# Development of alternative in vitro and ex vivo models for testing of inhalable antibiotics – “InhalAb”


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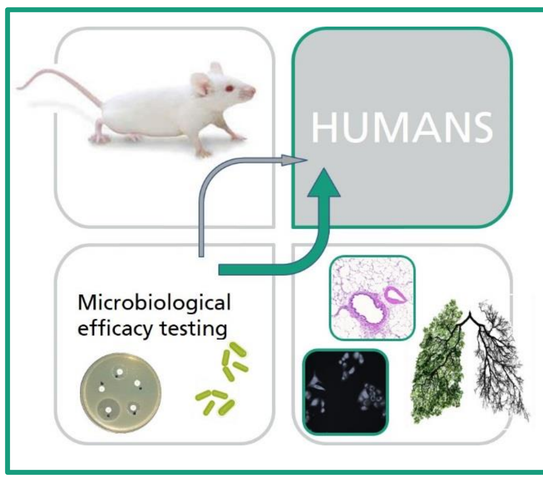
InhalAB (03L0135)

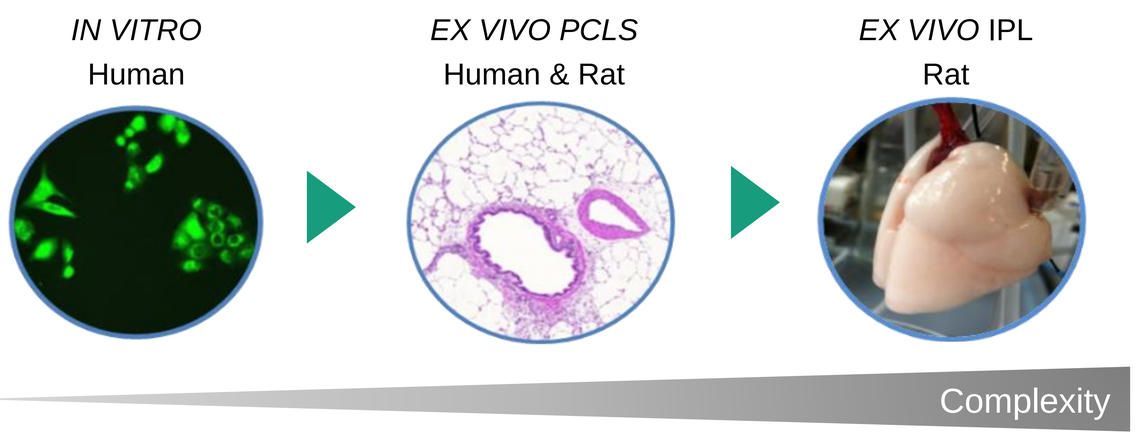

## Aim of the project

The aim of the project is the establishment of a test battery of *in vitro* and *ex vivo* lung models for preclinical testing of inhalable antibiotics. These shall be developed as replacement methods for regulatory required dose range finding studies as well as proof of concept studies of novel inhalable antibiotics and formulations aiming on optimized PK/PD parameters. So far this is only possible in animals *in vivo*. However, especially acute *in vivo* infection models are associated with a massive burden for test animals. Moreover, the regulatory application of alternatives to animal testing for inhalation toxicity and efficacy studies has lagged behind. Challenges for animal-free testing for the respiratory tract are e.g. use of relevant respiratory cells and tissues (standardization), dosimetry, and exposure technique.




Establishment of a test battery of *in vitro* and *ex vivo* lung models for preclinical testing of inhalable antibiotics for the replacement of animal studies

- **Toxicity testing**
- **Efficacy testing**
- **Pharmocokinetic analyses**

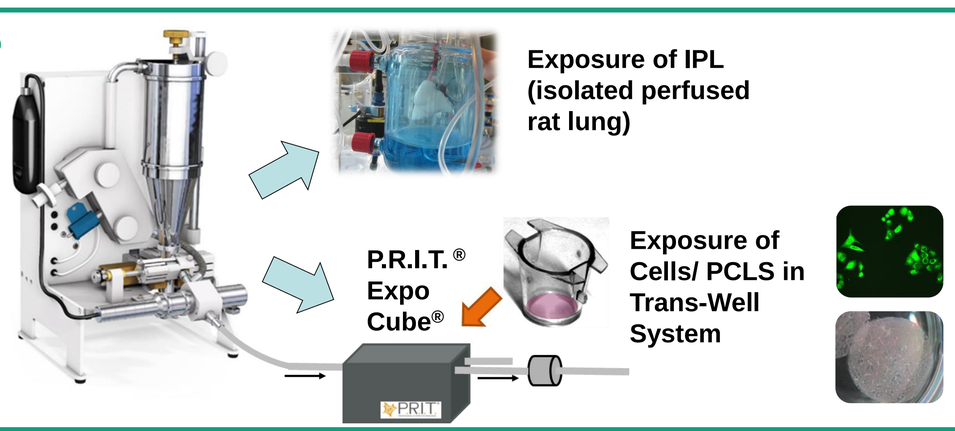



## Results

### ➢ *Aerosol Exposure*

**PreciseInhale™**

Generation and dosage of aerosol from powder



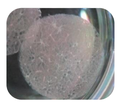

Aerosol generation for exposure of cells, precision-cut lung slices (PCLS) and isolated perfused rat lungs (IPL) was successfully established for ciprofloxacin using the PreciseInhale™ device.

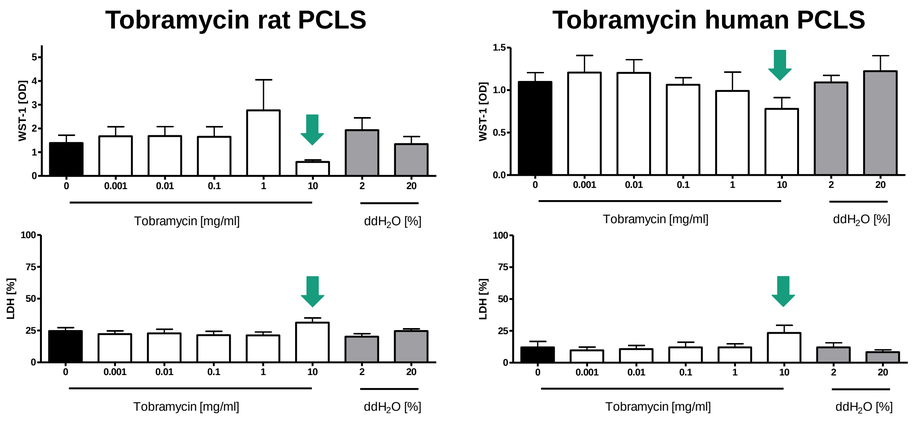
*In vitro* exposure of cells and PCLS was performed with the P.R.I.T.® ExpoCube® enabling a well-defined exposure with parallel non-exposed and vehicle (clean air) exposed samples.

Determination of ciprofloxacin in the respective sample matrices (e.g. IPL-buffer, cell medium) by HPLC-MS/MS has been successfully established. Method development for the determination of tobramycin at the relevant concentration in the respective sample media is currently under way.

### ➢ *Toxicity testing*

Determination of the local toxic dose was initially performed with submerged application in comparison between rat and human PCLS. A comparable non-toxic dose range up to 10mg/ml for tobramycin and 1mg/ml for ciprofloxacin, respectively, was observed in both species. As expected, acute respiratory toxicity was not induced in the IPL model, as no loss of lung function was observed as compared to the toxic positive control SDS.







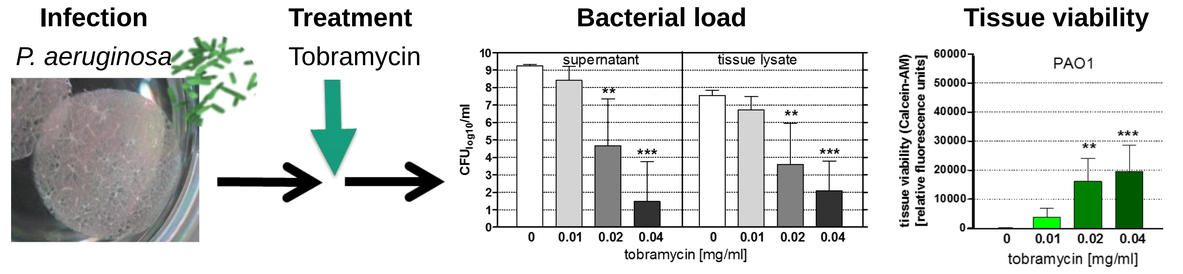




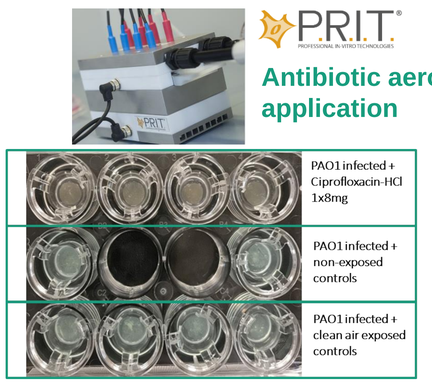


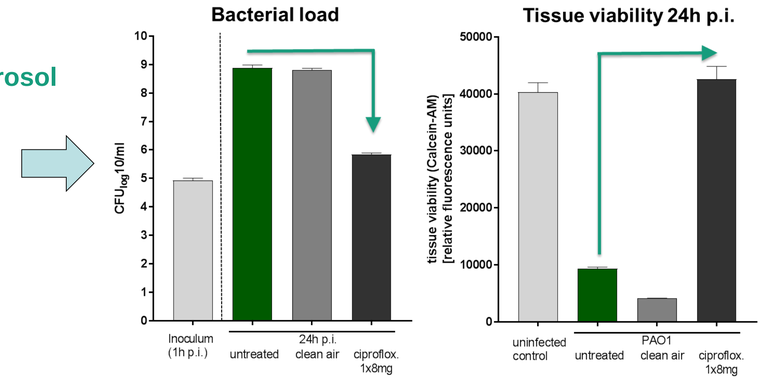
### ➢ *Efficacy testing*

For efficacy testing a PCLS model of *Pseudomonas aeruginosa* infection was established using tobramycin submerged application, leading to reduced bacterial load and improved tissue viability.




First experiments with ciprofloxacin aerosol exposure show proof-of-principle for efficacy of the inhalative treatment reducing the bacterial load and thereby rescuing tissue viability. Detailed dose-response efficacy studies are currently ongoing.




### ➢ *Pharmacokinetic analyses*

Uptake rates are investigated in human alveolar epithelial cells and rat IPL in order to generate data enabling modelling of uptake rates in human.

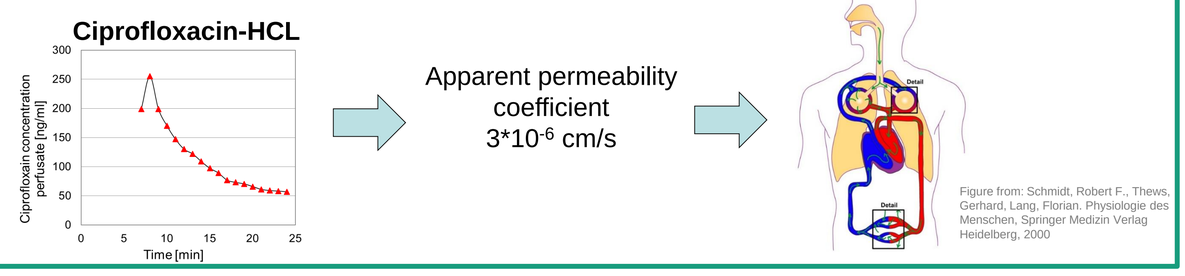



Figure from: Schmidt, Robert F., Thews, Gerhard, Lang, Florian. Physiologie des Menschen, Springer Medizin Verlag Heidelberg, 2000

## Summary and perspective

Up to now methods for antibiotic aerosol generation, *in vitro* exposure, infection, and analysis of transfer rates were successfully established, enabling us to now conduct detailed toxicity, pharmacokinetic and efficacy studies for the chosen antibiotics. In a next step, the obtained data will be compared between the models and correlated with available *in vivo* data to assess the predictivity of the established alternative methods.

## Contact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