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MAXIMIZING DRUG DEVELOPMENT EFFICIENCY USING PREDICTIVE IN VITRO & EX VIVO MODELS

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The SCIREQ & 3RsC webinar on *Maximizing Drug Development Efficiency Using Predictive in vitro & ex vivo Models*, held virtually on October 29, 2024 provided an in-depth look into the role of predictive models in expediting drug development, particularly for inhalational therapies addressing respiratory diseases. Dr. Katharina Schwarz, the lead speaker and head of the "Inhalation and Aerosol Sciences" department at the Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) in Hannover, Germany, brought her expertise to the session. Her insights provided an expert foundation for exploring strategies that bridge early-stage drug research with clinical applications.

This session presented an integrative approach using Precision Cut Lung Slices (PCLS), computational models, and the PRIT expoCube system, illustrating the substantial impact these tools can have on enhancing the speed, cost-effectiveness, and safety of preclinical testing.

BACKGROUND AND INDUSTRY CONTEXT

The burden of lower respiratory tract infections worldwide remains significant, with high incidence rates and a pressing need for effective therapeutic agents. The respiratory route is particularly promising for anti-infective drugs, as it allows for targeted drug delivery to the site of infection, optimizing the therapeutic effect by achieving the minimum inhibitory concentration directly within the respiratory tract.

Drug development, especially for inhalation therapies, involves a challenging process from research to patient application. Many potential drugs fail before they can reach clinical trials due to the "translational gap" between preclinical research and clinical outcomes. Current data indicates that only one in 5,000–10,000 compounds entering the drug pipeline achieves regulatory approval, underscoring the importance of adopting predictive and human-relevant models that can improve early-phase clarity on a drug's potential efficacy and safety.

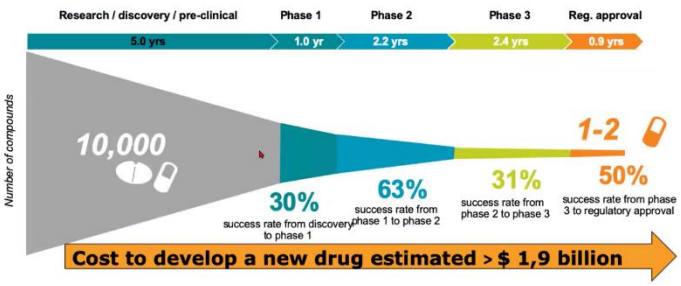

Figure 1 - DiMasi et al, Journal of Health Economics, January 2016

INNOVATIVE TOOLS AND MODELS IN PREDICTIVE TESTING

The webinar spotlighted several predictive tools and models that collectively help identify therapeutic windows, toxicity thresholds, and first-dose estimates, making them essential assets in preclinical development.

PRECISION CUT LUNG SLICES (PCLS) FOR DRUG EFFICACY AND TOXICITY

PCLS models are a cornerstone of respiratory research, offering an *ex vivo* approach to study both drug efficacy and respiratory toxicity. These ultra-thin slices (250 microns) of lung tissue can be derived from human or animal sources, allowing researchers to observe the drug's effects in a highly representative organotypic infection model.

Using human-derived PCLS, Dr. Schwarz's team assessed local toxicity and efficacy endpoints through markers like WST (a viability test), lactate dehydrogenase (LDH) for toxicity, and viral or bacterial load measurements for efficacy. This model effectively simulates respiratory environments, thereby providing insight into potential therapeutic windows and the lung-specific doses required to achieve efficacy without inducing local toxicity.

APPLICATION OF THE PRIT EXPOCUBE SYSTEM

The PRIT expoCube system is an advanced tool that allows dose-controlled air-liquid-interface (ALI) exposures, crucial for studying inhalable drugs in a relevant exposure environment. This compact and hermetically sealed system prevents secondary exposure routes, ensuring precise dosimetry by directly exposing individual wells with specific doses. The expoCube also includes a stagnation point flow design, which accommodates various deposition mechanisms - sedimentation and thermophoresis - thereby enhancing the deposition of even small particles and optimizing testing accuracy.

Figure 2 – PRIT expoCube

CASE STUDIES DEMONSTRATING PREDICTIVE MODEL EFFICACY

Several case studies presented in the webinar highlighted the practical applications of these predictive models in early proof-of-concept testing:

CASE STUDY #1: TOBRAMYCIN IN PCLS INFECTION MODEL

Dr. Schwarz's team developed an *ex vivo* infection model using PCLS slices infected with *Pseudomonas aeruginosa*, subsequently treating the slices with the antibiotic tobramycin at varying concentrations. The data demonstrated a clear reduction in bacterial load as tobramycin concentration increased, validating the model's reliability for testing bacterial efficacy. Furthermore, the decrease in bacterial presence correlated with a rise in tissue viability, reinforcing that the infection model effectively reflects real-world therapeutic responses.

This preliminary setup, though conducted in a submerged format, confirmed the PCLS's potential as a predictive model for exploring the therapeutic efficacy of inhalable drugs.

CASE STUDY #2: CIPROFLOXACIN'S THERAPEUTIC WINDOW IN AN INHALABLE MODEL

In exploring the local therapeutic window of ciprofloxacin, an additional study aimed to emulate an inhalable delivery scenario using the PRIT expoCube. In this experiment, PCLS slices were infected and subsequently exposed to aerosolized ciprofloxacin, with results showing a significant reduction in bacterial count as the antibiotic concentration increased. The study also identified a clear separation between effective doses and the onset of local toxicity, allowing the researchers to establish a wide therapeutic window. This highlighted the expoCube's utility in measuring efficacy and safety within inhalation models, a crucial insight for early-stage drug development.

ASSESSMENT OF PREDICTIVE MODEL RELIABILITY

To further gauge the accuracy of their model's predictions, the researchers compared ciprofloxacin and tobramycin. They noted that ciprofloxacin exhibited approximately five times the efficacy of tobramycin, achieving similar bacterial reduction at lower doses. This matched existing literature data on minimum inhibitory concentrations, encouraging confidence that the model can predict outcomes relevant to clinical applications.

CASE STUDY #3: NAFAMOSTAT'S POTENTIAL AGAINST SARS-COV-2

An additional case study was presented using Nafamostat Mesylate, an anti-inflammatory and antiviral candidate. This drug has shown promise against SARS-CoV-2 by preventing viral entry into lung cells. By using the A549 cell line, a reliable model for acute respiratory toxicity, cells were exposed to Nafamostat using the PRIT expoCube and identified key toxicity markers. Viability tests (e.g., WST, IL-8) indicated that the No Observed Adverse Effect Level (NOAEL) for Nafamostat was approximately 1.45 µg/cm^2. These results provided a robust basis for further Nafamostat testing *in vivo*.

IN VIVO & DOSIMETRY CORRELATION

A parallel *in vivo* study was performed to corroborate their *in vitro* results, employing a 28-day inhalation toxicological test in rats at low and high doses. Animals showed adverse effects in particular in the larynx/larger airways for a dose of 0.5 mg/kg bw.

In order to integrate and transfer the results of the different in-vitro and ex-vivo model and dose metrics to the in vivo situation, a conversion to a uniform dose metric was made using the MPPD model and detailed PK considerations. In vitro/ex vivo and in vivo data point towards comparable LOAEL values. These considerations also indicate that much lower doses of Nafamostat might be safe and still efficacious.

CONCLUSION AND IMPLICATIONS FOR DRUG DEVELOPMENT

This webinar emphasized the significant impact of predictive *in vitro* and *ex vivo* models on preclinical development, especially for inhalational drugs aimed at respiratory diseases. By leveraging tools like PCLS, expoCube, and computational dosimetry models, researchers can identify therapeutic windows, optimize dosing, and assess toxicity early, reducing the need for extensive *in vivo* testing.

These predictive models streamline the drug development process by mitigating risks, shortening timelines, and lowering costs—key advantages in a field where both safety and efficiency are essential. Ultimately, the approaches discussed outline a more reliable, ethical path in developing new inhalational therapies for respiratory conditions.

RESOURCES

Please find the below resources which may be helpful in preparation for your upcoming studies or grant submissions:

IN VITRO

- *Bridging the Gap Across Models: Integrating in vivo & in vitro Inhalation Toxicology Assessments*
- *Exploring ALI Exposures: From Cell Lines to Endpoints*
- *Aerosol Capabilities using the expoCube*
- *Advanced in vitro Models of Inhalation Toxicology*
- *In vitro Inhalation Testing: Revolutionizing Inhalation Product Safety*
- *Comparative In Vitro Toxicity of ENDS Aerosols: Impact on Macrophages*
- *Advantages of ALI Exposure – expoCube*
- *Moving from in vitro to in vivo*

EX VIVO

- *PCLS Resource hub*
- *Lung Slices as Translational Models of Airway Reactivity – Event Summary*
- *PCLS: An Effective ex vivo Platform for Studying Lung Physiology – Event Summary*
- *Reducing Variability in Lung Slice Analysis*
- *PCLS: Unraveling the Intricacies of Asthma Research*
- *PCLS: An ex-vivo Window into Lung Disease And Drug Discovery*

IN VIVO

- *Role of Inhaled Interferon-λ-loaded Nanoparticles Against Influenza A Virus*
- *Recent Advances in Inhaled Therapeutics for Influenza A Virus and SARS-CoV-2*
- *Preclinical Models of Smoke Exposure*
- *Treating Acute Inflammatory Lung Disease: The Promise of Trimannose-Coupled antimiR-21*
- *E-cigarette Induced Cardiac Arrythmia and Conduction Defects in Mice*
- *COVID-19, Vaping and EVALI*

Request more information about *in vitro, ex vivo* or *in vivo* exposure