***	138±17.32	75.77±4.71**

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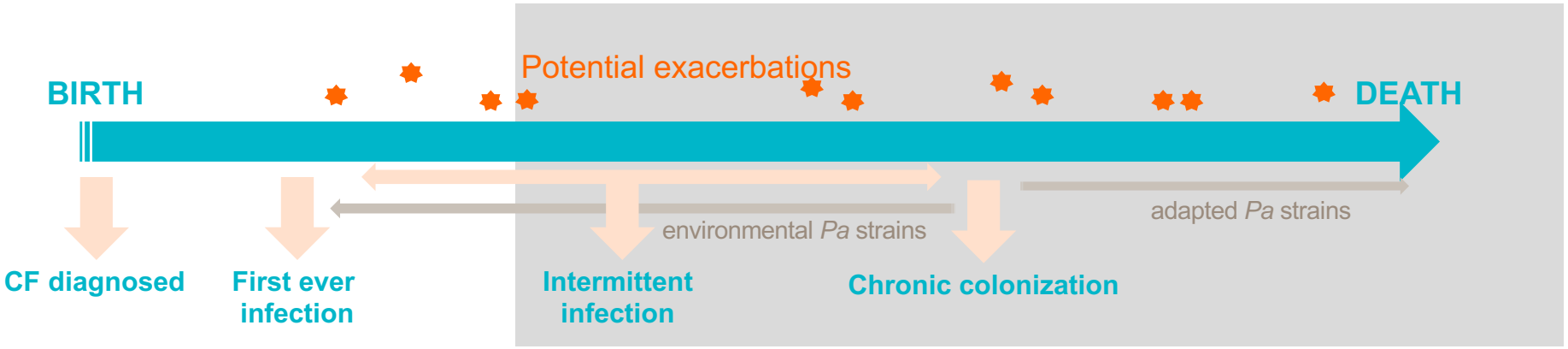
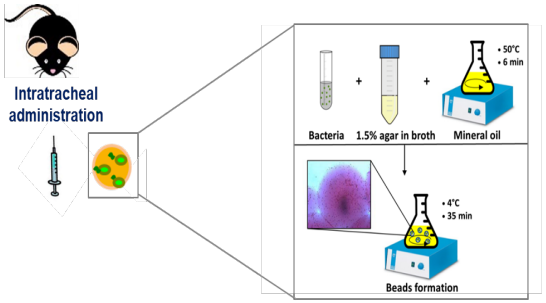
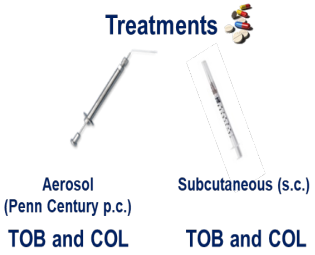
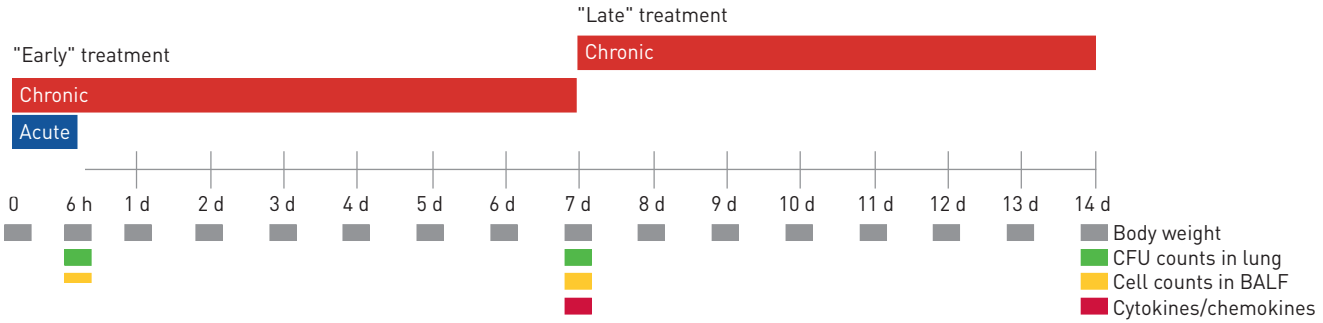
Mouse model of chronic infection and schedule of early and late treatment



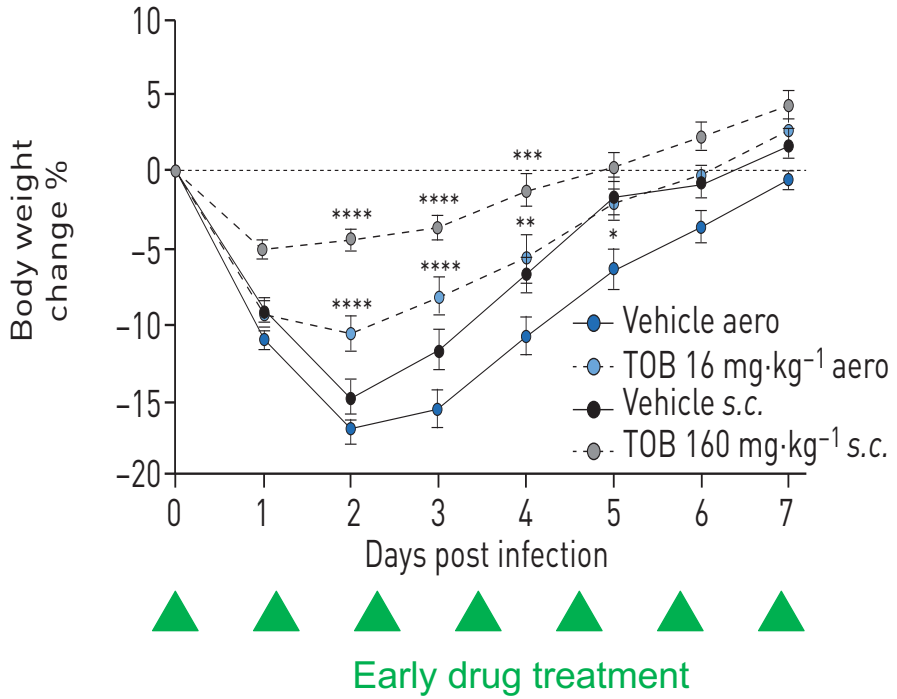


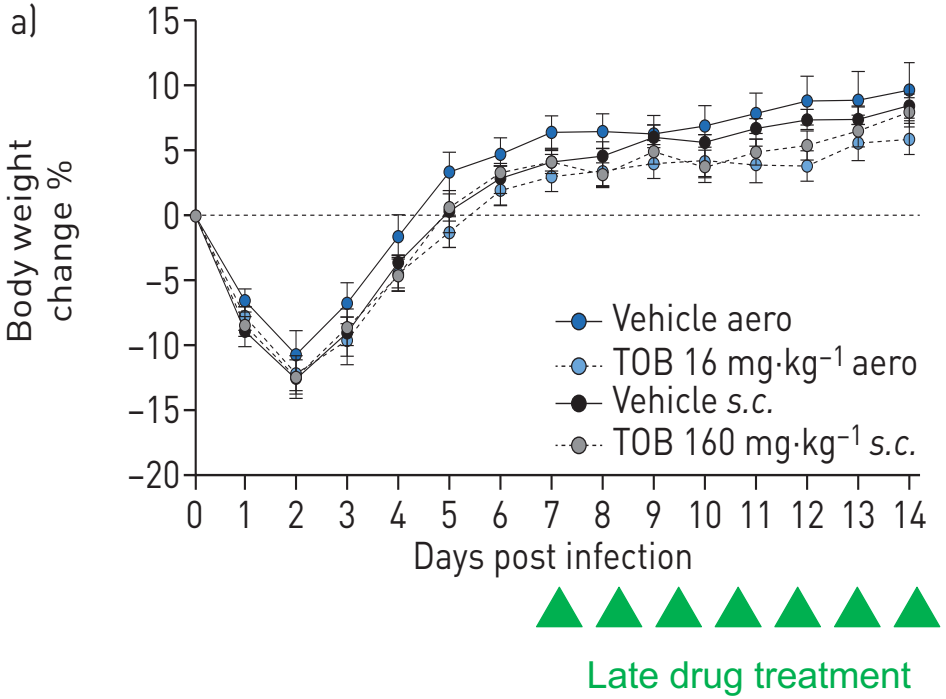




Early vs late treatment in mouse model of chronic infection

Body weight





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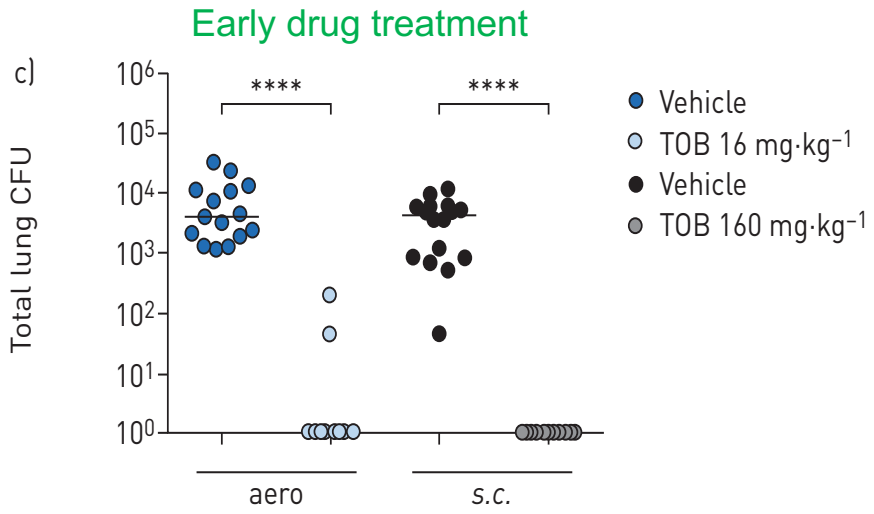
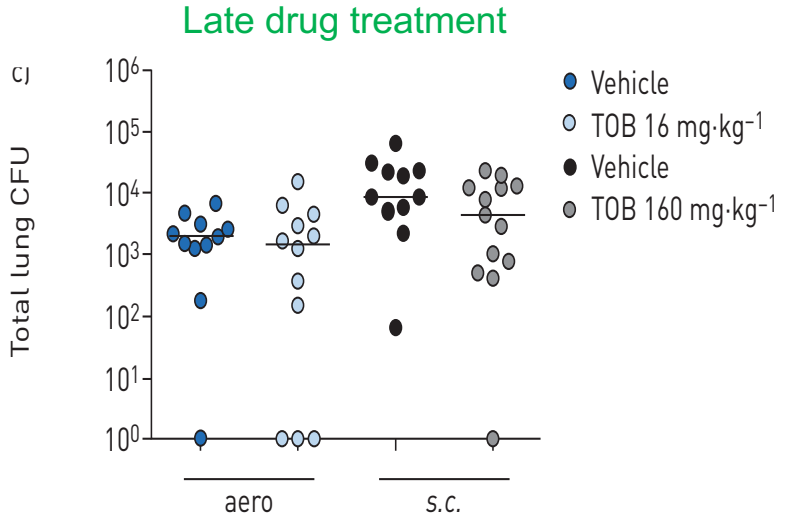
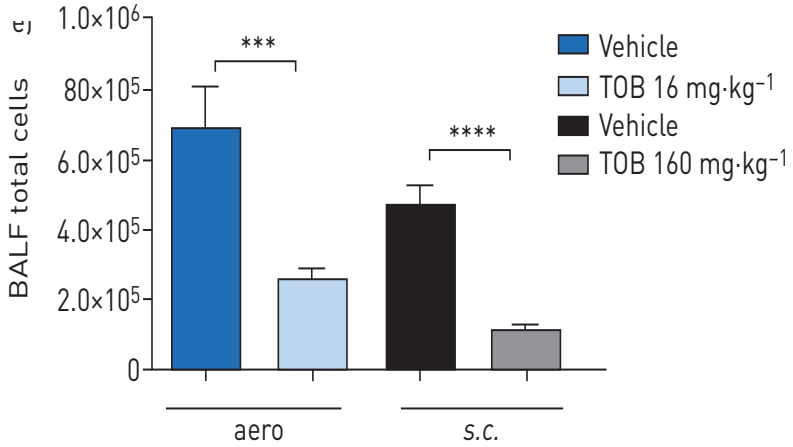
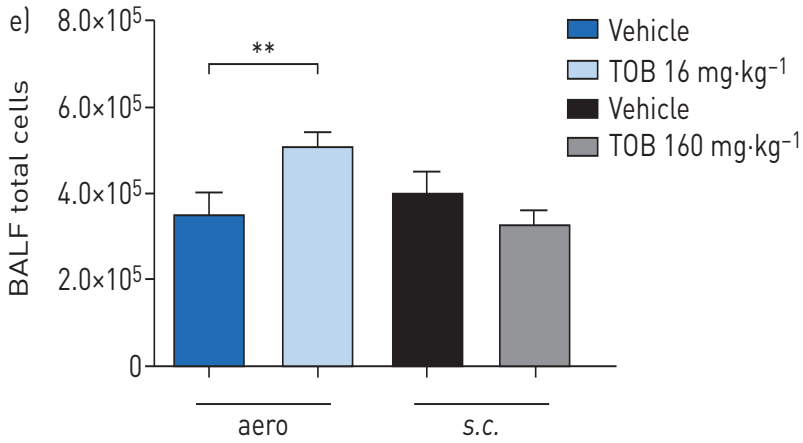
Early vs late treatment in mouse model of chronic infection

Bacterial load and cells in broncho-alveolar lavage









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Early vs late treatment in mouse model of chronic infection

Cytokines and chemokines

Cytokine / Chemokine	Level (mean pg/500 µg ± SEM)			
	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*
IL-1β	7.07±1.07	8.90±0.74	6.34±0.41	7.94±1.07
IL-2	4.33±0.09	7.49±0.89**	5.49±0.59	5.86±0.04
IL-3	2.24±0.14	2.52±0.18	2.44±0.17	2.89±0.07
IL-5	2.40±0.22	2.51±0.06	2.37±0.09	3.10±0.15**
IL-6	4.88±0.20	3.72±0.35*	4.45±0.34	3.15±0.08*
IL-9	16.49±0.48	19.44±1.21*	17.78±0.25	15.18±0.11
IL-10	9.44±0.53	9.65±0.83	10.65±0.60	10.41±0.47
IL-12p40	68.07±6.68	37.67±3.42***	59.37±4.66	32.50±2.47**
IL-12p70	26.77±2.45	20.46±1.77	26.56±2.86	22.80±1.42
IL-13	63.53±4.62	81.55±4.38*	74.97±3.83	83.52±3.25
IL-17A	15.08±2.24	6.01±0.17**	16.82±1.37	7.60±0.82**
eotaxin	637±67.26	489±59.16	634±31.45	448±47.34
G-CSF	8.50±0.95	7.26±0.00	8.26±0.51	7.26±0.00
GM-CSF	11.82±1.16	12.22±1.47	12.28±0.64	14.61±0.91
IFN-γ	24.84±1.72	28.83±1.84	26.24±0.94	23.24±0.36
KC	87.11±17.82	22.96±3.38***	49.11±7.62	23.85±3.43
MCP-1	150±15.00	108±12.14	123±10.31	121±9.36
MIP-1α	21.19±4.72	3.32±0.30**	17.06±4.22	2.56±0.27*
MIP-1β	27.40±1.99	26.89±3.08	30.13±2.78	20.10±0.89*
RANTES	324±67	69.49±6.66***	234±33.58	65.56±8.55*
TNF-α	44.31±4.31	34.57±1.71*	43.25±0.31	32.25±2.79*

Cytokine / Chemokine	Level (mean pg/500 µ			
	Vehicle p.c.	TOB p.c.	Vehicle s.c.	TOB s.c.
IL-1α	8.99±0.36	10.27±0.82	9.43±0.45	10.06±1.05
IL-1β	10.00±0.59	10.64±1.21	9.59±0.79	10.91±1.46
IL-2	9.35±0.57	9.72±0.75	8.27±0.46	9.31±1.19
IL-3	3.49±0.16	3.55±0.24	3.54±0.31	3.71±0.30
IL-5	3.14±0.13	3.03±0.18	2.80±0.48	3.11±0.39
IL-6	4.00±0.22	4.40±0.09	3.67±0.12	3.99±0.32
IL-9	23.52±1.54	25.82±1.11	21.11±1.16	21.67±1.09
IL-10	8.38±0.29	8.88±0.88	7.92±1.14	8.74±1.11
IL-12p40	39.70±3.41	47.98±2.46	48.51±2.99	44.41±6.32
IL-12p70	24.39±2.27	28.76±2.99	21.45±3.02	23.51±3.02
IL-13	71.57±2.65	80.26±4.35	72.43±4.10	73.37±6.23
IL-17A	10.31±0.52	9.25±0.66	12.59±0.51	11.31±1.10
eotaxin	451±20.08	482±48.88	397±20.91	394±28.88
G-CSF	7.73±0.37	7.36±0.00	7.36±0.00	7.36±0.00
GM-CSF	12.57±1.09	12.30±0.23	9.40±1.04	12.39±1.73
IFN-γ	34.24±1.36	38.43±1.71	30.13±1.81	32.32±2.49
KC	33.99±4.66	31.75±5.09	26.09±2.53	23.63±2.23
MCP-1	139±7.78	152±19.78	129±11.02	128±6.85
MIP-1α	2.83±0.46	4.64±0.77	4.74±0.68	3.49±0.54
MIP-1β	25.51±1.33	28.58±1.10	24.45±0.91	25.58±1.81
RANTES	76.33±6.34	75.65±3.13	75.11±4.74	77.92±7.76
TNF-α	54.38±4.27	59.62±2.66	50.12±2.79	51.15±4.00

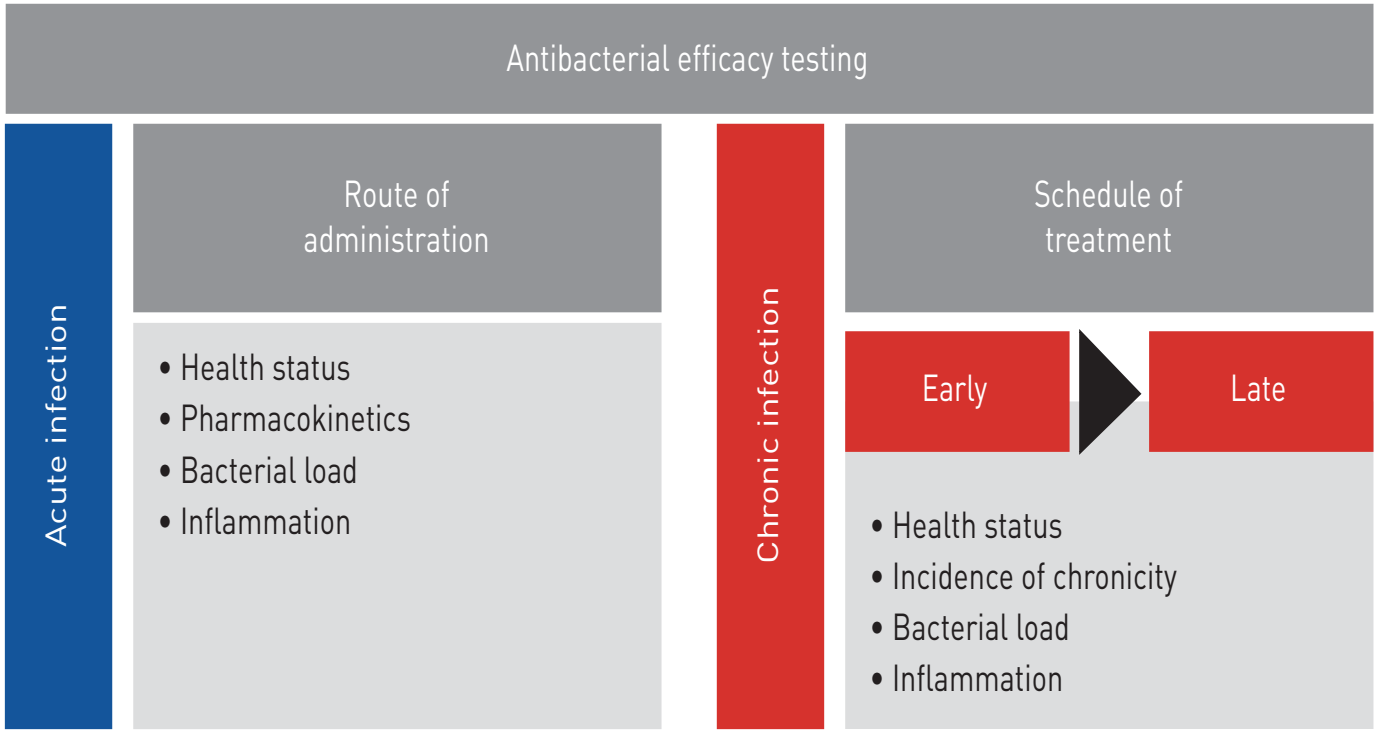
Cigana et al., ERJ 202

Conclusions

- Antibacterial efficacy is affected by the route of administration, type of antibiotic and mouse model of pulmonary infection
- The inflammatory response does not strictly reflect changes in bacterial load
- The schedule of treatment affects antibiotic efficacy during chronic *P. aeruginosa* infection

Multiple animal models and different treatment schedules are needed to properly translate results to the clinic and to optimize anti-*P. aeruginosa* drug development

Roadmap of antibacterial efficacy testing in mouse models of respiratory infection



Roadmap of Critical Milestones and Main Activities

Pre-clinical models of respiratory infection

- *Pseudomonas aeruginosa*
- *Burkholderia cenocepacia*
- *Staphylococcus aureus*
- *Nontuberculous mycobacteria*

2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021

Test antibiotic therapy

Test anti-inflammatory therapy

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Test novel vaccine

Chiesi

ANTABIO
Developing tomorrow's antibacterials

POLYPHOR

HELMHOLTZ
RESEARCH FOR GRAND CHALLENGES

University of Glasgow

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KING'S College LONDON

Aalto University

51 projects for efficacy testing studies

45 projects for CF mice

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