



Targeting the A-kinase Anchoring Function of PI3K γ for Treating Cystic Fibrosis

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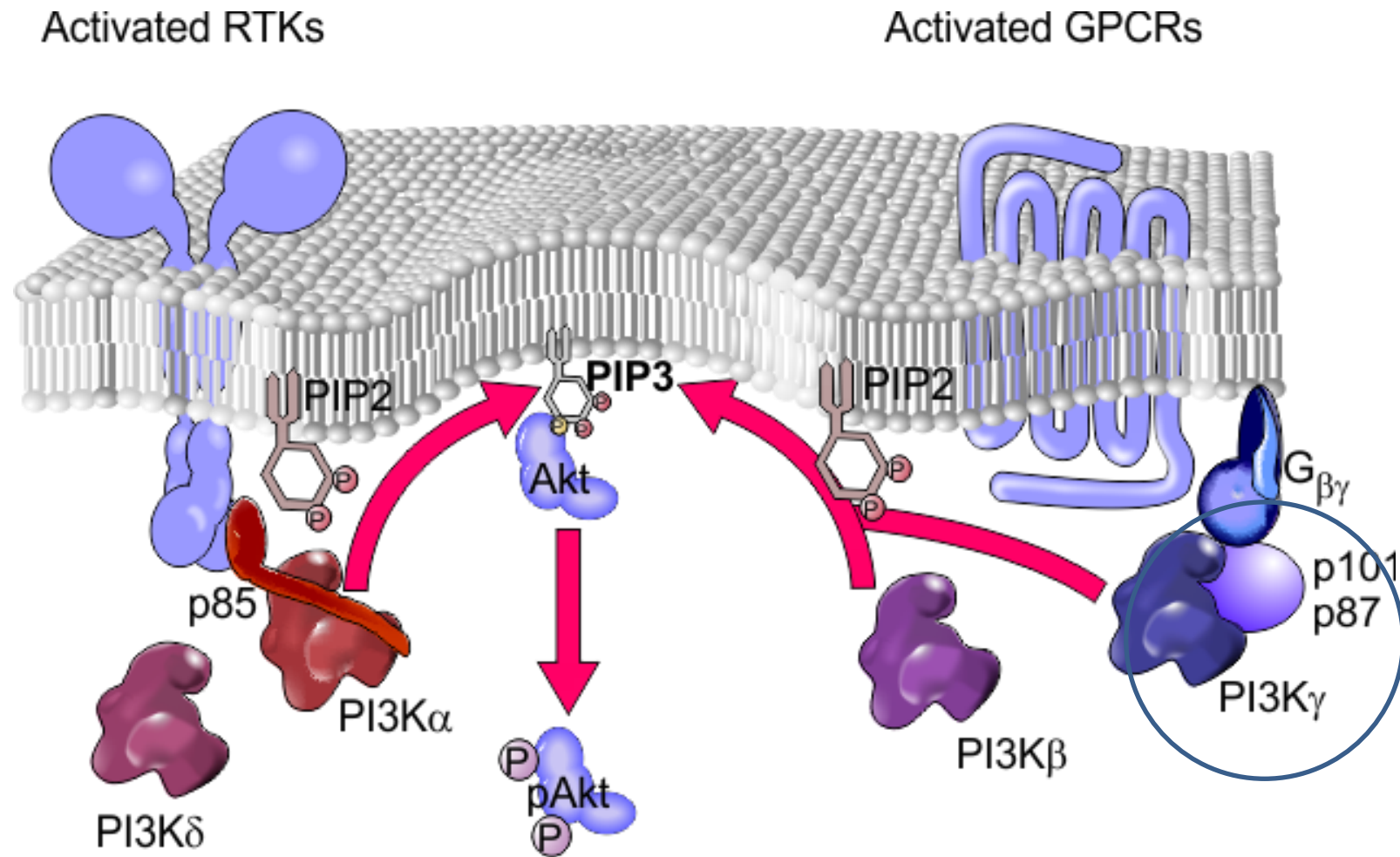
UNIVERSITÀ
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DI TORINO

Disclosure

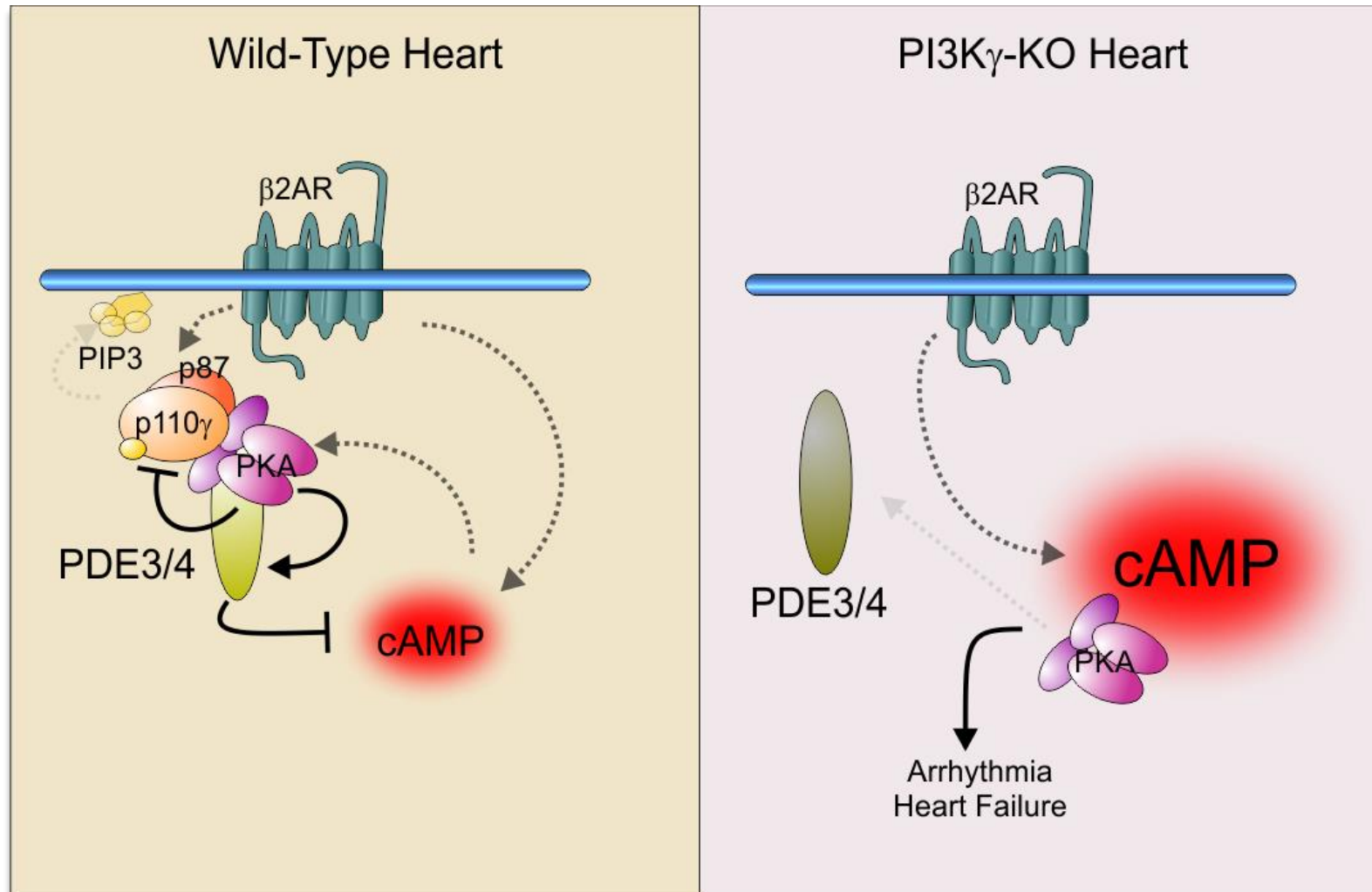
Co-founder and Board Member of Kither Biotech,
a spin-off company focused on the development
of the PI3K γ Mimetic Peptide for the treatment of
respiratory diseases



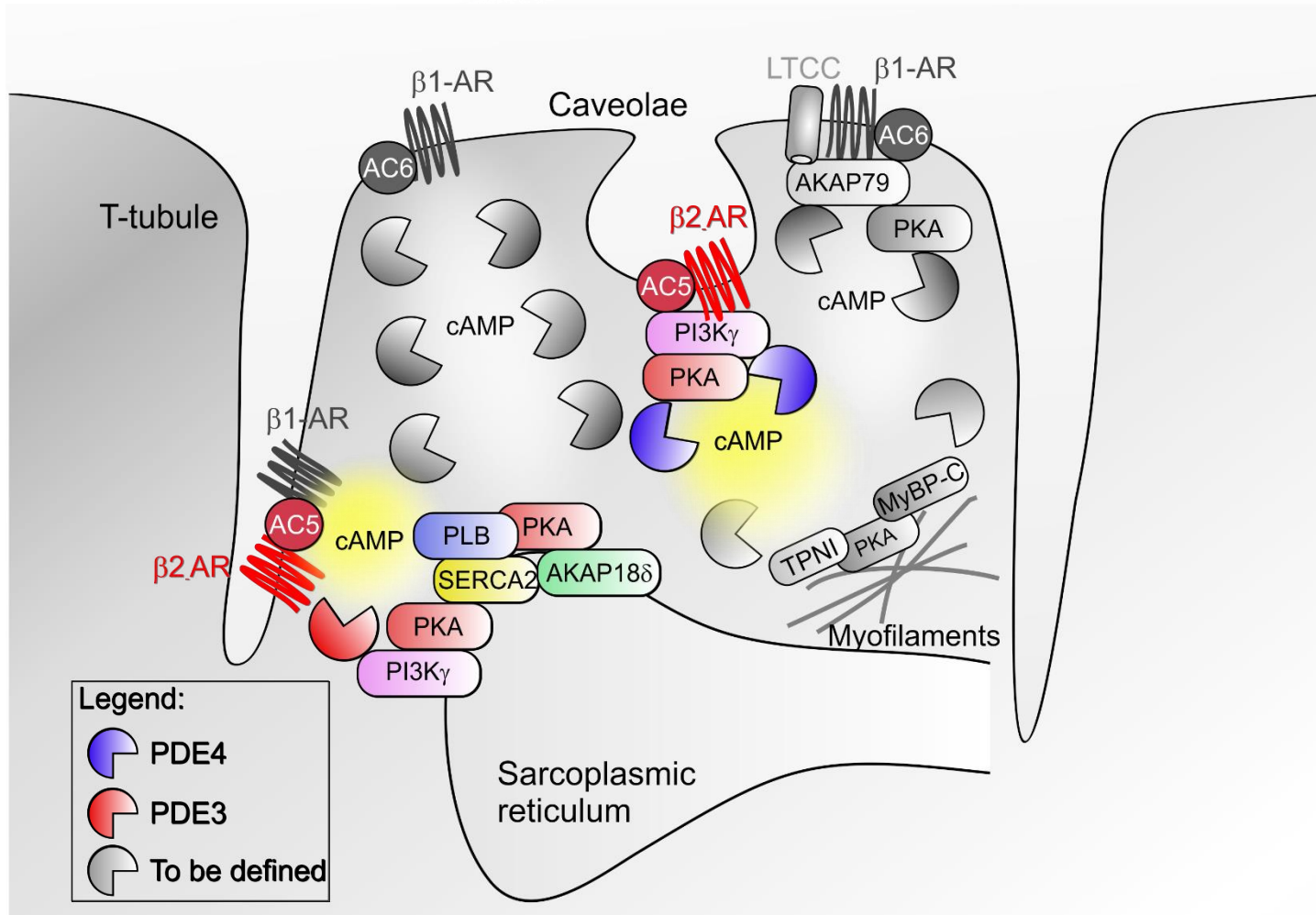
Class I Phosphatidylinositol 3-kinases (PI3Ks)



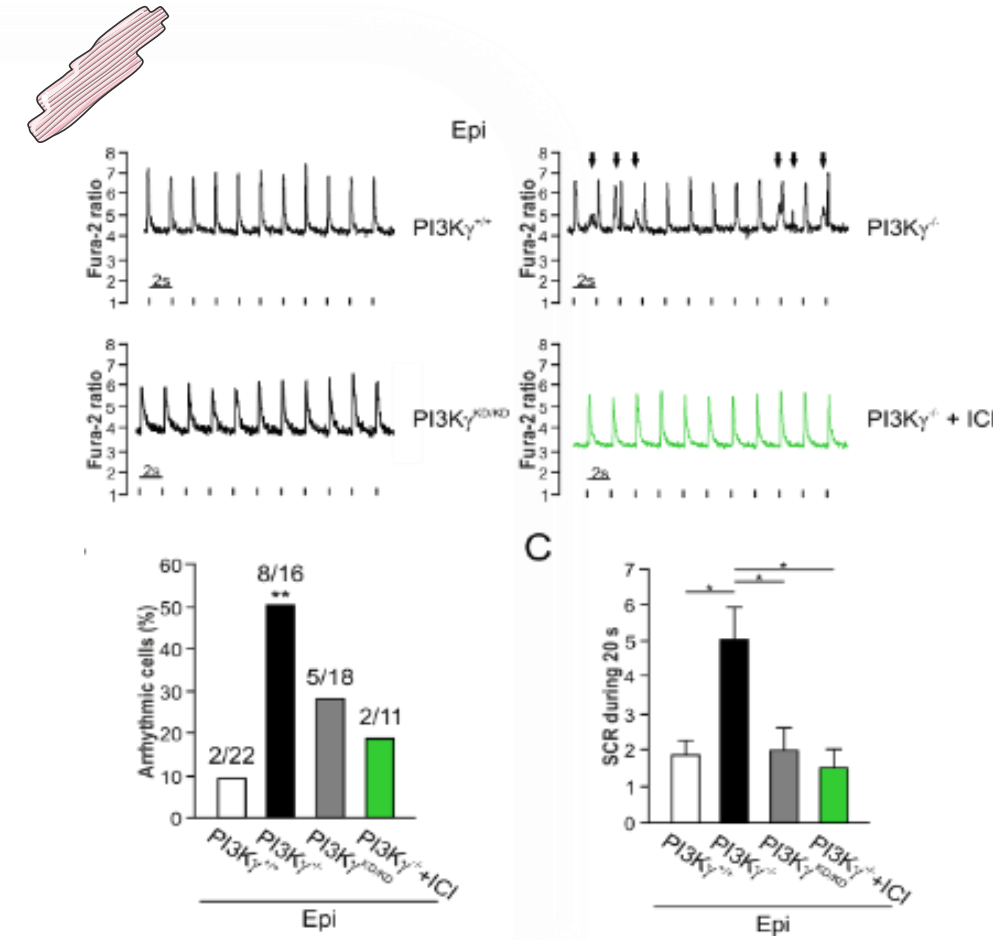
The Kinase-independent Function of PI3K γ



β_2 -AR-Induced Lethal Arrhythmias in PI3K γ KO but not PI3K γ KD Mice

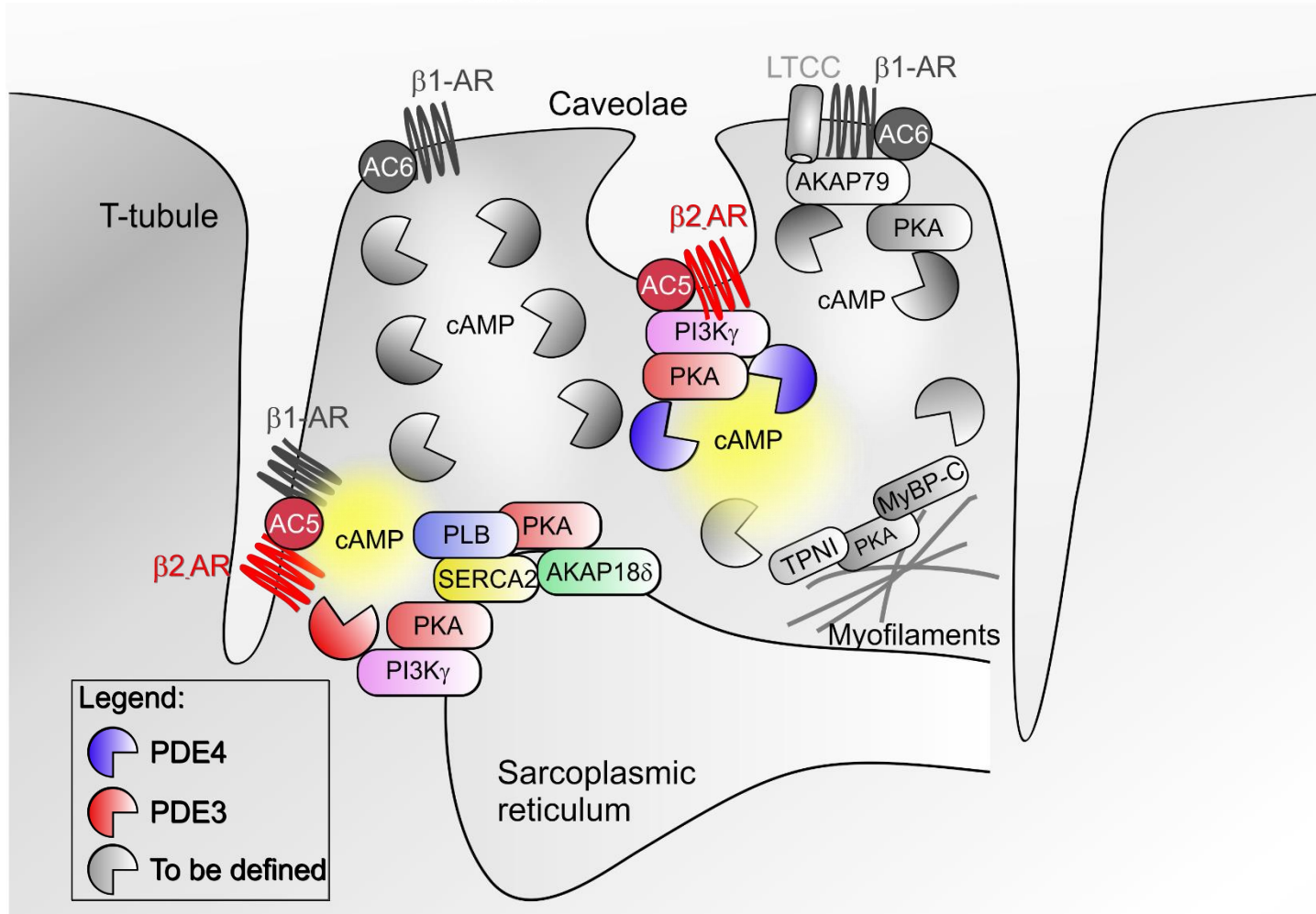


Ghigo & Mika, *J Mol Cell Cardiol.* 2019

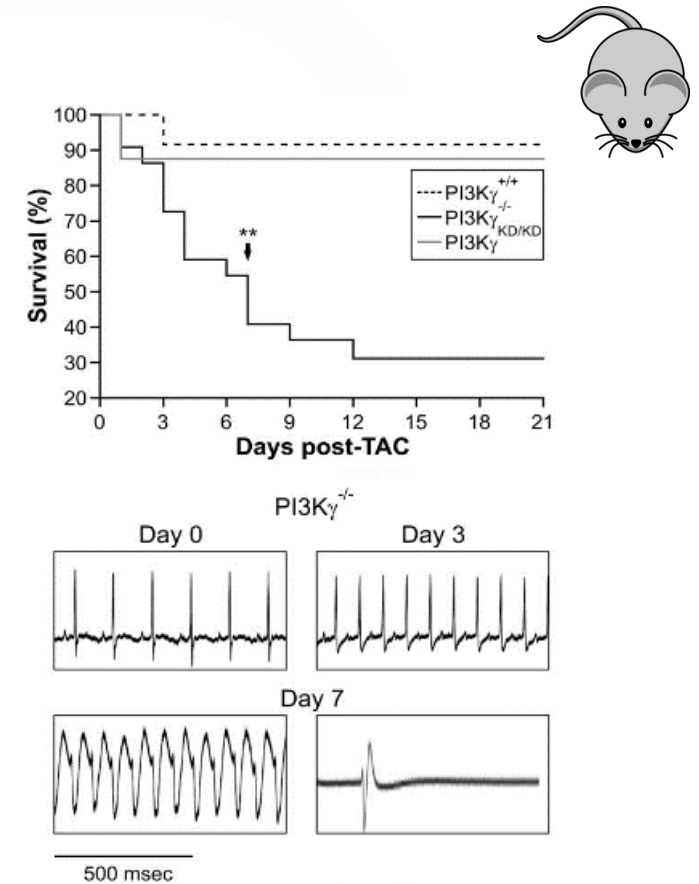


Ghigo et al., *Circulation* 2012

β_2 -AR-Induced Lethal Arrhythmias in PI3K γ KO but not PI3K γ KD Mice



Ghigo & Mika, *J Mol Cell Cardiol.* 2019



Ghigo et al., *Circulation* 2012

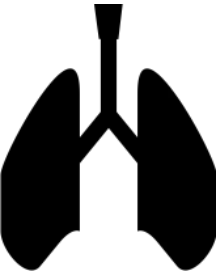
What's Bad for the Heart Might be Good for the Lungs!

cAMP is:

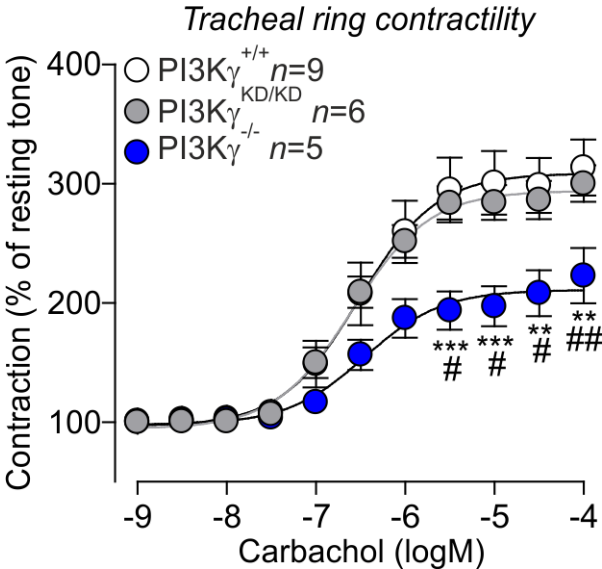


BAD
for the Heart
Heart failure:
β-blockers

BUT

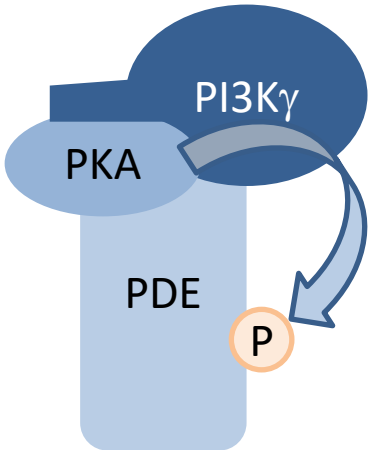


GOOD
for the LUNGS
Asthma:
Albuterol



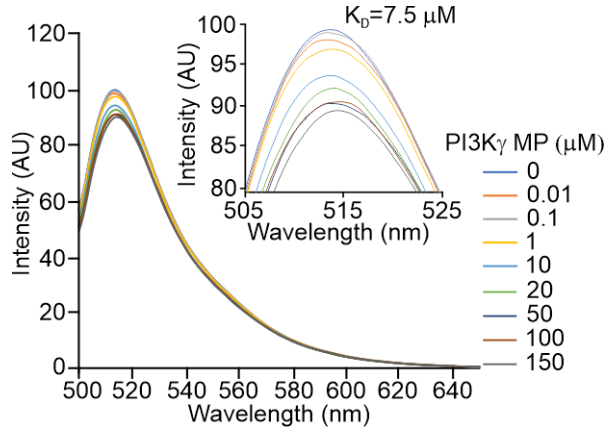
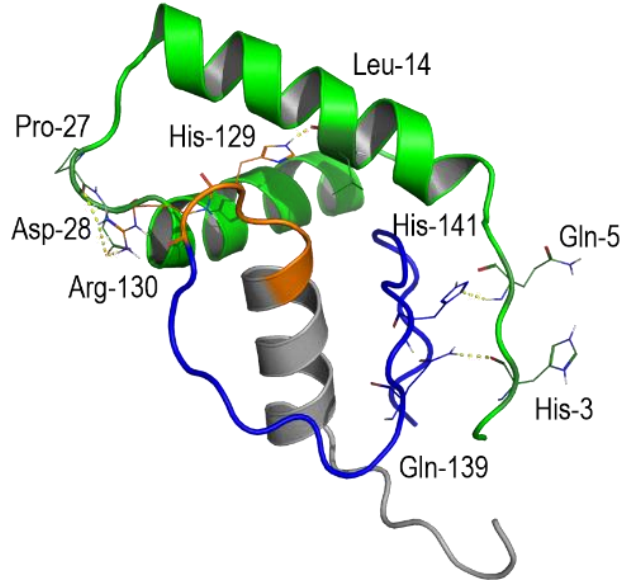
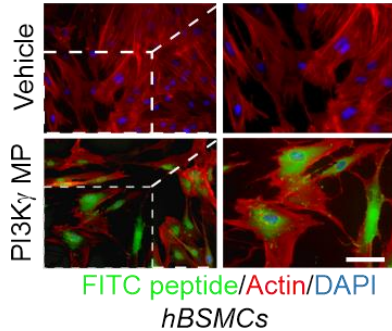
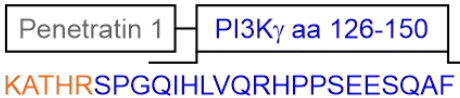
Targeting the AKAP function of PI3K γ with a Cell-Penetrating Mimetic Peptide

PI3K γ MP



PI3K γ MP internalization in Human Bronchial Smooth Muscle Cells

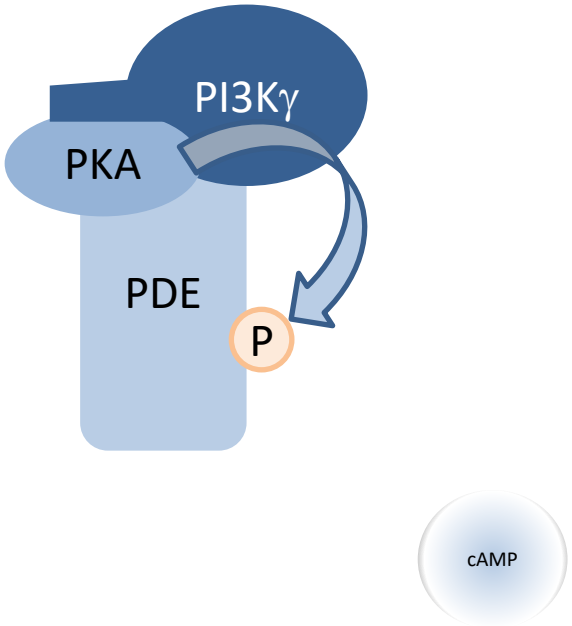
PI3K γ mimetic peptide (MP)



Hirsch et al., Science 2000
 Crackower et al., Cell 2002
 Patrucco et al., Cell 2004
 Perino et al. Mol Cell 2011
 Ghigo et al., Circulation 2012
 Ghigo et al. Circ Res 2017
 Lupieri et al. J Cell Sci 2020

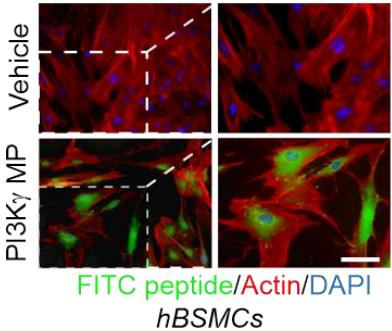
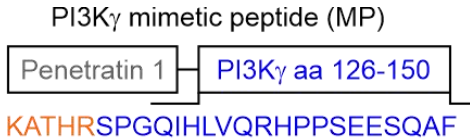
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PI3K γ MP

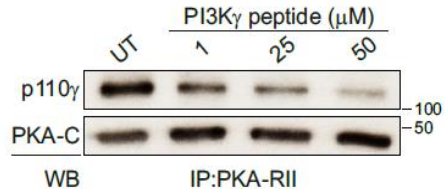


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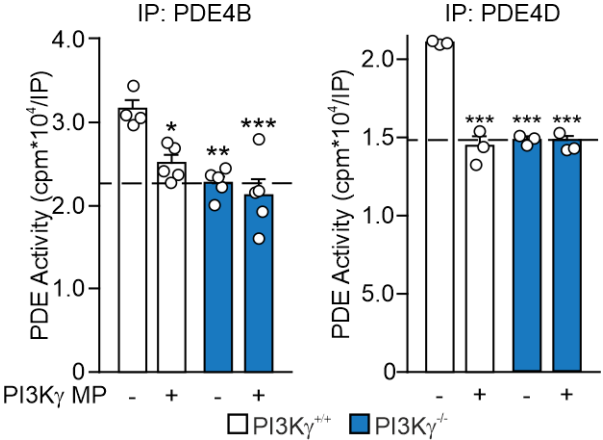
PI3K γ MP internalization in Human Bronchial Smooth Muscle Cells



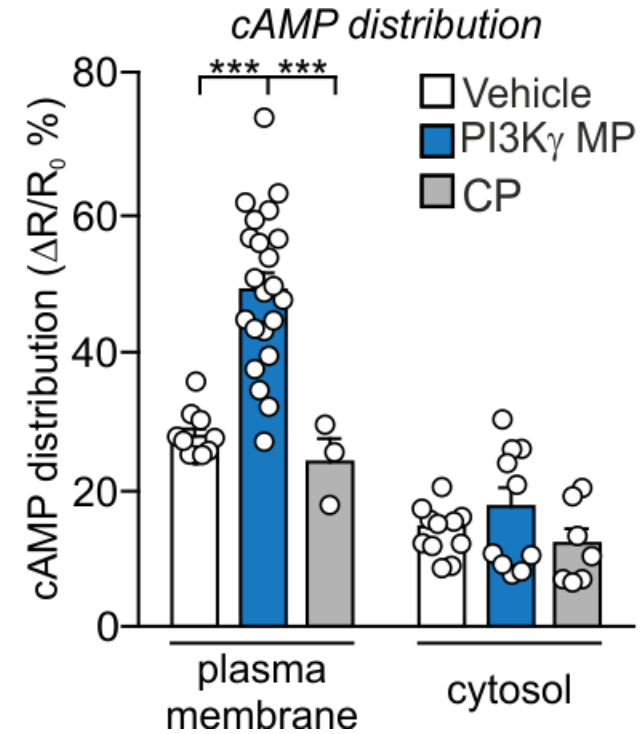
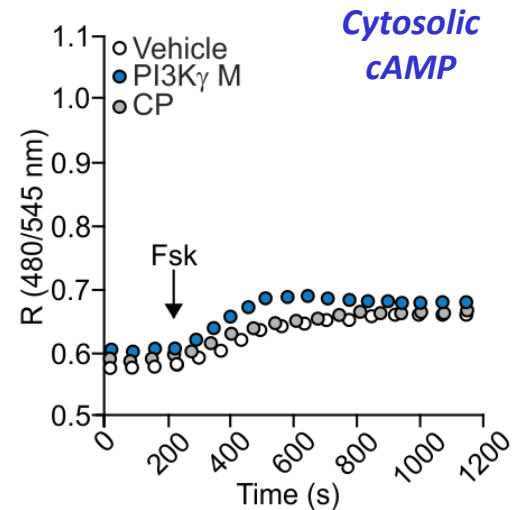
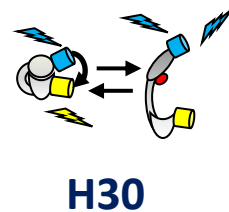
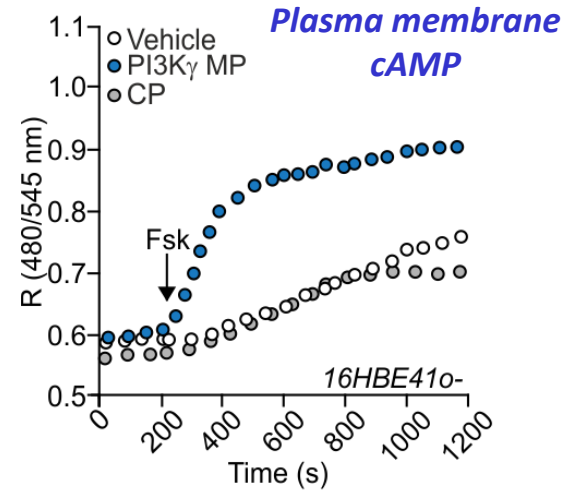
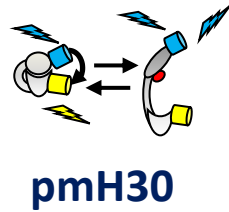
Disruption of PKA/PI3K γ binding by the PI3K γ MP



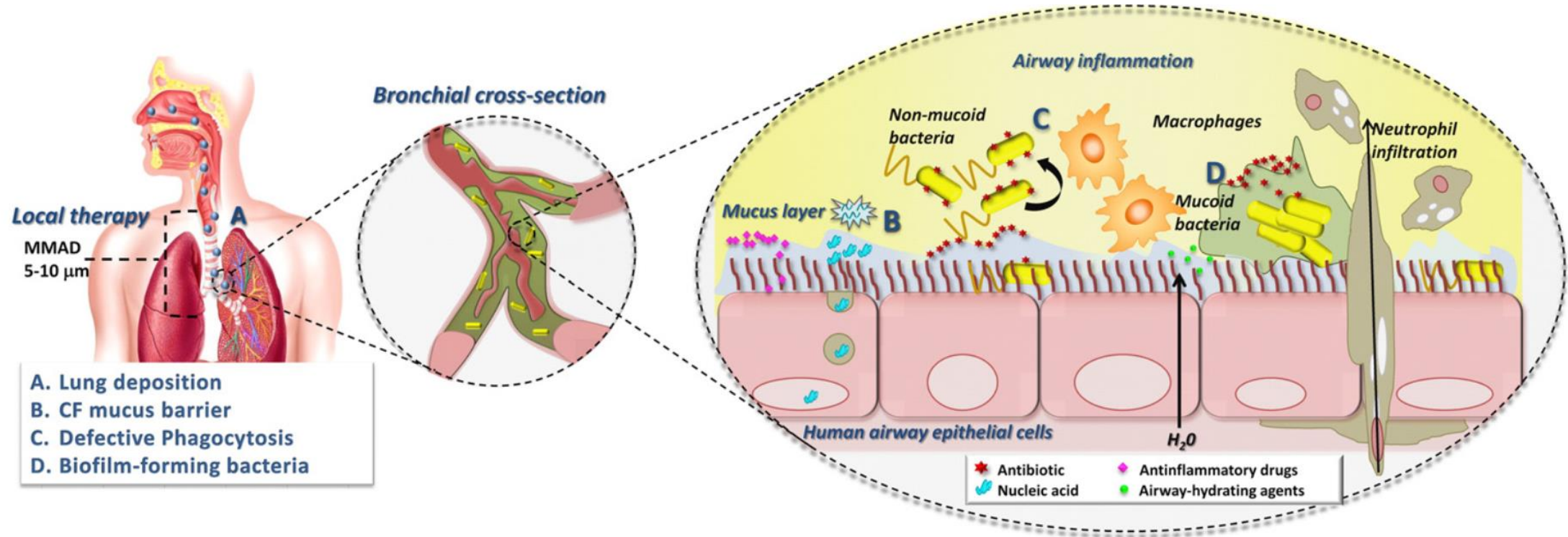
PDE4B and PDE4D inhibition by the PI3K γ MP



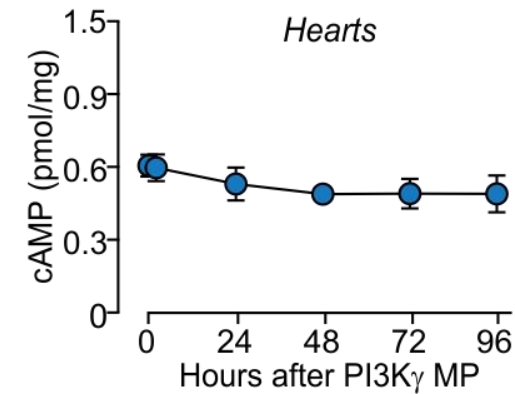
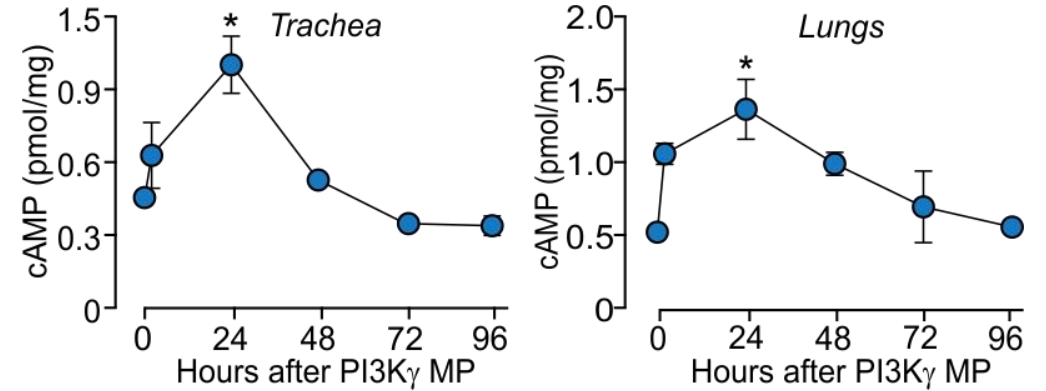
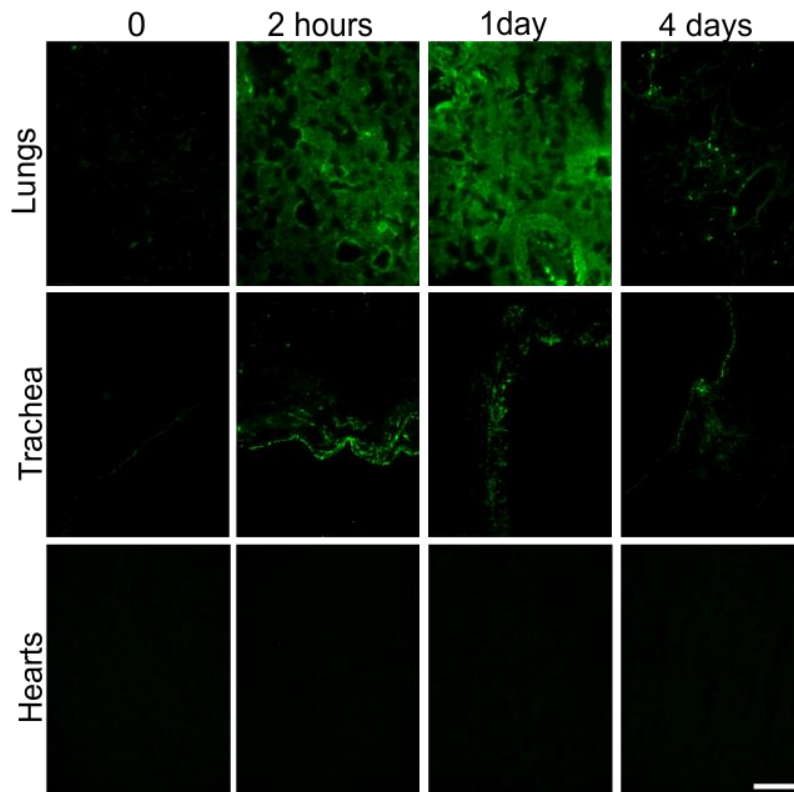
PI3K γ MP Triggers a Compartmentalized cAMP Elevation in Lung Cells



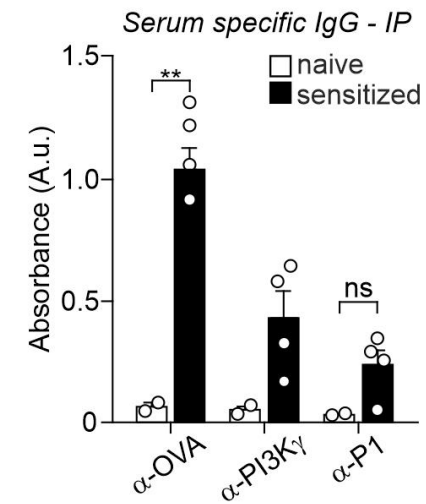
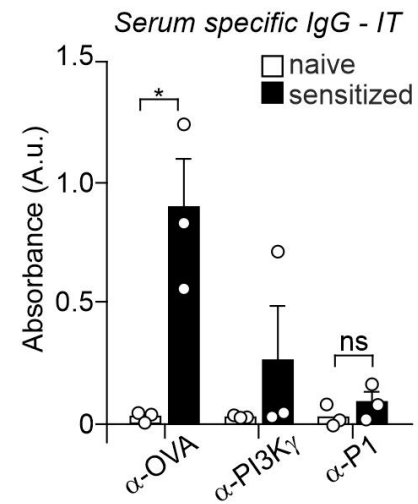
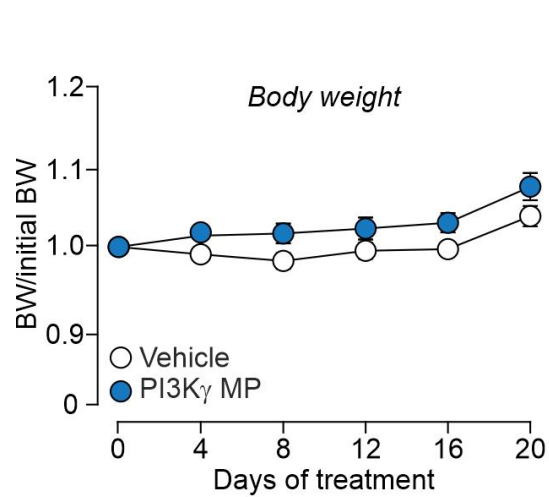
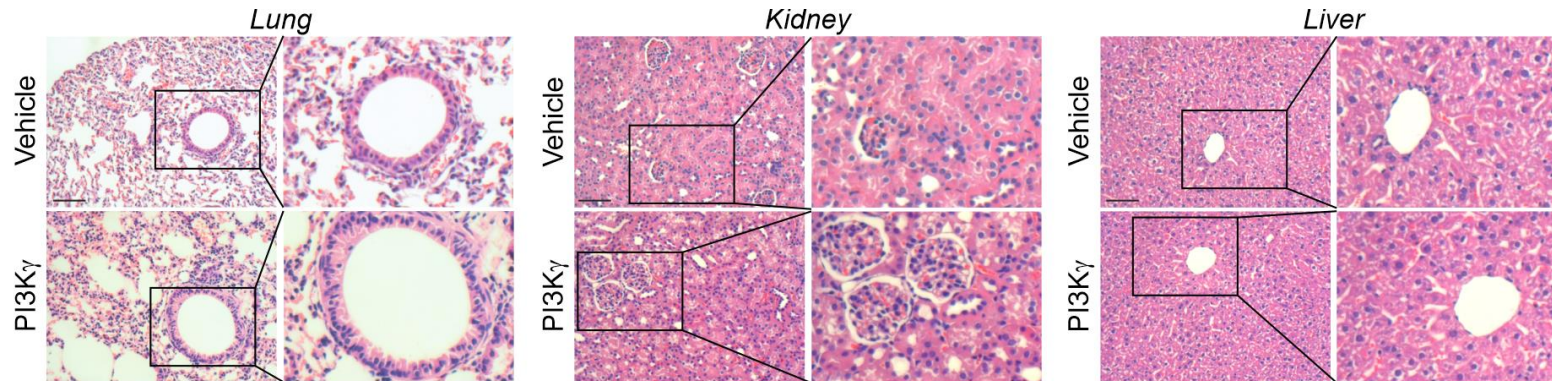
Challenges in the Development of Inhaled Treatments for Airway Diseases



Inhaled PI3K γ MP Promotes Long-lasting cAMP Elevation in the Lungs



PI3K γ MP has a Good Tolerability Profile



Therapeutic Effects of PI3K γ MP



Smooth Muscle Cells

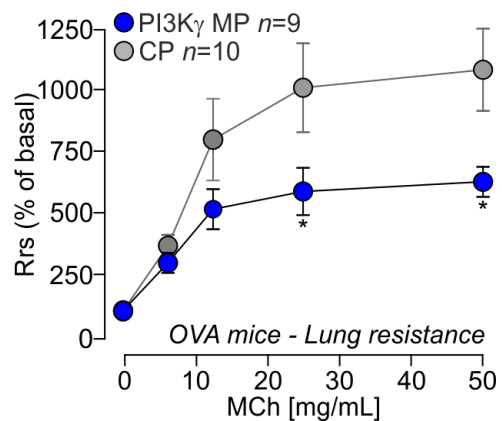


Inflammatory Cells

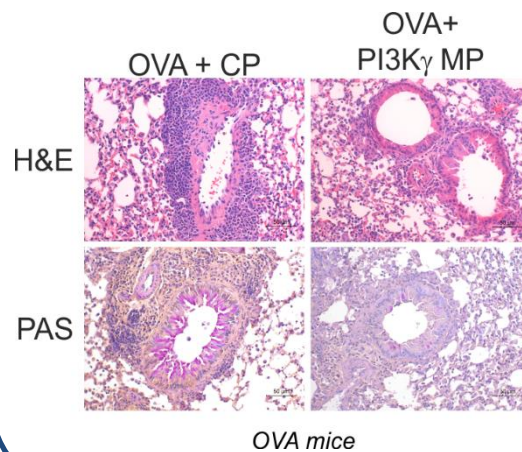


Epithelial Cells

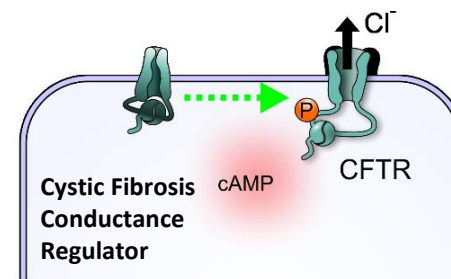
Bronchodilation



Anti-inflammatory effect



CFTR modulator



Therapeutic Effects of PI3K γ MP



Smooth Muscle Cells

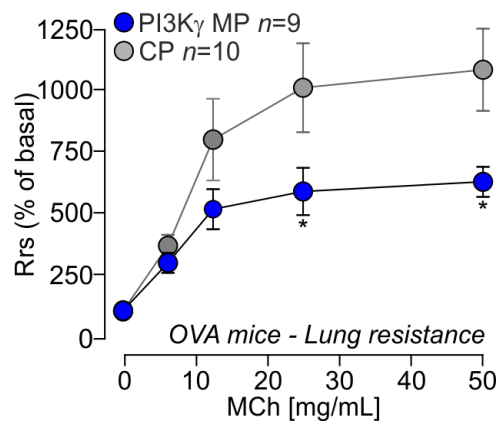


Inflammatory Cells

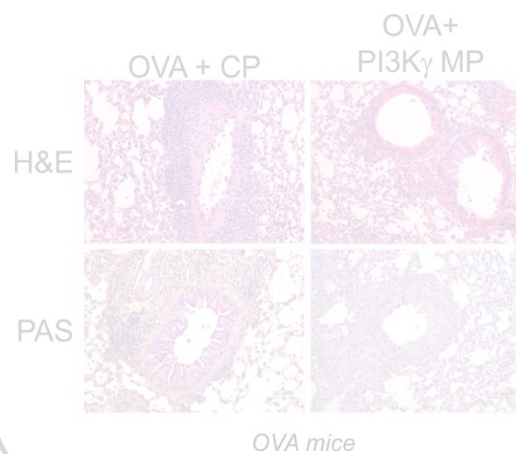


Epithelial Cells

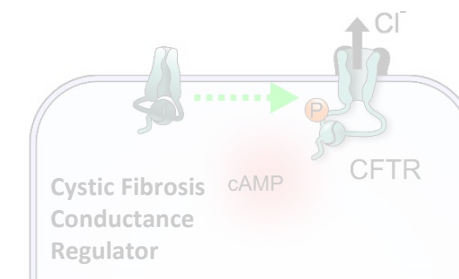
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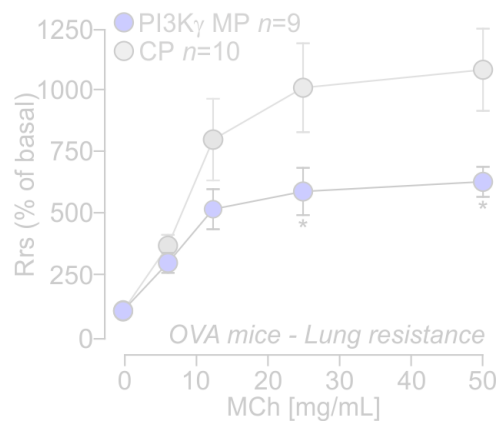


Inflammatory Cells

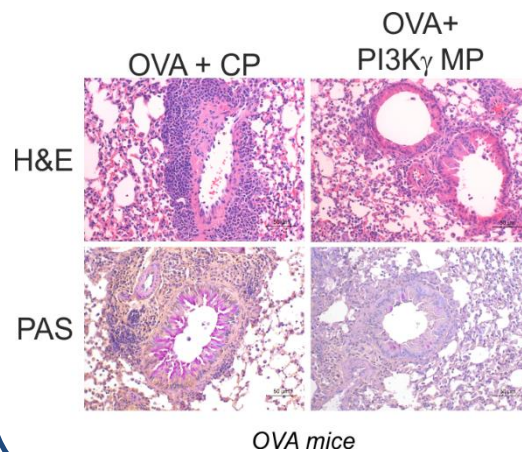


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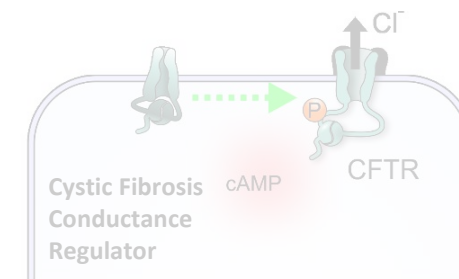
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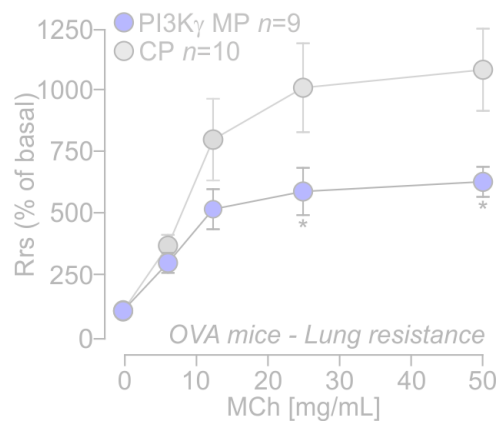


Inflammatory Cells

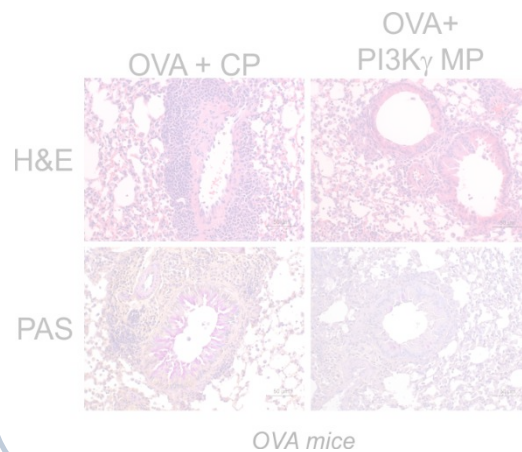


Epithelial Cells

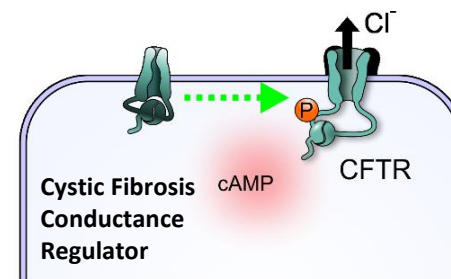
Bronchodilation



Anti-inflammatory effect



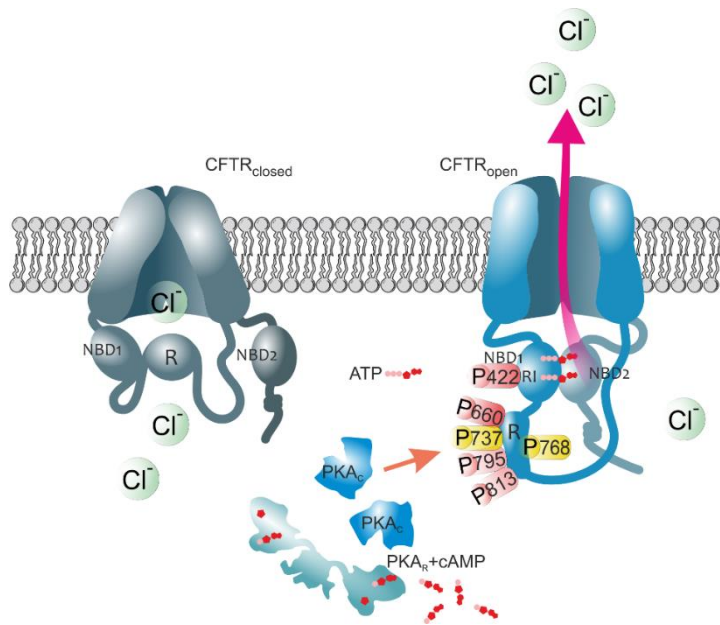
CFTR modulator



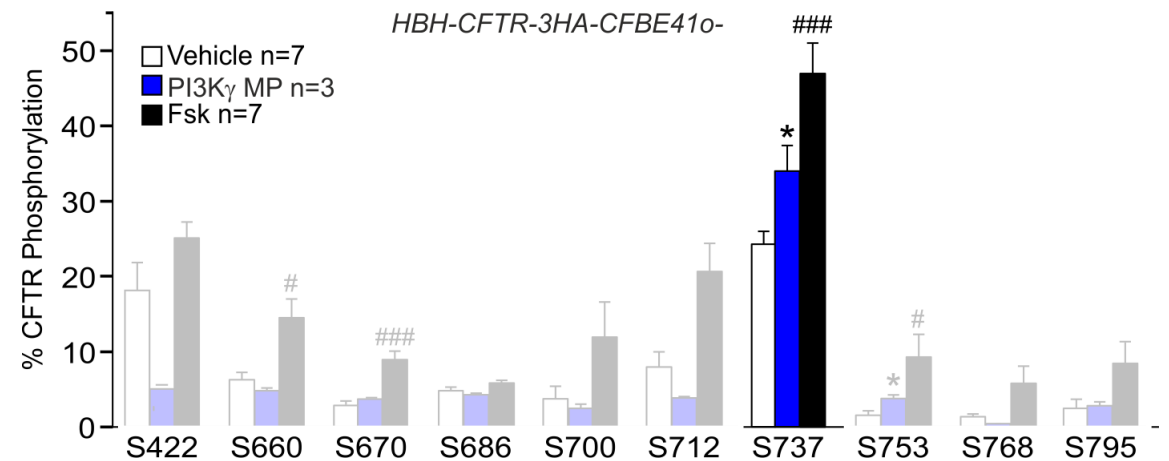
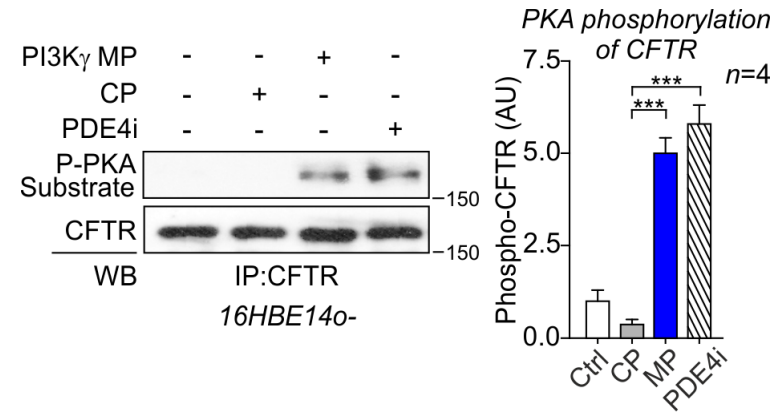
The PI3K γ MP promotes cAMP-mediated phosphorylation of the CFTR



Epithelial Cells



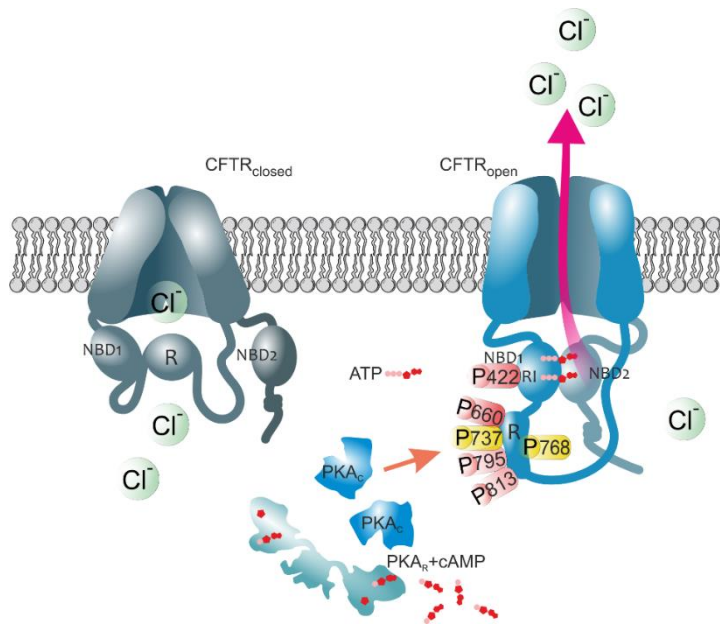
Della Sala A (and Ghigo) Front Physiol 2021



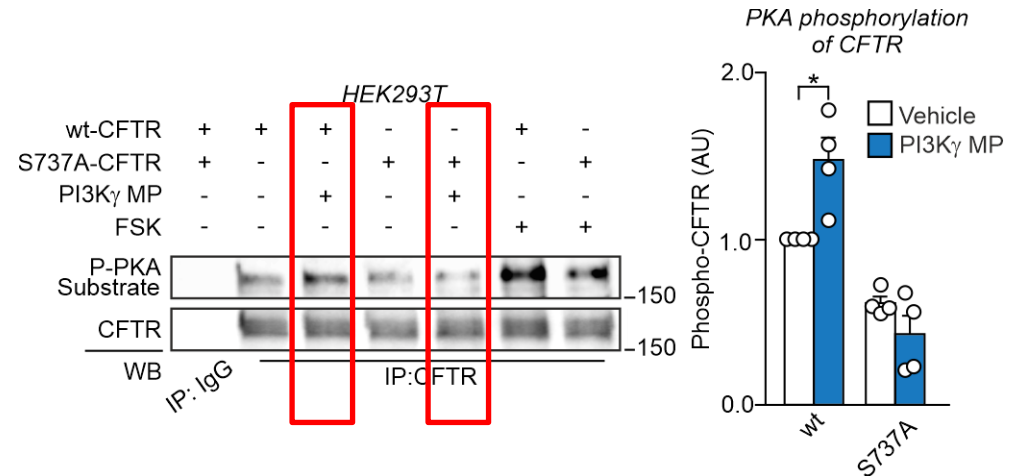
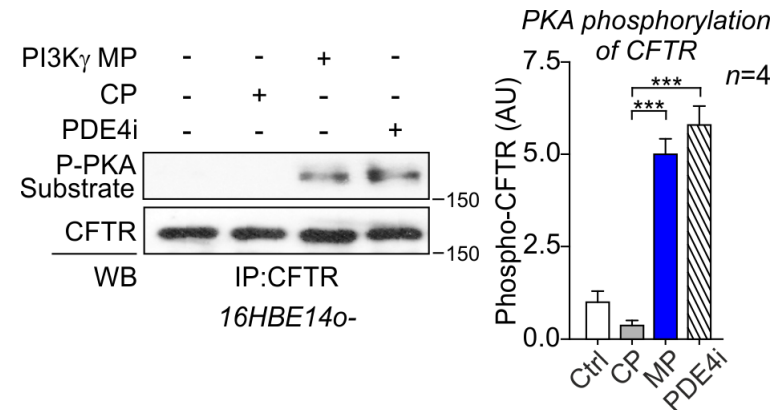
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Epithelial Cells



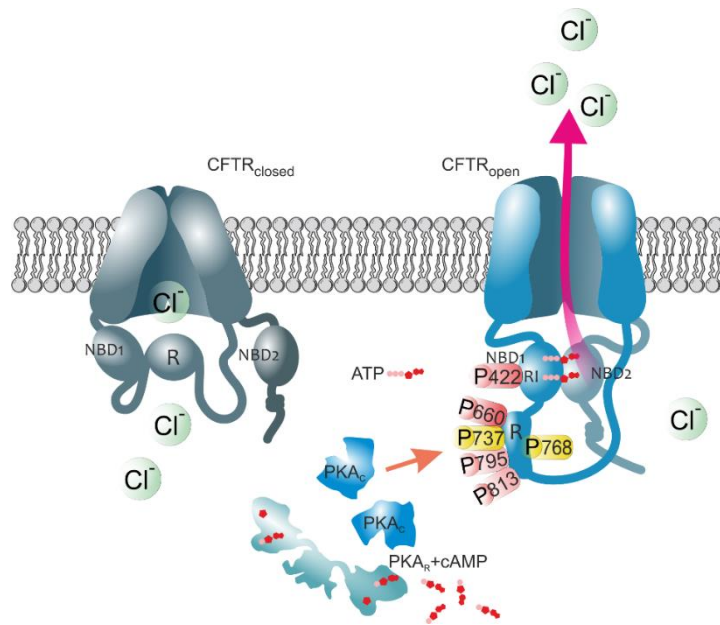
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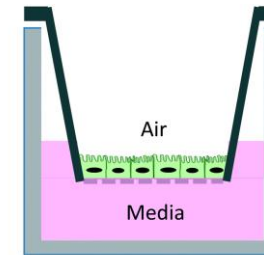
The PI3K γ MP Triggers CFTR Opening



Epithelial Cells

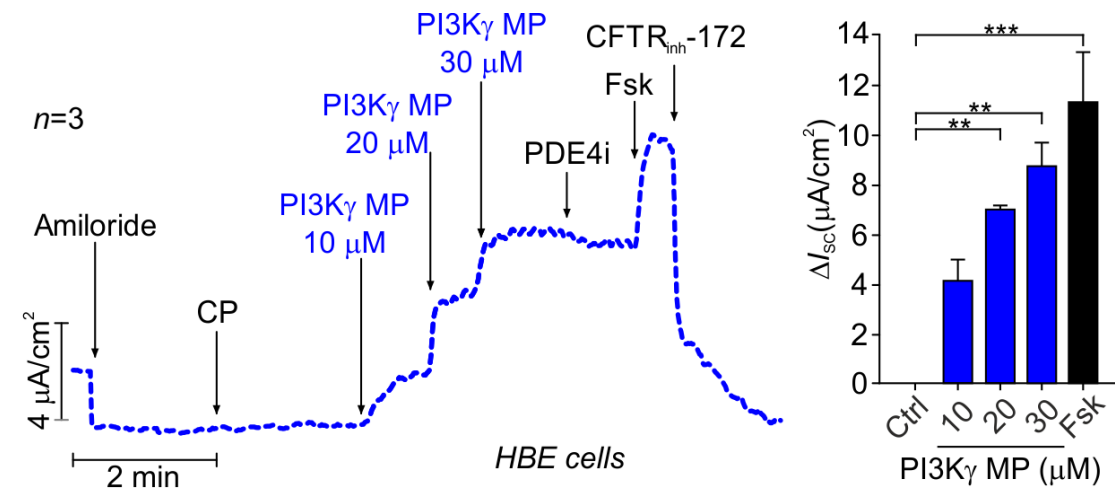


Della Sala A (and Ghigo) Front Physiol 2021



Air-Liquid Interface

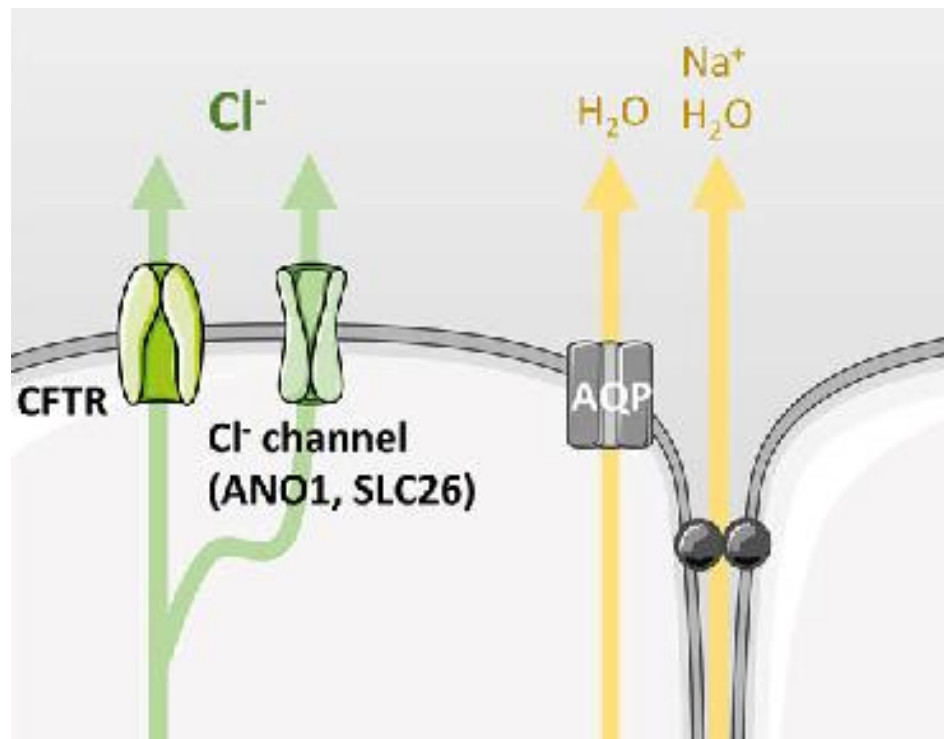
Short circuit current measurements in human primary bronchial epithelial cells



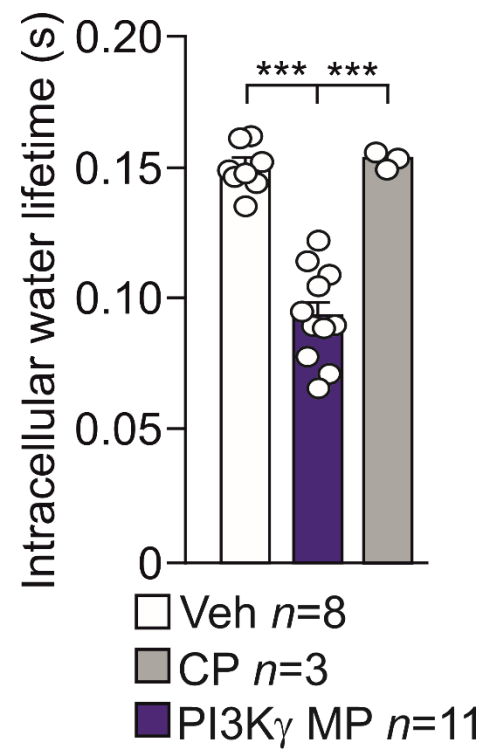
The PI3K γ MP Induces Water Secretion from Epithelial Cells



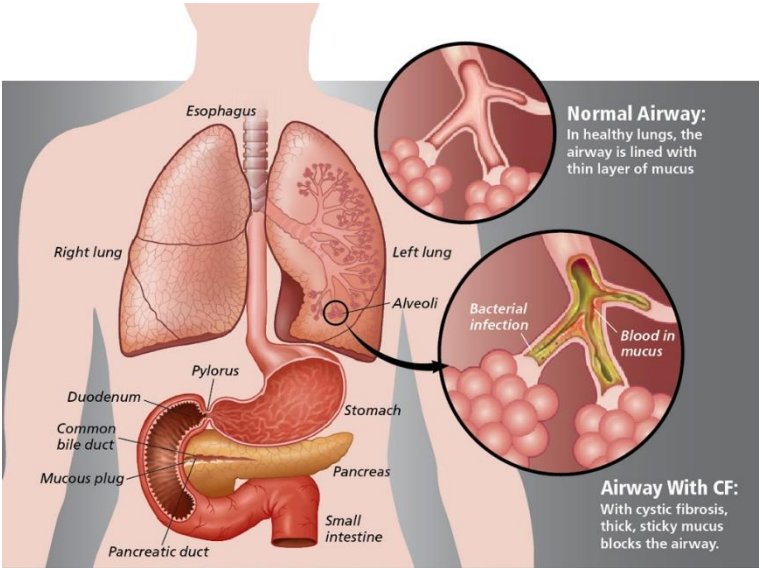
Mucus Hydration



Water residence time (τ_{in}) determined by ¹H NMR relaxometry



CFTR Dysfunction is the Basic Defect in Cystic Fibrosis

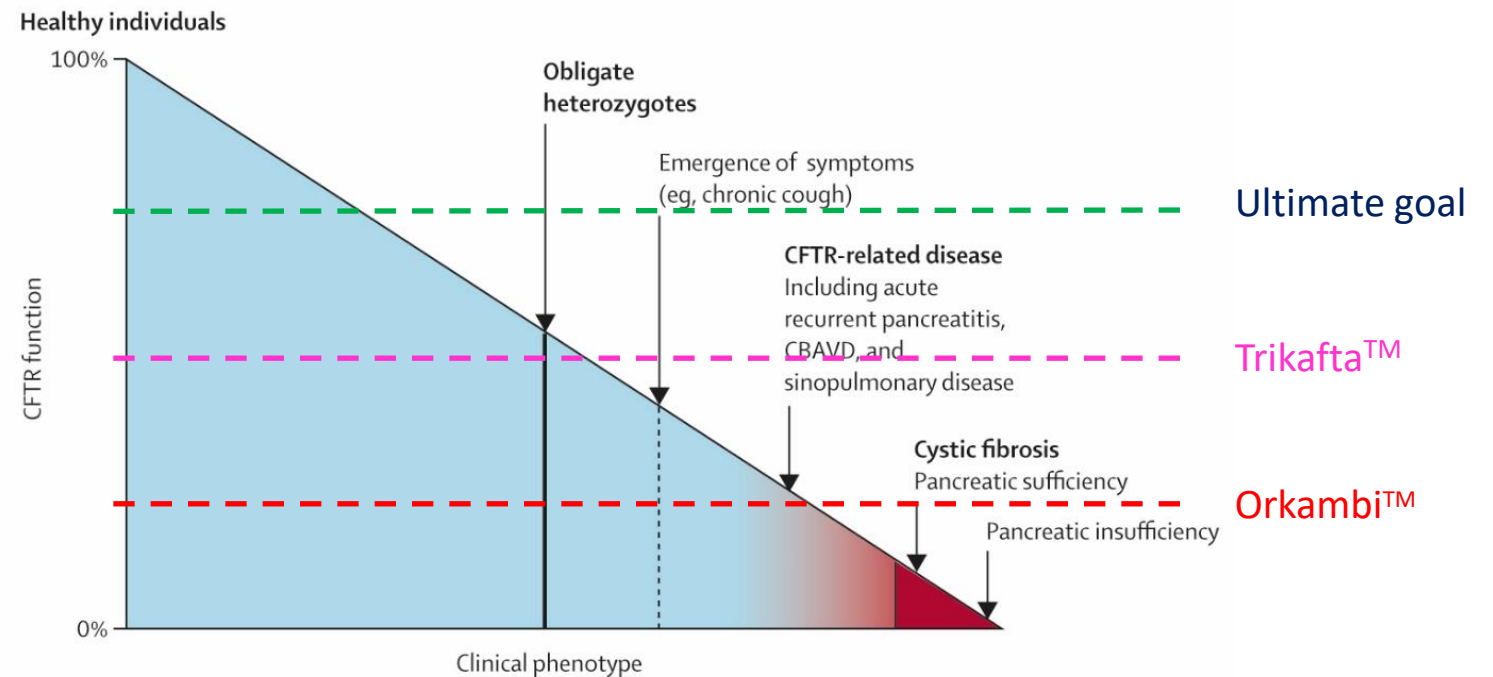
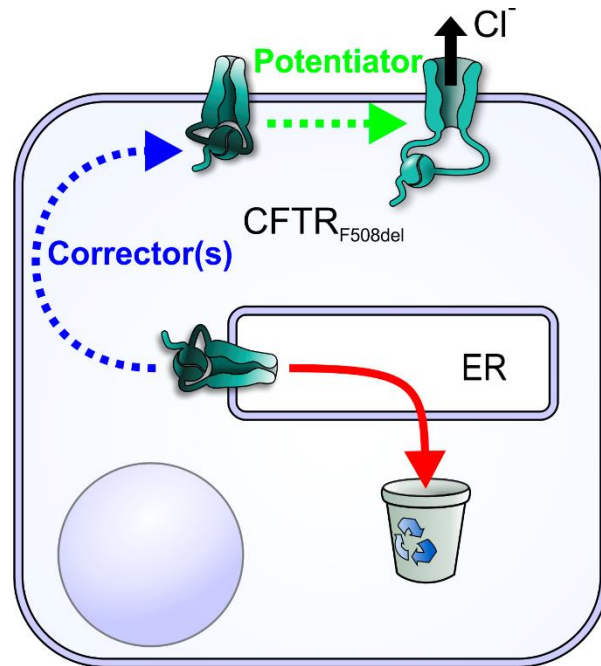


- Cystic Fibrosis is the **most common among rare genetic diseases**, affecting more than 80000 patients worldwide
- It is caused by mutations in the *CFTR* gene, encoding for a **cAMP-regulated chloride channel**
- It is a multiorgan syndrome, with respiratory failure being the major cause of death
- **Median predictive survival: 44 years**
- **No effective treatment available**

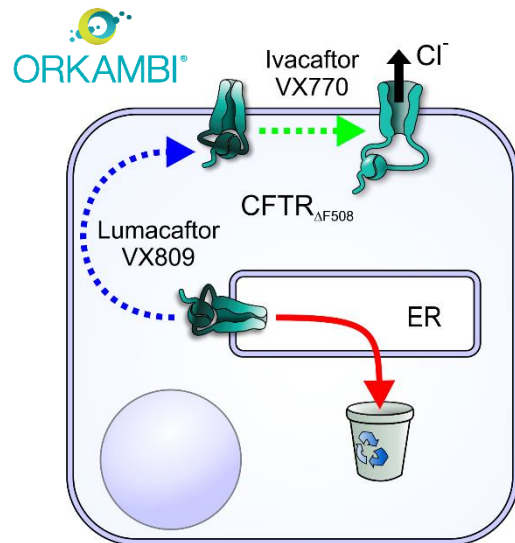
90% of patients:
F508del

	Class of mutation					
	Normal	I	II	III	IV	V
Molecular defect	No synthesis	Block in processing	Block in regulation	Reduced conductance	Reduced synthesis	Reduced half-life
Functional abnormality	Protein is not synthesized	Folding defect	Channel opening defect	Ion transport defect	Decreased protein synthesis	Decreased half-life of the protein
Main mutations	Gly542X Trp128X Arg553X 621+1G→T	Phe508del Asn1303Lys Ile507del Arg560Thr	Gly551Asp Gly178Arg Gly551Ser Ser549Asn	Arg117His Arg347Pro Arg117Cys Arg334Trp	3849+10kbC→T 2789+5G→A 3120+1G→A 5T	4326delTC Gln1412X 4279insA

Approved CFTR modulators only Partially Rescue F508del-CFTR Function

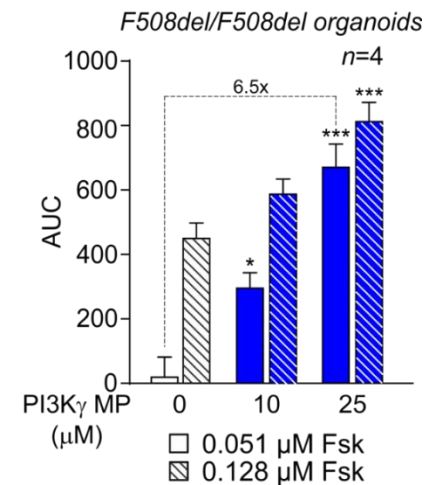
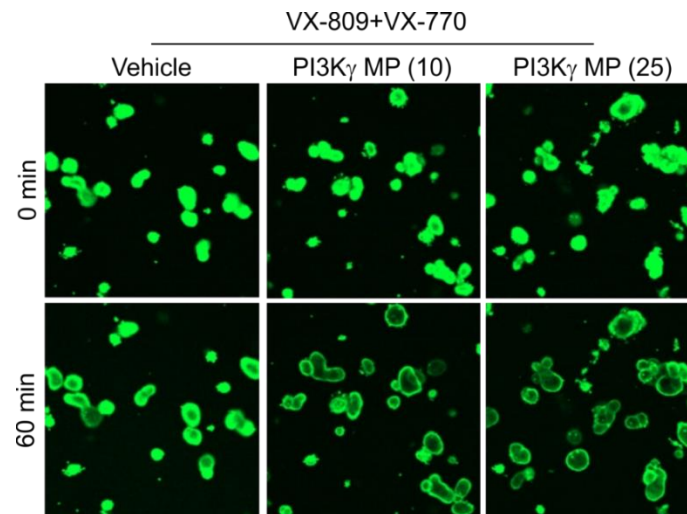
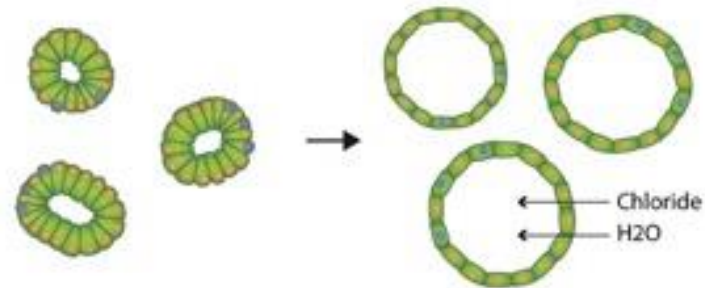


The PI3K γ MP Potentiates the Effect of CFTR Modulators by 6 folds in F508del Organoids

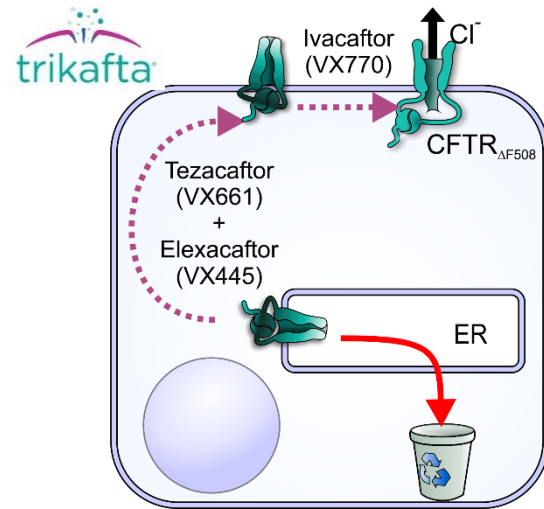


Approved by FDA in 2015 despite limited efficacy (<4% lung function improvement)

Forskolin-induced swelling assay in patient-derived organoids

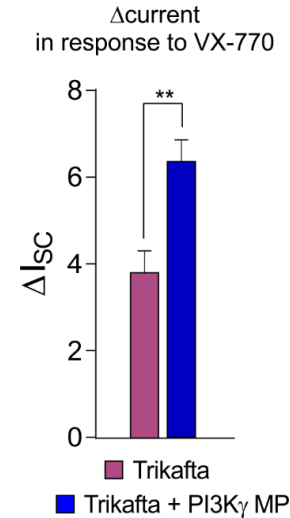
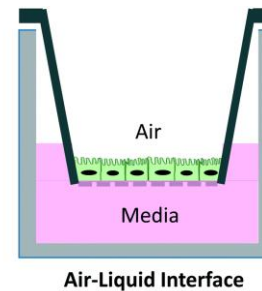


The PI3K γ MP Doubles the Effect of the Recently Approved Combination Trikafta™

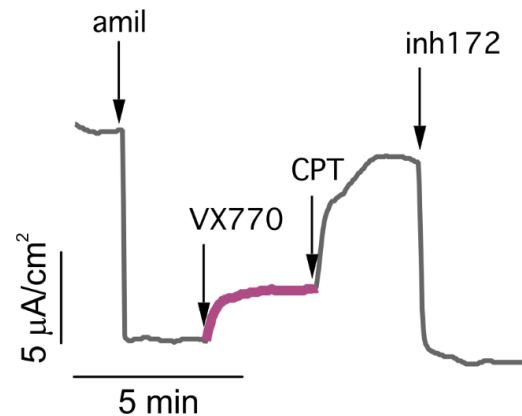


Approved by FDA in 2019
(by EMA in 2020)

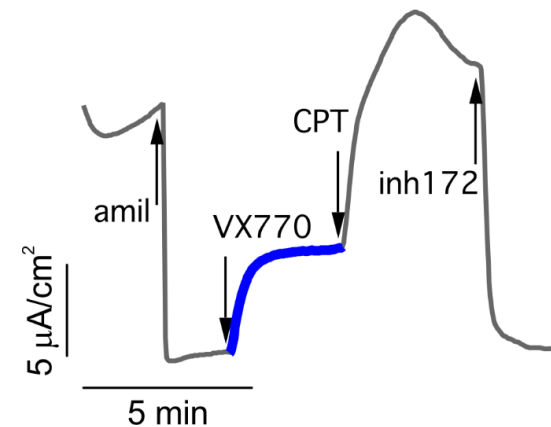
Short circuit current measurements in patient-derived bronchial epithelial cells (F508del/F508del)



Trikafta™ alone
VX-445+VX-661 (24h)

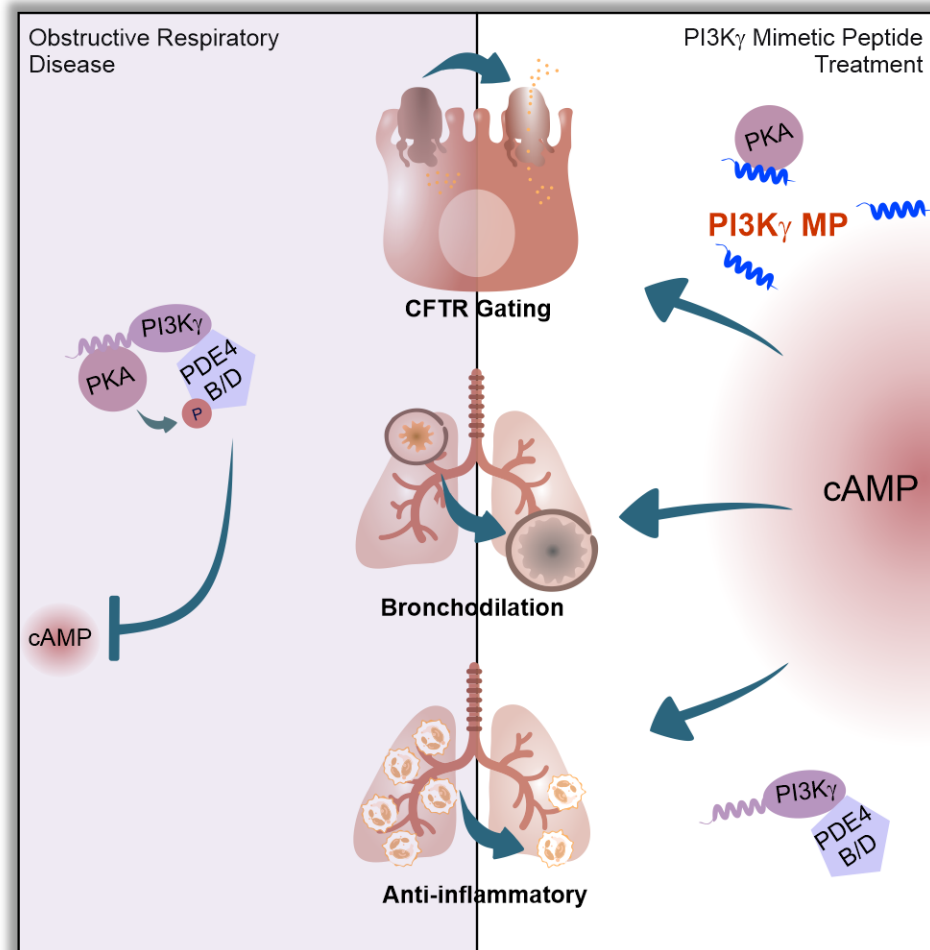


Trikafta™ + PI3K γ MP
VX-445+VX-661+peptide (24h)



N=2 independent patients

Conclusion I



- PI3K γ MP inhibits a specific pool of subcortical PDE4B and D increasing cAMP in a compartments that contain β 2-ARs
- Inhaled PI3K γ MP promotes long-lasting cAMP elevation in the lungs, triggering airway smooth muscle relaxation and reduced neutrophil infiltration
- In epithelial cells, PI3K γ MP elevates cAMP in the vicinity of CFTR triggering PKA-mediated phosphorylation of the activating serine 737
- PI3K γ MP potentiates the effects of existing CFTR modulators in rescuing F508del-CFTR function

Future directions:

- Does PI3K γ MP directly rescue the function of cAMP-responsive CFTR mutants, like class III-IV R117H, A455E, R334W, T338I, G551D, S549R?

KIT2014 as Orphan Drug

KIT2014 (Pat. Appl. WO 2016/103176)



Patent Approved in EU
& USA

- Orphan Drug Designation (Feb. 2017) by European Medicine Agency (EMA)

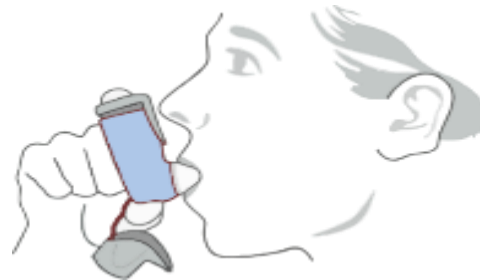


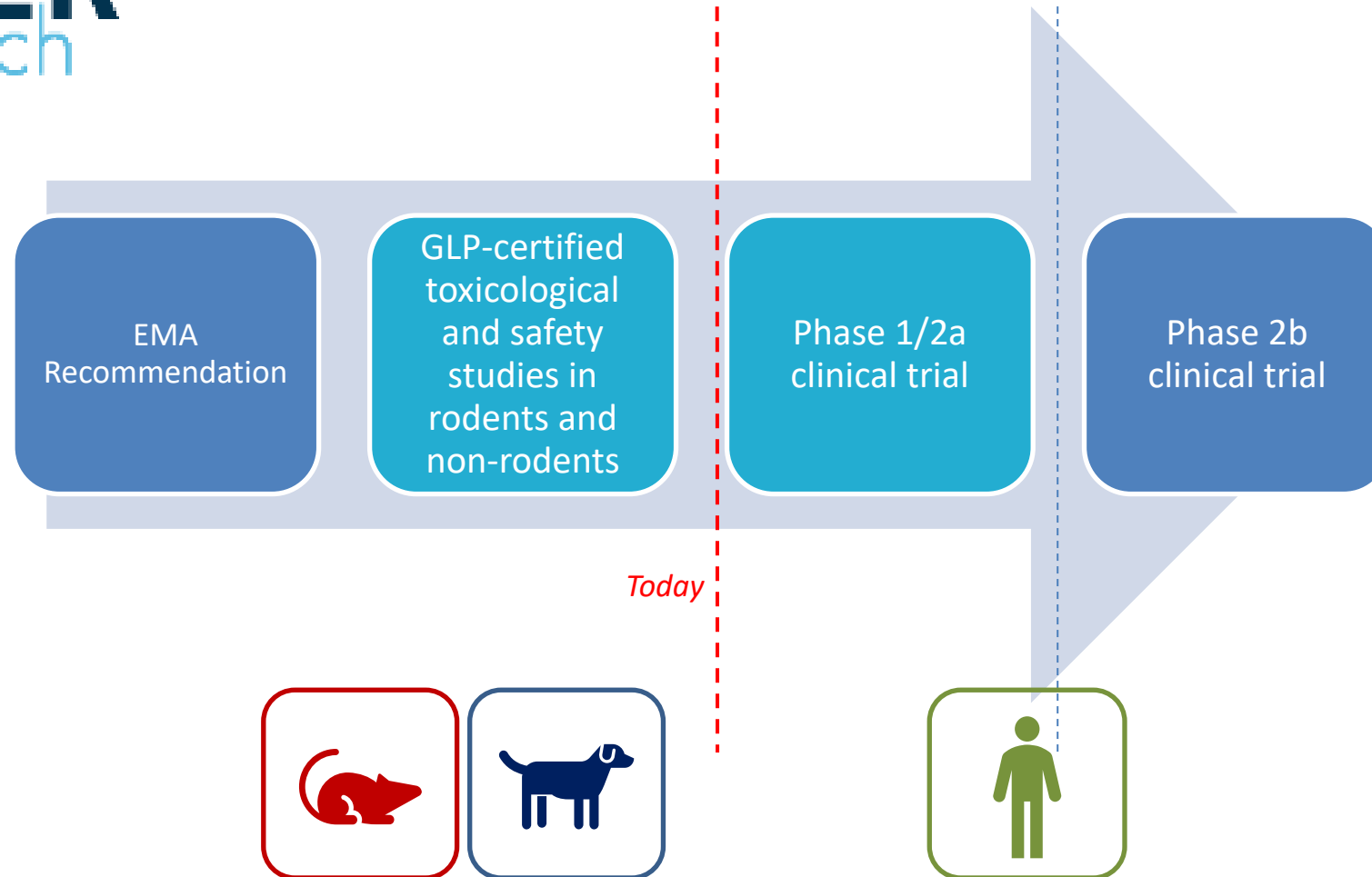
Orphan Drug
Designation

- “Scientific Advice” obtained from EMA for preclinical development (Oct. 2018)



Scientific Advice
from EMA





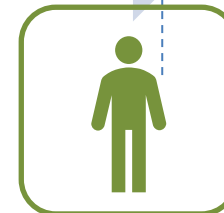
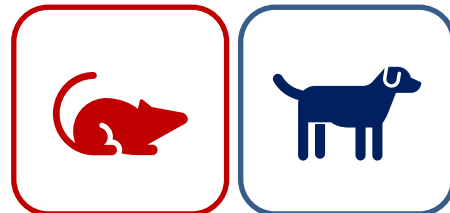
EMA
Recommendation

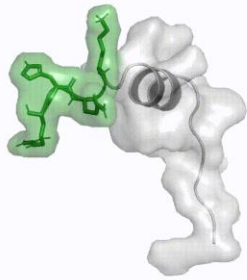
GLP-certified
toxicological
and safety
studies in
rodents and
non-rodents

Phase 1/2a
clinical trial

Phase 2b
clinical trial

Today





- PI3K γ /PKA peptide disruptors are valuable tools for manipulating β 2-AR/cAMP signaling in the lungs by mimicking the core of the native interaction of PI3K γ with PKA.
- PI3K γ /PKA peptide disruptors are suitable for inhalation and can overcome the biological barriers imposed by diseased lungs
- PI3K γ MP (KIT2014) is in advanced stage of preclinical development.
- Further preclinical testing is required to prove the feasibility and the safety for an inhaled therapy based on DRI-Pep#20.

Acknowledgements

University of Torino - IT

Mentor: Emilio Hirsch

Ghigo's lab

Alessandra Murabito

Angela Della Sala

Giulia Guerra

Marco Mergioti

Paola Peretto

Rebecca Priolo

Sophie Cnudde

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Aiswarya Premchandar

Charité Universitätsmedizin Berlin – GERMANY

Marcus Mall

University of Bonn – GERMANY

Bernd Fleischmann

FONDAZIONE



"Excellent Young PI" grant



fondazione per la ricerca
sulla fibrosi cistica - onlus
italian cystic fibrosis research foundation

