# ABSTRACT BOOK





International Seminar

Clinical Application of Stem Cells in Kidney Transplantation and Nephrology

19 October 2023 Kaunas, Lithuania

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#### Dr. Justinas Mačiulaitis

(Lithuanian University of Health Sciences, Department of Nephrology)

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# **Clinical Application of Stem Cells in Kidney Transplantation and Nephrology**

10:00 – 11:00	Registration of conference
11:00 – 11:15	Opening speech
11:15 – 15:00	PART I Moderators: prof. Romaldas Mačiulaitis, prof. Ryuichi Nishinakamura
11:15 – 11:45	Challenges translating promissing animal cell therapy results to humans <b>Prof. Romaldas Mačiulaitis,</b> Kaunas, Lithuania
11:45 – 11:50	Questions / Answers
11:50 – 12:30	Towards an optimized process of clinical cell therapy manufacturing <b>Prof. Petra Reinke</b> , Berlin, Germany
12:30 – 12:35	Questions / Answers
12:35 – 13:05	Coffee break
13:05 – 13:40	Round table discussion with audience "My challenges and lessons learned" Panelists and quality experts
13:40 – 14:20	Reconstructing kidney from human IPS cells <b>Prof. Ryuichi Nishinakamura</b> , Kumamoto, Japan
14:20 – 14:25	Questions / Answers
14:25 – 14:55	Perinatal stem cell efficacy in the prevention of acute kidney injury. Preclinical model <b>PhD student Agnė Gryguc</b> , Kaunas, Lithuania
14:55 – 15:00	Questions / Answers
15:00 – 16:00	Lunch break
16:00 – 17:50	PART II Moderators: prof. Inga Arūnė Bumblytė, prof. Paul Harden
16:00 - 16:30	Unmet needs in nephrology and transplantology <b>Prof. Inga Arūnė Bumblytė</b> , Kaunas, Lithuania
16:30 – 16:35	Questions / Answers
16:35 – 17:05	Experience of applying mesenchymal stem cell therapy in kidney injury Dr. Justinas Mačiulaitis, PhD student Rūta Insodaitė, Kaunas, Lithuania
17:05 – 17:10	Questions / Answers
17:10 – 17:40	Regulatory cell therapy in kidney transplantation <b>Prof. Paul Harden</b> , Oxford, United Kingdom
17:40 – 17:45	Questions / Answers
17:45 - 17:55	Best poster presentation <b>Paulius Valiukevičius,</b> Kaunas, Lithuania
17:55 – 18:00	End of conference and summary









# **INVITED SPEAKERS**



Prof. Petra Reinke Berlin, Germany

**Petra Reinke** is Professor of Internal Medicine and Nephrology; Head of the GMP-Facility and since 2017 Director of the Berlin Center for Advanced Therapies (BeCAT).

She has a great expertise in the fields of Nephrology, Transplantation, ATMP, GMP manufacturing cell therapeutics, performing investigator-initiated and pharma-sponsored trials (phase I-III) in transplantation, cell therapy, intensive care and has published more than 350 scientific papers (h-index: 71). Prof. Reinke is consultant and advisory board member in the field of cell and gene therapy for various pharmaceutical companies and scientific institutes, programme board member of the Medical Research Council (MRC, UKRMP), CMO & Head of Clinical Development – TCBalance Biopharmaceuticals GmbH and Co-initiator in establishing a Germany-wide academic QP network

#### Scientific Development/ CV:

**Since 2020** Member of the BIH Research Platform Multiscale Omics, Humanized Model Systems and Cell Engineering and Digital Medicine,

2019 - 2021 CART Coordinator (Charité) of the European University Hospital Alliance (EUHA),

**2019 - 2021** Steering committee member of the Charité/BIH Clinical Translational Sciences Platform (CTSP)

Since 2017 Director of the Berlin Center for Advanced Therapies (BeCAT),

**2006- 2021** Head of platform D "immune system" and Steering Committee Member of the BIH Center for Regenerative Therapies (BCRT)

Since 2006 Head of the GMP-Facility

**1995 – 2017** Head of the "Kidney Transplant Outpatient Clinic" at the Dept. Nephrology CVK

#### International consortia:

Project partner EU funded HORIZON-WIDERA-2022 consortium "CTGCT" (2023 – 2029) Project coordinator (Charité) AiF / ZIM / R&D cooperation project "T-CellMed-M1" (2023 – 2025) Project coordinator EU HORIZON-HLTH-2021 (HORIZON-RIA) consortium "geneTIGA", (2022-2026) External Project partner EU HORIZON 2020 / EIT Health - Education grant (2022 – 2024) Project coordinator EU H2020 consortium "ReSHAPE" (2019-2024) Steering board member large scale research initiative RESTORE (2018-) Project coordinator EU FP7 consortium "BIO-DrIM" (2012-2018) Work-package leader EU FP7 consortium "ONE- Study" (2010-2017) Steering committee / work-package leader EU FP6 consortium "RISET" (2005-2010)



# Prof. Ryuichi Nishinakamura

Kumamoto, Japan

**Ryuichi Nishinakamura** graduated from the University of Tokyo School of Medicine and worked as a clinical nephrologist for several years. After receiving his Ph.D. in 1996, he started working on kidney development at the University of Tokyo. In 2004, he moved to Kumamoto University as a professor at the Institute of Molecular Embryology and Genetics, Kumamoto University. He also served as the director of the institute for 4 years until 2020. He has generated knockout mice and identified several genes important for kidney development. He then determined the correct origin of kidney progenitors and used this knowledge to successfully generate kidney organoids from pluripotent stem cells. More recently, he has reported the modeling of kidney disease from patient-derived iPS cells and the generation of more complex, branched kidney organoids.



Prof. Paul Harden Oxford, United Kingdom

**Prof. Paul Harden** trained for six years as a nephrologist and transplant physician at the Western Infirmary in Glasgow. Subsequently he worked as a Consultant Nephrologist with a special interest in transplantation at the University Hospital of North Staffordshire. During this period he developed an interest in non-adherence in young adult transplant recipients and transition of care from paediatric to adult care. He established a joint transition process with Birmingham Children's Hospital.

In 2002, Dr Harden joined the Oxford Kidney Unit and Transplant Centre and has continued to pursue his interest in transition, having established joint clinics with Great Ormond Street and Evelina Children's Hospitals in London. He is clinical advisor to the National Health Service in the UK on adolescent transition and young adult services in patients with ESRD. He runs a unique community-based young adult transplant service in Oxford.

In addition Paul Harden is interested in malignancy post-transplantation and the impact of targeted immunosuppression reduction. He is Chief Investigator of the RESCUE (UK) trial of immunosuppression modulation for squamous cell skin cancer post-transplantation. He is currently working with a European consortium on development of a trial of cell therapy to allow immunosuppression reduction.

# ABSTRACTS

## CYTOTOXICITY OF MYCOTOXINS, THEIR COMBINATIONS AND THEIR INTERACTION TYPES (ADDITIVE, ANTAGONISTIC, SYNERGISTIC) ON MADIN-DARBY BOVINE KIDNEY CELL LINE

**Gintarė Vaičiulienė**<sup>1</sup>\*, Rimvydas Falkauskas<sup>2</sup>, Jurgita Jovaišienė<sup>3</sup>, Neringa Sutkevičienė<sup>1</sup>, Audronė Rekešiūtė<sup>1</sup>, Violeta Baliukonienė<sup>3</sup>

<sup>1</sup> Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės Str. 18, LT-47181 Kaunas, Lithuania <sup>2</sup> National Food and Veterinary Risk Assessment Institute, J. Kairiukščio Str. 10, LT-08409 Vilnius, Lithuania

<sup>3</sup> Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės Str. 18, LT-47181 Kaunas, Lithuania

\* Corresponding author: gintare.vaiciuliene@lsmu.lt

Moulds synthesize secondary toxic compounds – mycotoxins – that adversely affect human and animal organism systems [1]. Multiple mycotoxin contamination of feed is a major concern because of the effects on animal health and the operating principle, which may be additive, antagonistic or synergistic, meaning that the overall toxicity is not only the sum of the toxicity of the individual mycotoxins, but is also recurrent, and the evaluation of a single mycotoxin study may not be able to provide sufficient information on the risks associated with the feed or feed materials and requires a more focused consideration of the combined effect of mycotoxins [2, 3].

The aim of this study: determine the metabolic activity and interaction types (additive, antagonistic, synergistic) of mycotoxins (AFB<sub>1</sub>, ZEA, DON, T-2) and their combinations on MDBK cell line.

Exposure to mycotoxins: AFB<sub>1</sub> (aflatoxin B<sub>1</sub>), ZEA (zearalenone), DON (deoxynivalenol) and T-2 (T-2 toxin) and their combinations was assessed by the inhibition concentration (IC<sub>50</sub>) value. Stock solution of AFB<sub>1</sub>, ZEA, DON and T-2 were prepared in DMSO and the mycotoxins dilutions in the culture medium. MDBK (Madin-Darby Bovine Kidney) cells were maintained in MEM Eagle medium with L-glutamine, 50 mg/mL gentamicin and 10 % FCS inactivated. Cells were grown tissue culture flasks, 2.5 x 10<sup>5</sup> to 3.0 x 10<sup>5</sup> cells/well were seeded in 96-well tissue culture plates. When cells achieved 65 % confluence, AFB<sub>1</sub> (5  $\mu$ g/L and 7  $\mu$ g/L), ZEA (500  $\mu$ g/L and 1000  $\mu$ g/L), DON (600  $\mu$ g/L and 5000  $\mu$ g/L) and T-2 (250  $\mu$ g/L and 500  $\mu$ g/L) and the different combinations of these compounds at different concentrations were added and plates were incubated at 37 °C for 24, 48 and 72 h. Cell viability was assessed MTT. The absorbance was measured 540 nm and 580 nm on an ELISA microplate reader. IC5<sub>50</sub> values were calculated from full dose–response curves. The type of interaction between mycotoxins and combinations of mycotoxins individually was determined in cells by the Chou-Talalay method. Fa-CI and isobolograms were plotted using CompuSyn software (ComboSyn Inc, USA) to assess the pattern of action of the combination index (CI) for each concentration of the combination used.

The maximum and average concentrations of mycotoxins determined in feed were used. When assessing the effect of the three mycotoxin combinations on MDBK cells, the highest IC<sub>50</sub> was found after 72 h with the AFB<sub>1</sub>/ZEA/T-2 combination at a concentration of 7/1000/500 µg/L (p < 0.05). The lowest IC<sub>50</sub> after 72 h in MDBK cells were found after exposure to AFB<sub>1</sub>/ZEA/DON combination at 5/1000/5000 µg/L (p < 0.05). The combinations with the highest synergistic effect after 24 h on MDBK cells were found to be AFB<sub>1</sub>/ZEA/DON (5/500/600 µg/L) and AFB<sub>1</sub>/ZEA/T-2 (7/500/500 µg/L), and after 72 h AFB<sub>1</sub>/ZEA/T-2 (7/500/500 µg/L). ZEA/DON (1000/5000 µg/L) and ZEA/T-2 (500/250 µg/L) (p < 0.05). Mycotoxin combinations with the

MAX (ZEA/DON/AFB<sub>1</sub>/T-2 – 1000/600/7/500  $\mu$ g/L) and MRL (ZEA/DON/AFB<sub>1</sub>/T-2 – 500/5000/5/250  $\mu$ g/L) mycotoxin concentrations used were found to have a synergistic (p < 0.05) effect.

The highest IC<sub>50</sub> on the MDBK cell line was caused by AFB<sub>1</sub>/ZEA/T-2 combination at 7/1000/500  $\mu$ g/L (92.3 %) after 72 h (p < 0.05). The strong synergistic effect on the MDBK cell line was caused by ZEA/DON combination at 1000/5000  $\mu$ g/L concentration and ZEA/T-2 combination at 500/250  $\mu$ g/L (p < 0.05).

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# ALLOGENEIC MESENCHYMAL STROMAL CELLS AND THEIR EXTRACELLULAR VESICLES MODULATE INFLAMMATION VIA CYTOTOXIC AND REGULATORY T LYMPHOCYTE POPULATIONS. A PILOT IN-VITRO STUDY

**Paulius Valiukevičius**<sup>1</sup>, Dovydas Bagdonas<sup>2</sup>, Justinas Mačiulaitis<sup>3,4</sup>, Rūta Insodaitė<sup>3</sup>, Ieva Čiapienė<sup>4</sup>, Edita Gasiūnienė<sup>2</sup>, Brigita Gradauskienė<sup>2</sup>, Romaldas Mačiulaitis<sup>3</sup>

- <sup>1</sup> Medical Academy, Lithuanian University of Health Sciences (LUHS), Lithuania
- <sup>2</sup> Clinical Department of Immunology and Allergology, LUHS, Lithuania
- <sup>3</sup> Institute of Physiology and Pharmacology, LUHS, Lithuania
- <sup>4</sup> Institute of Cardiology, LUHS, Lithuania

**Introduction:** Mesenchymal stromal cells (MSC) and their extracellular vesicles (EV) have immunomodulatory properties (1,2), which are therapeutic in a wide spectrum of diseases (3). Potency assays are crucial to ensure consistency and quality, as well as to optimize dosage (4). One possible approach is the peripheral blood mononuclear cell (PBMC) co-culture assay. MSC and their exosomes can suppress stimulated PBMC proliferation; however, there is a lack of understanding of which populations of PBMC are affected the most. In this study, we focus on evaluating the effect of MSC or their EV on the proliferation of T regulatory and CD8+ lymphocytes, which are important in the pathogenesis of many inflammatory and autoimmune diseases (5,6).

**Methods:** A placenta was collected from a healthy volunteer who underwent a cesarean section after obtaining written informed consent. Venous blood samples were collected from three healthy volunteers with written informed consent. All procedures were performed in accordance with Lithuanian Bioethics Committee standards (approval no. BE-2-105). MSC were isolated from the placenta by collagenase digestion. Cells were cultured in MEM supplemented with 10% FBS and 0.1% gentamicin. Fourth passage cells were used in the experiments. The conditioned medium was collected, filtered through a 0.22 µm filter, and concentrated with a tangential flow filtration 500 kDa filter 30 times to concentrate the EV. PBMC co-culture was performed as described by Oliver-Vila et al. (7). One million cells from each donor were frozen for use as a baseline for the gene expression assay. PBMC (5×10^5 per well) were cultured with either 2×10^5 MSC, 12 µL of EV, or vehicle (control) in duplicate for five days. PBMC were collected, washed, and incubated with monoclonal anti-human antibodies CD3-eFluor450, CD4-Pacific Orange, CD8-APC-Cyanine7, CD25-APC, CD127-PE-Cyanine7, and viability dye 7-AAD (Invitrogen). At least 100 000 cells were acquired using a BD FACSLyric flow cytometer and analyzed with FlowJo. Fluorescence minus one (FMO) and isotype controls were used. The data were analyzed using FlowJo. PBMC gene expression for ACTB (reference gene) and IL-10 was analyzed using TaqMan<sup>®</sup> Gene Expression Assays (Applied Biosystems). Gene expression levels were calculated using the  $\Delta\Delta$ Ct method (8). Results are described as medians with the 25–75th interquartile range (IQR). Statistical analysis was performed using GraphPad Prism, with a significance level of p<0.05. Differences across groups were evaluated using the Kruskal-Wallis test, and Dunn's test was used for pairwise comparisons.

**Results:** Stimulation of PBMCs with PMA and ionomycin resulted in robust proliferation. Additionally, it increased the variability in lymphocyte size and granularity and modulated the expression of surface markers, making it difficult to separate the positive and negative populations. Therefore, FMO controls were used to select a cut-off value for negative populations. Viable CD3+ cells (lymphocytes) were selected and

further gated to CD4+CD8- (T helpers) and CD4-CD8+ (cytotoxic T lymphocytes) populations. T regulatory lymphocytes (Treg) were defined from the CD4+CD8- population by gating on cells with high expression of CD25 and low or negative expression of CD127 (CD4+CD25highCD127low/-) (9). The normalized proliferation of CD3+, CD4-CD8+, and Treg populations was analyzed in all groups. The proliferation rates in the control sample were normalized to a baseline value of 1 for accurate comparative analysis. Furthermore, the frequency of CD4-CD8+ in all CD3+ cells and Treg cells in CD4+CD8– cells was analyzed in all groups. CD3+ normalized proliferation in MSC samples was 0.75 (0.65-0.80), in EV samples - 0.64 (0.55-0.79), with a reduction in both treatment groups (p<0.05). CD4-CD8+ proliferation was also significantly reduced in both groups 0.46 (0.46-0.50) for MSC and 0.88 (0.87-0.93) for EV (p<0.05). Interestingly, only MSC statistically significantly (p<0.01) reduced the proliferation of Treg, resulting in a normalized proliferation of 0.12 (0.08-0.41). There was a trend of lower CD4-CD8+ frequency in the MSC group (15.0 (4.9-22.0) % control vs. 6.2 (4.8-13) % MSC), which did not reach statistical significance. Interestingly, there was also a trend of increased Treg frequency in the treatment groups (control 15.0 (9.0-20.0) %, MSC 19.0 (13.0-36.0) % and EV 18.0 (7.9-23.0) %), which did not reach statistical significance. IL-10 gene expression fold changes from baseline in control, MSC, and EV groups were 0.47 (0.35-0.50), 2.8 (2.8-6), and 0.91 (0.4-1.5), respectively, with a significant elevation only in the MSC group (p<0.01).

**Conclusions:** Both MSC and EV in selected doses reduced the proliferation of lymphocytes and their CD4-CD8+ population, but only MSC reduced the proliferation of Treg lymphocytes. Furthermore, MSC co-culture resulted in significant upregulation of IL-10, which is the main effector molecule of T regulatory lymphocytes (10). This suggests that MSC therapy may modulate Treg numbers not through increased proliferation but possibly by altering the relative proportions of both Treg and cytotoxic lymphocytes within the overall lymphocyte population and thus shifting the balance towards an anti-inflammatory immune response.

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## PERINATAL STEM CELLS EFFICACY IN THE PREVENTION OF ACUTE KIDNEY INJURY PROGRESSION TO CHRONIC KIDNEY INJURY. PRECLINICAL MODEL

**Agnė Gryguc**<sup>1</sup>, Justinas Mačiulaitis<sup>2</sup>, Lukas Mickevičius<sup>3</sup>, Arvydas Laurinavičius<sup>4</sup>, Neringa Sutkevičienė<sup>5</sup>, Ramunė Grigalevičiūtė<sup>6</sup>, Vilma Zigmantaitė<sup>6</sup>, Inga Arūnė Bumblytė<sup>1</sup>, Romaldas Mačiulaitis<sup>1,7</sup>

<sup>6</sup> Biological research centre, Veterinary academy, Lithuanian University of Health Sciences, Kaunas Lithuania;

**Introduction:** Acute kidney injury (AKI) is a dangerous condition. Its episodes can lead to chronic kidney disease (CKD). There is no effective and timely treatment. Therefore, it is very important to develop a new strategy for maintaining and improving the kidney function. Currently, regenerative medicine, especially human placental mesenchymal stem cells (hPSCs), is the most promising area.

**Methods:** Human placental amniotic and chorionic cells have been isolated. hPSCs have been cultivated and characterized by yield, viability, flow cytometry and potency in vitro. Rats underwent preclinical ischemia-reperfusion injury (IRI). Experimental group received 3x105 of hPSCS in each kidney, control group - phosphate buffer solution (PBS), untreated group - only induced IRI. Urine, blood serum samples and kidneys for histological analysis collected.

**Results:** hPSCs had a consistent yield, viability, mesenchymal stem cell markers expression and suppressed proliferation of T cells in a dose-dependent fashion. hPSCs increased survival and kidneys function by decreased creatinine, urea in serum in compare with surviving rats in control groups. Cells decreased renal injury scores and prevented chronic injury by reduced kidneys structural damage compare with control groups.

**Conclusion:** Although hPSCs are not use widely in preclinical and clinical trial yet, however, in the present study, we demonstrated that hPSCs have the potential to prevent initial kidney fibrosis cascade through ameliorating initial kidney damage and improving kidney function.

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<sup>&</sup>lt;sup>1</sup> Department of Nephrology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania;

<sup>&</sup>lt;sup>2</sup> Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas Lithuania;

<sup>&</sup>lt;sup>3</sup> Department of Urology, Hospital of Lithuanian University of Health Sciences, Kaunas Lithuania;

<sup>&</sup>lt;sup>4</sup> National Center of Pathology, Affiliate of Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania;

<sup>&</sup>lt;sup>5</sup> Large Animal Clinic, Veterinary academy, Lithuanian University of Health Sciences, Kaunas Lithuania;

<sup>&</sup>lt;sup>7</sup> Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas Lithuania.

### Hospital of Lithuanian University of Health sciences Kauno klinikos Structure of Nephrology Department



Nephrology Department

Unit of Nephrology (42 hospital beds)

Outpatient clinic (3 fulltime consulting rooms) Subunit of kidney transplantation

Subunit of resistant hypertension and renal disease

Subunit of urgent nephrology

Subunit of dialysis

Centre of Rare kidney disease

### Hospital of LUHS Kauno klinikos DEPARTMENT OF NEPHROLOGY FACILITIES IN NOWADAYS

- Established in 1992.
- Full 24-hours service for nephrological patients:
  - Clinical nephrology (42 beds, 1700 hospitalizations)
  - Hemodialysis (over 14000 HD procedures per year, from them – around 2000 in intensive care units (20 % slow continuous renal replacement therapy procedures)



- Peritoneal dialysis
- Kidney transplantation (median 40 Tx/per year)
- Outpatient clinic (over 14000 visits)
- Center of Rare kidney diseases
- 16 full-time nephrologists and 4 clinical toxicologists
- Academical staff:
  - 14 PhD ( 4 professors, 5 assoc. professors) 3 PhD students