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Late breaking abstracts

LP-58

Human Precision-Cut Lung Slices (hPCLS): A Powerful Tool for Drug Screening in Toxicology and Pulmonary Disease Modeling

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Pulmonary fibrosis is a progressive lung disease characterized by scarring and stiffening of lung tissue, ultimately leading to respiratory failure. Prior lung inflammation from viral infections (e.g., COVID-19) and interstitial lung diseases can be major contributors. Understanding the mechanisms behind its development is crucial, as currently no effective treatment exists to halt or reverse this condition.

To address this knowledge gap, researchers have used various *in vitro* models, including 2D cell cultures, 3D cell cultures, and *ex vivo* lung models like precision-cut lung slices (PCLS). PCLS offer a unique advantage by preserving the lung native 3D structure and containing all lung cell types. This makes them ideal for investigating lung diseases and assessing novel therapy safety, toxicity, and efficacy.

Generating human PCLS (hPCLS) requires meticulous technique. This presentation will highlight how to successfully prepare precise and reproducible hPCLS from surgical human lung resections. We will cover proven strategies for inducing fibrosis within this model using a specific cocktail of factors known to induce fibrosis. Additionally, we will discuss methodologies for precise hPCLS characterization, including a proteomic approach, histopathologic analysis, microscopic imaging, and gene expression analysis. These comprehensive methods allow for in-depth assessment of the hPCLS model and its response to anti-fibrotic treatments.

In conclusion, this presentation will focus on the valuable application of hPCLS for investigating respiratory diseases such as pulmonary fibrosis. Their viability for at least 14 days, along with the ability to mimic the lung natural structure though these detailed characterization techniques, makes hPCLS ideal for studying disease mechanisms, evaluating potential therapies, and assessing the effects of repeated dosing.

LP-59

Toxicokinetics of NIDO-361, a Potential Candidate to treat Spinal and Bulbar Muscular Atrophy (SBMA), in Rats and Monkeys Following Oral Dosing in a 4-Week Toxicity Study

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Introduction

While the underlying genetic causes of many rare diseases have been elucidated, there is still a gap between understanding disease conditions and translating this knowledge to effective treatments. In most circumstances, rare diseases have no cure and limited options for therapeutic intervention. Therefore, supporting the advancement of

preclinical rare disease drug programs is crucial for addressing this ongoing global health challenge. NIDO-361 is a novel small molecule that binds to a distinct site on the androgen receptor (AR) to regulate co-factor binding and thereby correct transcriptional dysregulation. NIDO-361 is currently being investigated for the treatment of patients suffering from Spinal and Bulbar Muscular Atrophy (SBMA); a rare inherited X-linked neuromuscular disorder caused by a genetic mutation of the AR resulting in the loss of skeletal muscle and motor neuron function. In this study, the toxicokinetics (TK) of NIDO-361 were assessed following once-daily oral dosing to male Sprague Dawley rats and male Cynomolgus monkeys.

Study Design/Methods

The rat study consisted of groups treated with NIDO-361 with once-daily oral dosing as follows:

- Group 1 – N=3, 0 mg/kg/day (control) for 28d
- Group 2 – N=6, 25 mg/kg/day for 28d
- Group 3 – N=6, 100 mg/kg/day for 28d
- Group 4 – N=10, 250 mg/kg/day for 4d, no dosing D5-D7, 150 mg/kg/day D8-D28

The monkey study consisted of groups treated with NIDO-361 with once-daily oral dosing as follows:

- Group 1 – N=7, 0 mg/kg/day (control) for 28d
- Group 2 – N=4, 45 mg/kg/day for 28d
- Group 3 – N=7, 200 mg/kg/day, reduced to 100 mg/kg/day on D5
- Group 4 – N=7, 600 mg/kg/day, dosing stopped on D5

Blood samples were collected at predetermined times from 3 rats/timepoint and all surviving monkeys on D1 and D28 and were used for measuring NIDO-361 plasma concentrations by validated HPLC/MS/MS assays. The TK parameters were determined by model independent methods (sparse sampling for the rat study and serial sampling for the monkey study).

Results/Conclusions

Following the completion of the outlined studies for both rat and monkey, the samples collected from animals on D1 and D28 were analyzed and the plasma concentrations of NIDO-361 quantitated. Tmax, Cmax, and AUC(0-24) values were calculated and the systemic exposure of NIDO-361 determined. For all dosed groups in the rat and monkey studies, D28 TK exposure was generally comparable to that of D1. NIDO-361 systemic exposure (Cmax and AUC(0-24)) displayed dose proportional increases with increasing dose. Overall, the pattern of NIDO-361 systemic exposure in treated animals appeared to be similar in both species, where the systemic exposure increased in an approximately dose proportional manner and exhibited no accumulation. Measuring the potential toxicity of NIDO-361 in relation to dose was a critical step in understanding the drug’s exposure profile and provided key information for dosing decisions prior to first-in-human studies.

LP-60

Fast-Tracking Antifibrotic Therapies: A Cell Migration and Gene Expression-Based Assay for Drug Screening

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Pulmonary fibrosis is a progressive, restrictive lung disease characterized by scarring and stiffening of lung tissue, ultimately leading to respiratory failure. This condition is driven by the excessive proliferation and migration of fibroblasts, specialized cells residing within the lung. These activated fibroblasts deposit excessive amounts of extracellular matrix (ECM) proteins, fundamentally disrupting the lung's architecture and hindering its ability to function properly.
Therefore, targeting fibroblast proliferation and associated ECM deposition represents a logical therapeutic strategy for both delaying disease onset and, potentially, reversal of disease pathology. In this study, we utilized primary human pulmonary fibroblasts to develop a functional screening assay for identifying novel anti-fibrotic drugs.
Primary human pulmonary fibroblasts (HPFs) were cultured in Ibidi® two-well inserts and treated with either nintedanib (a tyrosine kinase inhibitor) or pirfenidone (an anti-inflammatory drug), both FDA-approved treatments for pulmonary fibrosis. The wounded area of the inserts was imaged using a CytoSMART Omni brightfield live-cell imager for up to 24 hours. In parallel, separate cultures of HPFs were treated with either TGF-β alone or in combination with nintedanib. Untreated cells served as control. After 24 hours, mRNA expression of fibrosis markers was measured using RT-qPCR.
Our findings revealed that pirfenidone had minimal effect on HPF migration, while nintedanib significantly reduced their migratory capacity by 49%. Furthermore, treatment with TGF-β led to increased expression of fibrosis markers, particularly LRRC15 (11-fold increase), COL1A1 (5-fold increase), and FN1 (4-fold increase). Importantly, nintedanib treatment effectively countered this effect by decreasing the expression of TGF-β-induced fibrosis markers, particularly FN1 (22% decrease) and COL1A1 (37% decrease).
In conclusion, this study established a functional screening assay for identifying potential anti-fibrotic drugs. This assay combines cell migration and gene expression analysis is a simple, versatile, and cost-effective method for the screening of novel anti-fibrotic therapeutic treatments targeting pulmonary fibrosis.

LP-61

Towards a virtual Embryo: Computational modeling of neural tube closure defects

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Human embryonic development of the brain and spinal cord begins with the folding of neural tissue into a tube. This process, neural tube closure, is a critical event that occurs early in development, between day 23 and day 30 of human gestation. Its failure underlies neural tube defects (NTD) such as spina bifida. With an estimated global

incidence of two cases per 1000 births, NTDs are among the most severe and prevalent human birth defects. To understand how neural tube defects may occur and which genes are most important for successful neural tube closure, we developed a complex multicellular agent-based model (ABM) in the CompuCell3D modeling environment. This model recapitulates the dynamics of normal neural tube closure, such as tissue bending, fusion, proliferation, differentiation and cell delamination. These dynamics are driven by a gene regulatory network of neural tube closure, based on a previously created physiological map (Heusinkveld et al. 2021). This regulatory network allows us to introduce perturbations by varying the expression level of genes in the model. These perturbations lead to a wide variety of dynamic and structural phenotypes seen in both human and mice. Analyzing the phenotypes predicted by our ABM revealed mechanistic insights that are challenging to detect in animal models. Our ABM helps clarify these processes, providing a clearer understanding of how perturbations might disrupt neural tube closure at a cellular and molecular level. Future applications of our model include studying the effect of chemical related perturbations on neural tube closure. Changes in gene expression observed *in vitro* will be replicated as adjusted parameters in our ABM. Agent-based modeling provides a useful tool to mimic complex biological processes and demonstrates their potential to contribute to the prediction of chemical-induced disruptions of notoriously hard-to-study developmental processes.

Disclaimer: This abstract does not necessarily reflect USEPA policy.

LP-62

Toxicity of microplastics and additives in a pluricellular liver model

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Defined as particles smaller than 5 mm, microplastics (MPs) are currently a source of intense concern because of their presence in everyday products. Humans are exposed to MPs, as well as to their additives bisphenols (BPs) and perfluoroalkyl substances (PFAS), mainly by ingestion of contaminated food and water. Once ingested, these pollutants reach distant organs such as the liver and may cause damage related to metabolic dysfunction-associated steatotic liver disease (MASLD) [1]. This clinicopathological syndrome involves well-known steps from steatosis to the metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and finally cirrhosis and hepatocellular carcinoma (HCC).

The aim of our study is to evaluate the toxicological impacts of mixtures of MPs and their additives in order to decipher involved mechanisms in a complex 3D liver cellular model including the three cell types involved in MASLD steps: hepatocytes, stellate cells and Kupffer cells modelled respectively by HepaRG, LX-2 and THP-1 cell lines.

Our initial cytotoxicity results from the 3 cell types, independently grown in 2D, revealed differential susceptibility towards polyethylene (PE), or polypropylene (PP) MPs (5 - 150 µg/mL) and additives BPA, BPS, PFOS, PFOA (100 pM - 100 µM). Images obtained using scanning electron microscopy showed membrane interaction, with possible internalisation of MPs in HepaRG cells.

Lipid droplet quantification assay by microscopy performed on HepaRG cells also showed a disruption in lipid metabolism following a 24-hour or 5-day exposure to MPs and additives, whether administered individually or in

combination. The mixture of PE-MPs and PFAS in combination with oleic acid (OA) at 100 µM resulted in a notable exacerbation of steatosis after a 24-hour exposure.
Some of these pollutants induced a preferential polarisation of undifferentiated THP-1 macrophages towards a pro-inflammatory M1 phenotype, but are also capable of inducing inflammatory reprogramming of THP-1 macrophages from M2 to M1 phenotype, thereby promoting the evolution towards a MASH stage.
Our spheroids generated by mixing HepaRG, LX-2 and THP-1 cells in agarose micro-molds is being developed and is currently being validated in terms of viability, hepatic marker gene expression assessed by RT-qPCR, and spatial distribution of the different cell types visualised by confocal microscopy analyses. The main prospect of these results is the use of our complex 3D liver model to investigate the adverse impacts of MPs and their additives, alone or in combination on several readouts such as induction of steatosis, inflammation and fibrosis.

References

[1] Cheng, Wei, 2023 'Combined effect of polystyrene microplastics and bisphenol A on the human embryonic stem cells-derived liver organoids: The hepatotoxicity and lipid accumulation', *Science of the Total Environment*, 854, 158585.

LP-63

Machine learning-based potential hazards prediction based on the combinatorial use of chemical structure and biological activity

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Prediction of potential hazards with high confidence for chemical substances, for which there is little experimental data, is a primary goal of computational toxicology. Although chemical structure-based prediction is the first application of computational approaches, biological activity patterns can offer complementary insights for the risk assessment to overcome activity cliffs. The predictive performance of such a hybrid approach remains debated, therefore comprehensive investigation on several hazard classes is desired. In this study, we developed machine learning-based predictive models for several hazard classes (CMR; carcinogenicity, mutagenicity, or reproductive toxicity) based on both structural and biological features. The objective was to investigate an impact of the hybrid approach on the hazard predictive performance.
We employed chemicals tested in the ToxCast/Tox21 program. The harmonized classification of CMR labelling were obtained from Annex VI to the ECHA CLP Regulation. The listed chemicals whose CMR labeling are unavailable were considered non-CMR substances. SMILES strings were obtained using EPA CompTox Chemical Dashboard. Biological activities were acquired from the ToxCast/Tox21 InvitroDB (version 4.1). The chemicals with hit-call ≥ 0.9 were considered active, and < 0.9 were considered inactive. Subsequent data analyses were performed on KNIME Analytics Platform. SMILES strings were desalted, then converted into MACCS fingerprint. Averaged Tanimoto coefficients with 5-nearest neighbors were used as a measure of structural or biological similarity of each chemical. Two models were developed based on the XGBoost algorithm using MACCS fingerprint (FP-model) or biological activity pattern (Bio-model) for each CMR hazard class. The predictive performance was calculated by 5-fold cross-validation.Regarding carcinogenicity prediction, FP-model showed better performance in sensitivity (40.1%) and comparable specificity (93.6%) compared to Bio-model (14.2% and 94.9%, respectively), indicating that predictive performance using the structural feature solely was superior to that using biological activity pattern solely for the curated chemicals. In addition, due to class imbalance in the dataset (only 20% chemicals were carcinogens), both models showed poor sensitivity. However, when limited to double-hit chemicals (i.e, predicted positive or negative in both models), although specificity deteriorated slightly (86.1%), sensitivity was drastically improved (75.0%). Moreover, for single-hit chemicals (i.e., chemicals with different predictions in each model), a similarity-weighted

approach also improved the predictive performance. Similar trends were also observed in the mutagenicity and reproductive toxicity prediction. These results suggest that the combinatorial use of structural and biological features on potential hazards prediction has advantages compared to sole use of these features.

LP-64

Co-cultures of pneumocytes types I and II with endothelial cells to study the impact of nanoparticles on lung barrier function.

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The respiratory tract is continuously exposed to all the possible components of the air, including particles derived from human activities. The size of particles dictates the region of the respiratory tract that may be exposed. In the case of nanoparticles (NPs), they can reach the alveoli[1]. To evaluate the safety of NPs, it is important to have a human cells-based model mimicking the human lung barrier. We developed a human lung barrier model by co-culturing type I (hAELVi) and type II-like (A549) pneumocytes with endothelial (EA.hy926) cells. The model was challenged with silver NPs (AgNPs) to assess their impact on the alveolar barrier function and cellular health.
Mono-, double- or triple- cultures of the different cell types were seeded on 0.4 µm pore inserts, allowed to grow at air-liquid interface (ALI), and characterized by measuring the transepithelial electrical resistance (TEER), permeability to Lucifer Yellow (LY), cellular metabolism (resazurin and calcein-AM), and LDH leakage throughout time in culture. The expression of relevant markers of barrier formation and cell type [e.g. tight junctions, surfactant production, and others] was studied by immunocytochemistry. Exposure to AgNPs for 72 h was performed in submerged (SUB) versus quasi-ALI (qALI) conditions.
Monocultures of A549 cells displayed very low TEER values (<35 Ω.cm^2) throughout the culturing period, but high metabolism and a punctuated pattern of the tight junction protein ZO-1. In contrast, hAELVI monocultures stably exhibited TEER values above 1000 Ω.cm^2 after one week in culture, with low metabolism rates and widespread, continuous, expression of ZO-1. When double (A549 + hAELVi) or triple (A549 + hAELVi + EA.hy926) cultures were prepared, the TEER values were initially low, but increased after 10 days in ALI, reaching interesting resistance values (>300 Ω.cm^2)towards the third week of culture. After 21 days in ALI, the elevated TEER values (~1000 Ω.cm^2) were accompanied by low permeability to LY in the triple cultures (average P*app*=1.34x10^6 cm/s), without major impact on cell viability. Exposure to AgNPs resulted in a decrease in the TEER values, compared to the controls, which was significant in the qALI condition ($p<0.05$). However, the P*app* of the cultures did not change, and cell viability did not seem to be affected. Triton X-100 (positive control) dramatically disrupted the barrier function, as demonstrated by the decreased TEER values ($p<0.001$) and increased barrier permeability ($p<0.05$).
Subsequent steps will be to challenge the model with different types of NPs and to grow the triple culture in an organ-on-chip with breathing motion ability, to evaluate its impact on barrier formation.

References

[1] Ramos-Godínez, M 2013, 'TiO2 nanoparticles induce endothelial cell activation in a pneumocyte–endothelial co-culture model.', *Toxicology In Vitro*, 27(2), 774

LP-65

Pyrogen detection using primed THP-1 instaCELLs

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Testing for pyrogen contamination is particularly important for regulatory approval. Pyrogens are substances that can induce an immunological response in the body, accompanied by fever. For the development of new pharmaceuticals or medical devices, it is necessary to ensure that these substances are not present.
Quality control for pyrogen contamination aims to ensure that products are free of pyrogens. Therefore, highly sensitive tests are required to predict the pyrogenic potential of a product. In the past, the rabbit pyrogen test was used to determine the risk potential. However, there is a trend to reduce animal testing, and this test is being replaced by cell-based assays. A cell-based alternative, such as the monocyte activation test, has been accepted by the pharmacopoeias and is therefore becoming increasingly important.
The Monocyte Activation Test can be used to reliably measure the amount of pyrogens in a product. It uses cells that respond to pyrogens by secreting pro-inflammatory substances such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α).
We have generated primed THP-1 cells, which are particularly suitable for the QC test. THP-1 cells were primed into a macrophage-like state by the addition of phorbol 12-myristate 13-acetate. In the MAT assay they show precise specificity for the detection of non-endotoxin pyrogens (NEPs) and endotoxins.
The developed primed THP-1 cells have been cryopreserved as assay-ready cells, meaning no prior cultivation is required. These pre-qualified assay-ready cells are more accurate than continuous cultures because there is no variation due to handling and passage of the cells, ensuring consistent quality. The characteristic surface markers CD11b and CD14 were also successfully detected without alteration.
The primed THP-1 cells can be used in the MAT assay like a reagents in and were neither less nor more sensitive to endotoxin and non-endotoxin pyrogens than cells from continuous culture.

LP-66

Histopathological examination of liver damage caused by Tripterygium wilfordii extract powder

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Objective: Tripterygium wilfordii, characterized by its bitter and pungent taste and cool nature, is highly toxic and targets the liver and kidney meridians. It is reputed for its therapeutic effects, including dispelling wind and dampness, promoting blood circulation and collateral unblocking, reducing swelling and pain, as well as possessing insecticidal and detoxifying properties. With the advancement in research on its active components and pharmacological effects, coupled with its expanding clinical applications, there has been an increasing focus on its toxic side effects and adverse reactions. The liver, as a pivotal organ in human metabolism, is particularly susceptible to drug-induced injury. This study, utilizing animal models and histopathological techniques, aims to investigate the hepatotoxic effects of Tripterygium wilfordii extract powder.

Methods: Male and female Sprague-Dawley (SD) rats were randomly assigned to three groups: a solvent control group (0.5% CMC-Na) and two treatment groups receiving Tripterygium wilfordii extract powder at low (0.203 g/kg) and high (0.610 g/kg) doses. Following oral administration for 29 days, 92 days, and a 30-day recovery period, the rats were anesthetized and dissected. The liver tissues were excised and fixed in pre-prepared formalin solution. The specimens were then transferred to the pathology lab for further histopathological examination.
Results: In the pathological examination conducted after 29 days of gavage administration, the high-dose Tripterygium wilfordii group exhibited notable vacuolation in liver tissue compared to the solvent control group and the low-dose Tripterygium wilfordii group. Following 92 days of gavage administration, liver tissues from both the low-dose and high-dose Tripterygium wilfordii groups displayed varying degrees of vacuolation, multifocal mononuclear cell infiltrates, and sinusoidal congestion, with the high-dose group showing the most pronounced changes. During the 30-day recovery period, the incidence and severity of these lesions in liver tissue from both the low-dose and high-dose Tripterygium wilfordii groups decreased, indicating a partial alleviation of the pathological changes.
Conclusion: Based on the pathological examination results at 29 days, 92 days, and a 30-day recovery period post-administration, it is evident that Tripterygium wilfordii exhibits a degree of hepatotoxicity. Following cessation of the medication, the liver demonstrates a moderate capacity for recovery over time.

References

[1] Li Yan, Fu Bin, Liang Bingnan. Research progress on the mechanism of hepatotoxicity of Tripterygium wilfordii Hook. f. [J]. Tianjin Journal of Traditional Chinese Medicine, 2017, 34 (5): 3.
[2] Cao Lingjuan, Yan Miao, Li Huande, et al. Research progress on liver injury caused by Tripterygium wilfordii and the mechanism of detoxification in combination with licorice [J]. Chinese Journal of Traditional Chinese Medicine, 2015, 40 (13): 5.

LP-67

HeLLO: Hepatocyte-like liver organoids for drug toxicity prediction

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Using whole transcriptome and cell identity analysis, we previously found that many liver *in vitro* models do not fully represent hepatocytes, rendering the cells incapable of modeling a substantial proportion of liver functions. We developed an improved liver organoid culture protocol to allow establishment, expansion, and biobanking of hepatocyte-like liver organoids (HeLLOs) from any liver tissue. Whole transcriptomic analysis showed that HeLLOs displayed high liver resemblance in important hepatic functions such as drug metabolism, fatty acid metabolism, and bile secretion. Accordingly, HeLLOs display high enzymatic activities for drug metabolism and fatty acid metabolism and robustly recapitulate functional bile acid transport compared to the established liver organoid model. Furthermore, we demonstrated that HeLLOs predicted hepatotoxic drugs with twice higher sensitivity than 2D cultured primary human hepatocytes. We believe that HeLLOs represent an interesting model for drug toxicity prediction especially in the early phase of drug development where high throughput and reliable models are needed.