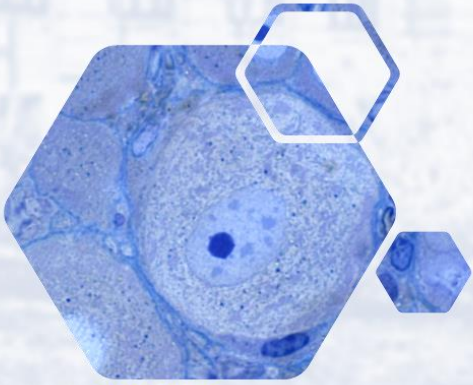
„Victor Babeş" National Institute of Pathology

Institutul Naţional de Cercetare-Dezvoltare
în Domeniul Patologiei şi Ştiinţelor Biomedicale
"Victor Babeş"

International Pathology Conference of the Victor Babeș Institute Bucharest

17 - 19 November 2022

BOOK OF ABSTRACTS



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INTERNATIONAL PATHOLOGY CONFERENCE OF THE „VICTOR BABEȘ" INSTITUTE BUCHAREST

BOOK OF ABSTRACTS

2022

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Editing: Victor-Eduard Peteu, Mihaela Gherghiceanu, "Victor Babeș" National Institute of Pathology, Bucharest

CONTENT

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PROGRAM

Thursday 17, November	
	CHANNEL1
9:30-11:00	Opening The changing face of biomedical research Prof. Mihail Eugen Hinescu, General Director of „Victor Babeș" National Institute of Pathology Bucharest, Romania Prof. Ștefan Constantinescu, President of Federation of European Academies of Medicine, Victor Babeș Honorary Scientist Award 2020 Prof. Univ. Dr. Ing. Adrian Curaj, General Director of UEFISDCI Prof. Viorel Jinga, Rector of „Carol Davila" University of Medicine and Pharmacy Bucharest, Romania
11:15-13:15	Session 1: Victor Babeș Institute - research news Session Chair – Prof. Mihail Eugen Hinescu - 3D tumor spheroid models for therapeutic screening. Ana-Maria Enciu - Molecular approaches in adjuvant therapy with natural bioactive compounds. Cristiana Tănase - Telocytes in cardiovascular system. Mihaela Gherghiceanu - Portrayal of antigen presenting cells in cutaneous melanoma - innovative pillars for harnessing immunotherapy. Monica Neagu
13:15 13:45	Diagnosis of MSI status with the Gold Standard markers using OncoMate™ MSI Dx Analysis System CE-IVD. Kseniya Carr, Sr Key Account Manager CEE. Promega GmbH, Germany
15:00-16:00	Keynote Lecture - Victor Babeș Honorary Fellow Award Targeting B-cell Survival in Lymphoid Malignancies Alexandru Almăşan Department of Cancer Biology, Cleveland Clinic, Cleveland, USA

Thursday 17, November	
16:15-17:15	Keynote Lecture: Update on Mesothelioma - Pathogenesis and Diagnostic Evaluation Lucian R. Chirieac Brigham and Women's Hospital, Harvard Medical School, Boston, USA
17:30-19:00	Session 2: Next generation pathology Session Chair – Octavian Bucur - Expansion Pathology. Octavian Bucur, Victor Babeș National Institute of Pathology Bucharest, Romania - AI in hematolymphoid disorders. Rajan Dewar, McLaren Greater Lansing, Michigan State University, USA - The application of artificial intelligence in pathology and issues related to deployment. Liron Pantanowitz, University of Michigan, USA

Friday 18, November		
	CHANNEL 1	CHANNEL 2
09:00-11:00	Session 3: Immunology Covid-19 lessons for the post-pandemic era Session Chair – Monica Neagu - How can genomic studies inform public health policies? Simona Ruță, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania - Preparing for the next wave: toxicodynamics and immunosurveilance of SARS-CoV-2. Konstantinos Nikolouzakis, University of Crete, Greece - Development of SARS-CoV-2 IgM following vaccination predicts longer immunity. Donato Zipeto, University of Verona, Italy - Therapeutic delivery efficacy of nanovectors. Giuseppe Bardi, Istituto Italiano di Tecnologia, Genova, Italy	Session 4: Genomic and proteomic technologies in research and diagnosis of complex diseases Session Chairs – Cristiana Tănase & Aurora Arghir - Clinical utility of periconceptional carrier screening for prevention of neurodevelopmental disorders. Anita Rauch, Institute of Medical Genetics, Zürich, Switzerland - Proteomic and Phosphoproteomic Analysis of Clear Cell Renal Cell Carcinoma. Nurhan Özlü, Department of Molecular Biology and Genetics, Koç University, Istanbul, Turkey
11:15-13:15	Session 5: Cardiovascular pathology Session Chair – Elisa Anamaria Liehn - Cardiac-targeted delivery of a novel Drp1 inhibitor for cardioprotection. Shiang (Max) Lim, St. Vincent's Institute Medical Research, Melbourne, Australia - Human platelet mitochondria improve the mitochondrial and cardiac function of donor heart. Yin Hua Zhang, Seoul National	Session 6: Short communication - Biomedical research Session Chair – Anca Hermenean & Laura Cristina Ceafalan - The inhibition of Galectin-1: new insights for treatment of diabetic complications. Maria Consiglia Trotta et al. University of Campania

Friday 18, November		
	CHANNEL 1	CHANNEL 2
	University College of Medicine, Korea - Mitochondrial DJ-1 as therapeutic target for cardioprotection. Sauri Hernandez-Resendiz et al. National Heart Centre Singapore - Collagen synthesis involves distinct functions of Vitamin C. Mihaela Rusu et al. Institute for Molecular Cardiovascular Research, University Hospital Aachen, Germany - The effect of vitamin C on oxidative stress in ischemic conditions. Yichen Xu, et al. Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark - Targeting mitochondria to prevent vascular restenosis. Gustavo Enrique Crespo Avilan et al. Duke-NUS Medical School Singapore, Cardiovascular and Metabolic Disorders, Singapore, Singapore	"Luigi Vanvitelli", Naples, Italy - Role of pro-resolving mediators in retinal diseases. Carlo Gesualdo et al. University of Campania "Luigi Vanvitelli", Naples, Italy - Investigation of the biological effects of β-cyclodextrins. Ferenc Fenyvesi, University of Debrecen, Debrecen, Hungary - Investigating the chemopreventive potential of natural products on breast cancer in vitro models. Sevinci Pop et al. - Generation of a reporter cell line to study IL-1β-mediated inflammation. Gabriela Chiritoiu et al. Institute of Biochemistry of the Romanian Academy, Bucharest, Romania
13:30-14:30	Keynote Lecture: Extracellular Vesicles in Cardiovascular Theranostics Prof. Lucio Barile - Università della Svizzera Italiana, Lugano, TI, CH	
14:45-16:45	Session 7: Nephropathology Session Chairs – Mihaela Gherghiceanu & Gener Ismail - Advances in understanding of nephrotic lesions. Vanesa Bijol, North Shore University Hospital	Session 8: Neurosciences Session Chair – Bogdan O. Popescu - Neuro … perspectives. Emil C. Toescu, Institute of Transdisciplinary

Friday 18, November		
	CHANNEL 1	CHANNEL 2
	and Long Island Jewish Medical Center, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, New York, USA. - Unexpected kidney biopsy finding in a patient with AKD. Nicoleta Petre, Carol Davila Clinical Hospital & „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania - Urinary soluble CD163 in ANCA associated vasculitis. George Terinte Balcan - Victor Babeș National Institute of Pathology Bucharest, Romania - A novel activity and chronicity index for histologic assessment of renal biopsies in ANCA-associated vasculitis. Bogdan Obrișcă, Fundeni Clinical Institute &„Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania - Nefrita lupus-like: O complicatie autoimuna rara a infectiei cu virusul hepatitei C. Andreea Andronesi, Fundeni Clinical Institute &„Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania	Discoveries, Medical School, University of Pecs, Hungary & Liberal Arts and Natural Sciences, College of Medical and Dental Sciences, The University of Birmingham, UK Victor Babeș Honorary Fellow Award - Pain, from Aristotle to the Gate Theory, via Descartes. Alexandru Babeș, University of Bucharest, Romania - Neuroethics, neuromodulation and the neurologic catastrophe of our era. Bogdan O. Popescu, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
17:00-19:00	Session 9: Short communication - Young Pathologists Session Chair – Gabriel Becheanu & Adelina Maria Cohn - Diagnostic Challenges in Primary Rhabdoid Cutaneous Melanomas - a Case Series. Dana Tapoi, et al. „Carol Davila" University of	Session 10: Muscle pathology Session Chair – Laura Cristina Ceafalan - Muscle systemic signaling via myokines. Fabio Demontis, Comprehensive Cancer Center, St. Jude

Friday 18, November		
	CHANNEL 1	CHANNEL 2
	Medicine and Pharmacy, Bucharest, Romania - Mesonephric-like adenocarcinoma of the female genital tract - an underdiagnosed entity. Antonia-Carmen Georgescu, et al. Carol Davila Nephrology Clinical Hospital &„Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania - Analysis of the tumor microenvironment in gastric adenocarcinoma with the emphasis on LAG-3. Florina Almarii, Fundeni Clinical Institute, Bucharest, Romania - Vein invasion, a rare morphological feature of breast cancer. Adelina Baltan, et al. Poundbury Cancer institute, Poundbury, Dorchester, UK - Differential diagnosis of bone sarcomas using immunohistochemistry. Oana Neagu, Gr. Alexandrescu Hospital, Bucharest - A rare cause of rectal mucosal ulceration. Adelina Maria Cohn, Gabriel Becheanu „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania - Difficult case in nephropathology. George Terinte Balcan, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania	Graduate School of Biomedical Sciences, Memphis, TN, USA - Diagnostic problems in pediatric muscle pathology. Magda Budișteanu, Spitalul Clinic de Psihiatrie, Prof. Dr. Alexandru Obregia, Bucharest, Romania - Charcot-Marie-Tooth Neuropathy and Neuromyotonia - a rare association, Diana Anamaria Epure et al. Neurologie Pediatrica, Sp. Clinic de Copii Dr Victor Gomoiu, Bucharest, Romania - The two different faces of Interleukin-6, as a myokine and as a cytokine. Emilia Manole et al. Victor Babes National Institute of Pathology, Bucharest, Romania - Challenges in muscle biopsy evaluation. Alexandra Bastian et al. Colentina Clinical Hospital, Pathology Department, Bucharest

Saturday 19, November	
	CHANNEL 1
09:00-11:00	Course – Human induced pluripotent stem cell-based models for development and disease Prof. Irina Roxana Deleanu, Department of Anatomy, Histology and Embryology, Medical University of Innsbruck, Austria
11:15-12:00	Intracellular signaling through ITAM-motif receptors Loredana Săveanu, INSERM U1149, Centre de Recherche sur l'Inflammation, Faculté de Médecine, Paris, France.
12:15-14:00	Session 11: Short communication - young researchers Best Presentation Award - Motic microscope sponsored by HypoTech&Consulting Session Chairs – Ana-Maria Enciu & Tudor Emanuel Fertig open for submission - Multimodal Micro-CT Imaging for 3D Pathology. Victor Gabriel Ungureanu, Antoanela Tanca, Daniel Anghel, Elisa Anamaria Liehn, Octavian Bucur - Tumor cells under irradiation. Maria Dobre, Elena-Mihaela Dragnea, Ionela Victoria Neagoe - Tumor spheroids as models for in vitro testing. Iulia Costache, Lucian Albulescu, Ionela Daniela Popescu, Elena Codrici, Maria Dudau, Ana-Maria Enciu, Cristiana Tănase - Application of CRISPR/Cas-9-Mediated Genome Editing – first steps to generate a K.O. cell line. Maria Dudau, Tobia Fantoni, Michele Bissoli, Zipeto Donato, Maria Teresa Valenti
14:30-15:30	Communication (K)now Session Chairs – Mihail Eugen Hinescu & Dan Anton Vasiliu Marina Hanganu & Alexandru Baltă
15:45-16:00	Closing remarks

DAY 1 – THURSDAY, NOVEMBER 17

Opening Ceremony: The changing face of biomedical research

Session 1: Research news

Lecture: OncoMate™ MSI Dx Analysis System CE-IVD

Keynote Lecture: Victor Babeș Honorary Fellow Award

Keynote Lecture: Update on Mesothelioma

Session 2: Next generation pathology

Opening Ceremony

Prof. Mihail Eugen Hinescu, General Director of „Victor Babeș" National Institute of Pathology Bucharest, Romania

Prof. Ștefan Constantinescu, President of Federation of European Academies of Medicine, Victor Babeș Honorary Scientist Award 2020

Prof. Univ. Dr. Ing. Adrian Curaj, General Director of UEFISDCI

Prof. Viorel Jinga, Rector of „Carol Davila" University of Medicine and Pharmacy Bucharest, Romania

Session 1

Session Chair – Prof. Mihail Eugen Hinescu

3D TUMOR SPHEROID MODELS FOR THERAPEUTIC SCREENING

Ana-Maria Enciu[1,2] ,Lucian Albulescu[2], Ionela Daniela Popescu[2], Elena Codrici[2], Maria Dudau[1,2], Cristiana Tănase [2,3]

1. "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
2. „Victor Babeș" National Institute of Pathology, Bucharest, Romania
3. "Titu Maiorescu" University, Bucharest, Romania

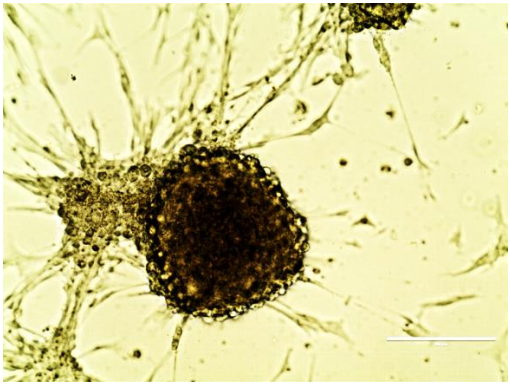
Tumor spheroids are 3D conglomerates of cancer cells, aiming at a better mimicking an *in vivo* tumor than a 2D cell culture. These spheroids have a higher resistance to chemotherapies than conventional 2D cultures and are increasingly preferred for cytotoxic screening of new drugs.

The research projects implemented in the Biochemistry Laboratory during the last 2-3 years gradually revealed the differences in protein signaling expression between 2D and 3D cell cultures and the need to change the standard cytotoxicity protocols, by implementing spheroids as *in vitro* models. To this end, we implemented a hydrogel-based spheroid generation protocol, which can yield viable spheroids in 5 days. As viability assay, we are currently performing a qualitative screening using live/dead assay and we are optimizing spectrophotometric and fluorometric assays for quantification.

We are also editing a tumor cell line to assess the impact of CD36 on tumorigenesis, by assessing cell adhesion, migration and invasion, as well as the ability to form spheroids.

As future directions, the Biochemistry-Proteomics lab aims at generating tumor edited spheroids as personalized models of therapeutic responses and continuously improve in vitro models to increase translatability of research results toward clinical practice.

Acknowledgments: This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-3141, within PNCDI III, POC A 1.2.3., ID: P_40_197/2016, 31PFE/2021 and PN.19.29.01.04.



Glioblastoma U87 cell culture; it forms tumoral spheroids by being kept in culture for longer. Phase cointrast ob. 20x

PN-III-P2-2.1-PED-2019-3141- Tehnologie bazată pe substrat nanostructurat și funcționalizat anti-CD36, pentru captarea celulelor tumorale metastatice circulante CTNanoScan
https://www.ivb.ro/pn-iii-p2-2-1-ped-2019-3141

POC A 1.2.3., ID: P_40_197/2016 - Implementarea expertizei de cercetare biomedicală prin transfer de cunoștințe către mediul privat pentru validarea de produse și servicii în domeniile biotehnologii medicale și sănătate – INTELBIOMED
http://www.intelbiomed.ro/

31PFE/2021- Dezvoltarea excelenței INCD „Victor Babeș" în cercetarea pentru sănătate – EXCELSAN
https://www.ivb.ro/excelsan

PN.19.29.01.04.- Evaluarea profilului kinomic și miRNomic, pentru explorarea de noi ținte moleculare și terapii combinatorii în glioblastom; abordări omice
https://www.ivb.ro/evaluarea-profilului-kinomic-si-mirnomic-pentru-explorarea-de-noi-tinte-moleculare-si-terapii-combinatorii-in-glioblastom-abordari-omice

MOLECULAR APPROACHES IN ADJUVANT THERAPY WITH NATURAL BIOACTIVE COMPOUNDS

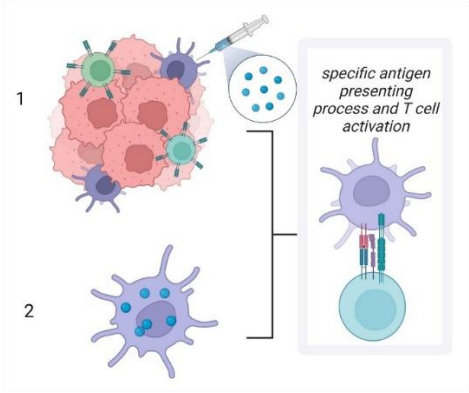
Cristiana Tănase[1]

1. „Victor Babeș" National Institute of Pathology, Bucharest, Romania

PORTRAYAL OF ANTIGEN PRESENTING CELLS IN CUTANEOUS MELANOMA - INNOVATIVE PILLARS FOR HARNESSING IMMUNOTHERAPY

Monica Neagu[1]

1. „Victor Babeș" National Institute of Pathology, Bucharest, Romania



Therapeutic approaches using LCs

1) In situ activation of LCs with specific antigenic peptides;
2) Ex vivo manipulation of LCs using specific antigenic peptides; both approaches induce antigen presentation enhancement and further T-cell specific activation

PN-III-P4-PCE-2021-0549
Portrayal of antigen presenting cells in cutaneous melanoma - innovative pillars for harnessing immunotherapy
Website:
https://www.ivb.ro/pce9_2022

TELOCYTES IN CARDIOVASCULAR SYSTEM

Mihaela Gherghiceanu[12]

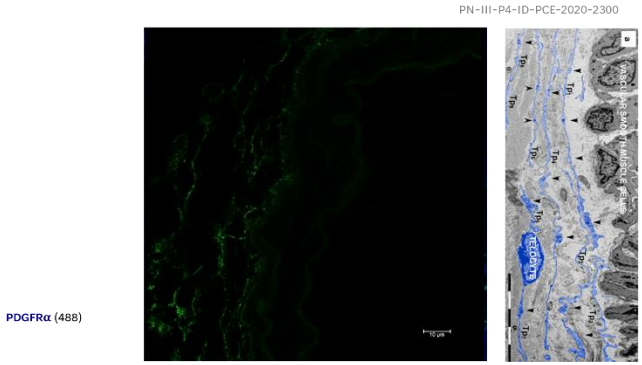
1. "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
2. „Victor Babeș" National Institute of Pathology, Bucharest, Romania

The strategies to stimulate endogenous cardiomyocyte renewal depend on our understanding of molecular and cellular mechanisms involved, in situ. To contribute to this effort, this study aims to investigate the identity of telocytes (TCs) as a type of cardiac interstitial cell.

Although the role of the mesenchymal compartment in tissue homeostasis or disease is increasingly recognized, the relative contribution of distinct cell subsets to these processes remains poorly understood. The main limitation has been the lack of specific markers able to discriminate between the different interstitial cells. Despite the importance of this field, there have been limited efforts to systematically recognize the identity of cells involved in tissue renewal and regeneration, or fibrosis and degeneration.

TCs are essential to heart function through multiple interactions with cardiac progenitors, cardiomyocytes or other cardiac cells, but appreciation of their contribution has too suffered from the incomplete characterization of their molecular identity. We found out that PDGFRα may be used to describe TCs in the mouse heart. Even if FOXL1 seems to be a reliable marker for TCs as a subset of PDGFRα+ cells in the intestine it appears not to be the case for PDGFRα+ cells in the heart.

Acknowledgments: This work was supported by a grant of the Romanian MCID, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-2300 (Ctr. 43PCE/2021)



Lecture

DIAGNOSIS OF MSI STATUS WITH THE GOLD STANDARD MARKERS USING OncoMate™ MSI Dx ANALYSIS SYSTEM CE-IVD

Kseniya Carr[1]

1. *Sr Key Account Manager CEE. Promega GmbH, Germany*

www.promega.de; kseniya.carr@promega.com

Keynote Lecture

TARGETING B-CELL SURVIVAL IN LYMPHOID MALIGNANCIES

Alexandru Almășan[1]

1. Department of Cancer Biology, Cleveland Clinic, Cleveland, USA

Defects in apoptosis can promote tumorigenesis and impair responses of malignant B-cells to chemotherapeutics. Overexpression of antiapoptotic BCL-2 family proteins is associated with treatment resistance and poor prognosis. Venetoclax is a highly selective BCL-2 inhibitor currently widely used in treatment of chronic lymphocytic leukemia (CLL). Chronic activation of the BTK-mediated B-cell receptor signaling is a hallmark of many B-cell lymphoid malignancies, including CLL and DLBCL. The BTK inhibitor ibrutinib demonstrated high response rates. Despite impressive clinical activity, monotherapies with these therapeutics can lead to drug resistance.

The ibrutinib-resistant (IB-R) cell lines we generated showed decreased FOXO3a and PTEN levels and activation of AKT. Inhibition of PI3K and AKT increased ibrutinib-induced apoptosis in IB-R cells by downregulation of $pAKT^{473}$ and restoring FOXO3a levels. The exportin 1 inhibitor, selinexor synergized with ibrutinib in IB-R cells and restored nuclear abundance of FOXO3a and PTEN. Thus, reactivation of FOXO3a nuclear function enhances the efficacy of ibrutinib and overcomes acquired resistance to ibrutinib. These findings reveal a novel mechanism that confers ibrutinib resistance via aberrant nuclear/cytoplasmic subcellular localization of FOXO3a.

Two different BTK inhibitors downregulated miRNAs located in the 14q32 miRNA cluster region. BTK IB-R CLL and DLBCL cells had striking had reduced PTEN expression. Overexpression of a miR-494 mimic abrogated both PTEN mRNA and protein levels. Conversely, overexpression of a miR-494 inhibitor in IB-R cells restored PTEN mRNA and protein levels, thereby sensitizing cells to ibrutinib-induced apoptosis. Inhibition of miR-494 and miR-495 sensitized cells by cooperative targeting of *pten*, with additional miRNAs in the 14q32 cluster that target *pten* able to contribute to its regulation. Therefore, targeting 14q32 cluster miRNAs may have therapeutic value in acquired BTK-resistant patients via regulation of the PTEN/AKT/mTOR signaling axis.

IB-R cells had STAT3-induced, elevated BCL-2 levels, thus providing a rationale for combined use of BCL-2 and BTK inhibitors.

Keynote Lecture

Update on Mesothelioma - Pathogenesis and Diagnostic Evaluation

Lucian R. Chirieac[1]

1. Brigham and Women's Hospital, Harvard Medical School, Boston, USA

https://www.dfhcc.harvard.edu/insider/member-detail/member/lucian-r-chirieac-md/

Session 2

Session Chair – Octavian Bucur

EXPANSION PATHOLOGY

Octavian Bucur[1]

1. „Victor Babeș" National Institute of Pathology, Bucharest, Romania

AI IN HEMATOLYMPHOID DISORDERS

Rajan Dewar[1]

1. McLaren Greater Lansing, Michigan State University, USA

THE APPLICATION OF ARTIFICIAL INTELLIGENCE IN PATHOLOGY AND ISSUES RELATED TO DEPLOYMENT

Liron Pantanowitz[1]

1. University of Michigan, USA

Now that whole slide imaging is mature and there is increased global interest in adoption for clinical use, digital pathology is poised for an era of artificial intelligence (AI). AI in Anatomical Pathology can help detect rare events (e.g. microorganisms), automatically quantify features in digital images (e.g. mitoses), diagnose diseases (e.g. cancer) from WSI, make prognostic predictions by analyzing pixels, and help with discovery (e.g. biomarker research, clinical trials). This presentation will focus on the prerequisites needed for clinical deployment of AI, discuss where to insert AI tools in pathology workflow, and review the considerations for validation of AI use in clinical practice.

DAY 2 – FRIDAY, NOVEMBER 18

Session 3: Immunology

Session 4: Genomics and Proteomics

Session 5: Cardiovascular pathology

Session 6: Short communication - Biomedical research

Keynote Lecture: Extracellular Vesicles

Session 7: Nephropathology

Session 8: Neurosciences

Session 9: Short communication - Young Pathologists

Session 10: Muscle pathology

Session 3

Session Chair – Monica Neagu

HOW CAN GENOMIC STUDIES INFORM PUBLIC HEALTH POLICIES ?

Simona Ruță[1]

1. „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

PREPARING FOR THE NEXT WAVE: TOXICODYNAMICS AND IMMUNOSURVEILANCE OF SARS-CoV-2

Konstantinos Nikolouzakis[1]

1. University of Crete, Greece

In December 2019 a new viral strain occurred in Wuhan, China, that rabidly took over the globe resulting in the first pandemic of the last 100 years. Soon, this strain was identified to be a member of the coronaviridae family; the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the associated disease was named corona virus disease 19 (COVID-19). As a result to this pandemic, a number of groundbreaking changes have occurred affecting the global socioeconomic structure and healthcare systems. Soon after the identification of the genomic and proteomic structure of the SARS-CoV-2, numerous research teams across the globe focused their interest on unveiling the kinetics and dynamics of COVIC-19 in order to produce effective treatments and vaccines. As of October 2022 more than 609.247.000 confirmed cases of COVID-19 have been reported and 6.364.000.000 doses of all vaccines have been administered globally. However, despite the fact that a large portion of the global population has been vaccinated, infectiousness remains high with a relative trend of seasonal increase (approximately during the middle of December, April and August) which resembles that of other viral diseases of the respiratory tract. Thus, re-appreciation of the underlying toxicodynamics of SARS-CoV-2 infection and the available vaccines is still needed in order to understand possible individual and/or environmental predispositions explaining these trends.

DEVELOPMENT OF SARS-COV-2 IGM FOLLOWING VACCINATION PREDICTS LONGER IMMUNITY

Chiara Piubelli[1], Alessandra Ruggiero[2], Lucia Calciano[3], Cristina Mazzi[1], Concetta Castilletti[1], Natalia Tiberti[1], Sara Caldrer[1], Matteo Verzè[1], Silvia Stefania Longoni[1], Simone Accordini[3], Zeno Bisoffi[1], Donato Zipeto[2]

1. Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar (Verona), Italy
2. Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
3. Department of Diagnostics and Public Health, University of Verona, Verona, Italy

Background. The development of SARS-CoV-2-specific IgM after vaccination with BNT162b2 is associated with higher levels of SARS-CoV-2 IgG. In this study, we tested whether the development of SARS-CoV-2 IgM predicts longer immunity even after the third booster dose.

Methods. We longitudinally analyzed anti-SARS-CoV-2 spike protein receptor binding domain RBD (IgG-S) and anti-S IgM (IgM-S) in 1872 health care workers (HCWs) recipients of BNT162b2 before the first administration of the vaccine (D1, week 0, W0), at administration of the second dose (D2, W3), 6 weeks after D2 (W6), 6 months after D2 (W27), at administration of the third dose (D3, W39), 3 weeks after D3 (W42) and 6 months after D3 (W63). Two-level linear regression models were used to assess the differences in IgG-S concentration by time of examination and IgM-S group.

Findings. In subjects immunologically naïve to SARS-CoV-2 (IN), the development of IgM-S after the first vaccination dose is associated with higher levels of IgG-S at short (W6, $p<0.0001$) and long follow-up (W27, $p<0.001$) after the vaccine dose, as well as also after the booster dose ($IgM\text{-}S^{POS}$w3 VS $IgM\text{-}S^{NEG}$ $p<0.001$, at W39). Even in previously infected subjects (PI), a tendency for higher IgG-S levels was observed in subjects who developed Ig-M. Of note, among the IN who never experienced infection, 28/33 (85%) had developed IgM after vaccination, showing a significant lower probability of getting infected ($p=0.016$).

Interpretation. The development of anti-SARS-CoV-2 IgM following the first vaccine doses is predictive of higher IgG-S levels even after the administration of a booster dose almost one year later. Many individuals who developed IgM in response to vaccination never get infected, suggesting that development of vaccine-induced IgM-S may have a protective role in preventing SARS-CoV-2 infection. This protection does not appear to be mediated by IgM persistence but rather by higher IgG-S levels.

THERAPEUTIC DELIVERY EFFICACY OF NANOVECTORS

Giuseppe Bardi[1]

1. Istituto Italiano di Tecnologia, Genova, Italy

Drug delivery carriers have greatly evolved in the last decades. The possibility to manipulate organic or inorganic materials at the nanoscale and their application to biomedicine dramatically expanded the possibility to release therapeutics in defined tissues and cells. Nanovectors are broadly employed in cancer treatments and vaccination, as shown by the different vaccine preparations to counteract the Covid-19 global pandemic. Independently of the cargo, whether a drug or a mRNA, the nanocarrier can add therapeutic advantages, such as specific targeting, immune system escape, solubility to insoluble molecules, dose efficacy and, eventually, adjuvant properties. Nanoparticles of several bulk materials, shape and sizes are currently available with the possibility to modify their surface chemistry on purpose. Although some applications, especially in cancer drug delivery, could allow the use of inorganic (i.e. metallic) nanoparticles to also exploit their imaging properties, biodegradable nanovectors are often preferred for lower toxicity and biocompatibility. Liposomes, solid-lipid or polymer nanoparticles are the mainly used drug or vaccine carriers among organic nanovectors. The improved targeting and delivery of the treatment can be, however, limited by concerns regarding the immune response to alien materials. The vector surface modification aimed at the specific targeting using active biomolecules can be recognized as non-self and trigger unexpected inflammatory reactions or antibody release. For example, the widely used PEGylation of drugs or particles can stimulate anti-PEG IgE production followed by complement activation-related pseudo-allergy (CARPA) and accelerated blood clearance (ABC) of the PEGylated delivery system, ultimately reducing its efficacy. Many research observations are currently available but more clinical and epidemiological data are needed to tailor effective nanovectors for different pathologies.

Session 4

Session Chairs – Cristiana Tănase & Aurora Arghir

CLINICAL UTILITY OF PERICONCEPTIONAL CARRIER SCREENING FOR PREVENTION OF NEURODEVELOPMENTAL DISORDERS

Anita Rauch[1]

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Targeted carrier screening in high-risk populations has been shown to reduce the incidence of several diseases, such as Tay-Sachs in Ashkenazi Jews, cystic fibrosis in White Europeans, and alpha/beta-thalassemia in Sardinians. However, expanded carrier screening (ECS), where more genes or pathogenic variants are tested in a pan-ethnic manner, seems to be more cost-effective. Additionally, clinical utility of preconception ECS has been shown regarding the reproductive decision making in couples found to be at-risk of having an affected child, of which 62–77% pursued actions to avoid an affected pregnancy. Moreover, all of the 0.2–2.6% of couples found to be at-risk in other studies took action and opted for PGT-M (Preimplantation Genetic Testing for Monogenic disease). Nevertheless, the question of residual general risk after expanded carrier screening remains open and hampers genetic counselling.

Despite existing recommendations for gene panel design, most ECS studies differ greatly in numbers of genes tested ranging from tens to a few hundreds with little overlap. In addition, although most of these studies attempted to adhere to the current guidelines, consensus variant selection and definition of pathogenic or likely pathogenic (P/LP) classifications remain vague, further exacerbating the disparity and hindering systematic comparison. Moreover, a large number of unclassified missense variants is often neglected. Accordingly, the carrier and (estimated) at-risk-couple frequencies vary.

The recently released ACMG guidelines provide criteria on gene selection for expanded carrier screening, but recommend replacing this term with "Tier 1–4 carrier screening". Tier 1 includes screening of CFTR, SMN1 and medically and family-based risk genes, Tier 2 includes Tier 1 plus genes with carrier frequency ≥1/100, Tier 3 includes Tier 2 plus genes with carrier frequency ≥1/200 and X-linked conditions, and Tier 4 includes Tier 3 plus genes with carrier frequency <1/200. The ACMG recommends to offer Tier-3 screening to all pregnant women and those planning a pregnancy, while for consanguineous pregnancies Tier-4 screening should be considered. Thereby, all tiers should be restricted to disease genes with at least moderately severe phenotypes.

Nevertheless, the recent ACMG guidelines do not recommend to provide a residual carrier-risk after ECS, because exact carrier frequencies and precise test

sensitivities are not known. Independent of the residual carrier-risk for specific genes, which may be reasonably well estimated, the general question of how much of the reproductive risk can be detected by ECS remains vague. Since neurodevelopmental disorders (NDDs), especially intellectual disability (ID), in the offspring are a major concern of couples who wish to have children, we wondered how much of the risk for a child with disabling NDD could be detected by ECS. The prevalence of ID is about 2–3% in Western countries, and 0.3–0.5% of the population fulfills the criteria of severe ID with IQs below 50. ID has significant comorbidity with other NDDs and is most commonly accompanied by epilepsy, cerebral palsy, anxiety disorder, oppositional defiant disorder, and autistic disorder and many affected individuals have serious long-term health problems. According to a large-scale exome sequencing study in NDD patients of European ancestry, 3.6% of cases are attributable to recessive coding variants, while 50% are explained by de novo coding variants. In contrast, in NDD patients with consanguineous parents these figures are reversed with about 50% inherited recessive and 6% de novo likely causative coding variants.

Previous ECS studies were commonly performed in general populations or fertility clinics in order to avoid bias through enrichment of disease risk. While this approach is reasonable, it does not allow to address the question of residual ID-risk, which would require huge cohort numbers and long-term follow-up data. We therefore retrospectively studied the parental samples of a cohort of children with developmental delay or ID, who previously had undergone extensive diagnostic work-up, in order to assess the sensitivity of ECS in detecting the NDD-risk in relation to various gene contents and variant classification approaches, as well as parental consanguinity status. By blindly screening for carrier-alleles in up to 3,046 recessive/X-linked genes and depending on various variant pathogenicity thresholds and gene content, NDD-risk-reduction potential was up to 43.5% in consanguineous, and 5.1% in nonconsanguineous couples. The risk-reduction-potential was compromised by underestimation of pathogenicity of missense variants by automatized tools (false-negative-rate 4.6%), inherited copy-number variants and compound heterozygosity of one inherited and one de novo variant (0.9% each). Adherence to the ACMG recommendations of restricting ECS to high-frequency genes in nonconsanguineous couples would more than halve the detectable inherited NDD-risk. Thus, for optimized clinical utility of ECS, screening in recessive/X-linked genes regardless of their frequency (ACMG Tier-4) and sensible pathogenicity thresholds should be considered for all couples seeking ECS.

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PROTEOMIC AND PHOSPHOPROTEOMIC ANALYSIS OF CLEAR CELL RENAL CELL CARCINOMA

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Clear cell renal cell carcinoma (ccRCC) is the third most common and most malignant urological cancer. In this study, we took dimethyl labeling based quantitative proteomic, to determine mis-regulated proteins and post-translational modifications in tumor cells. Our analysis identified 10,160 unique proteins, of which 955 proteins were significantly regulated between tumor and normal adjacent tissues. We verified four putatively secreted biomarker candidates, namely, PLOD2, FERMT3, SPARC, and SIRPα, as highly expressed proteins in tumor tissues. Based on molecular expression profiles, we propose a biomarker panel for the robust classification of ccRCC tumors into two main clusters, which significantly differed in patient outcome with an almost three times higher risk of death for cluster 1 tumors compared with cluster 2 tumors. In order to elucidate the underlying molecular mechanisms orchestrated by phosphorylation modifications, we extended our proteomic profiling into phosphorylation differences in ccRCC tumor and normal adjacent tissues. Our in-depth analysis revealed 1,336 phosphopeptides to be differentially regulated between tumor and normal tissues. Our rigorous characterization of the renal phosphoproteome also suggests that both EGFR and VEGFR are the most important mediators of phospho signaling in RCC pathogenesis. Furthermore, we determined the kinases PAK2, CDK1, Erk1 and Erk2 to be master kinases that are responsible for phosphorylation of many substrates associated with cell proliferation, inflammation, and migration. Our rigorous proteomics approach identified promising diagnostic and tumor-discriminative biomarker candidates which can serve as therapeutic targets for the treatment of ccRCC.

Session 5

Session Chair – Elisa Anamaria Liehn

CARDIAC-TARGETED DELIVERY OF A NOVEL DRP1 INHIBITOR FOR CARDIOPROTECTION

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Introduction: Heart disease is the leading cause of death worldwide. Treatments that protect the heart from myocardial ischaemia-reperfusion injury (MIRI) are urgently needed. The mitochondrial fission protein Drp1 is a viable target for cardioprotection. However, specific, potent inhibitors of human Drp1 are currently not available. Here, we reported a novel inhibitor of human Drp1 (DRP1i1), delivered using a new cardiac-targeted nanoparticle system, as an advanced approach in achieving ischaemic cardioprotection.

Methods: DRP1i1 was identified as a hit compound from a virtual screen of compound libraries against the active site of human Drp1. Surface plasmon resonance and GTPase assays were employed to assess Drp1 protein binding and enzymatic activity, respectively. Cell-based assays were conducted to assess Drp1-mediated mitochondrial fission. To improve the bioavailability and reduce off-target tissue effects, DRP1i1 was encapsulated in cubosome lipid nanoparticles with conjugated cardiac-homing peptide (Nano-DRP1i1). Nano-DRP1i1 was characterised by dynamic light scattering, transmission electron microscopy and dynamic dialysis (for DRP1i1 entrapment and release). Finally, the cardioprotective effect of Nano-DRP1i1 was evaluated in a clinically-relevant murine (8–10-week-old, male, C57BL/6) model of MIRI and in human heart tissues engineered from induced pluripotent stem cells (iPSC-cardiac organoids).

Results: DRP1i1 is a drug-like small molecule that binds to human Drp1 protein (Kd=2.58μM) and significantly inhibits the GTPase activity of Drp1 at 5μM (0.54±0.05 vs. 1.0±0.05 in DMSO, n=3, $P<0.05$). DRP1i1 promoted mitochondrial fusion in mouse and human fibroblasts in a Drp1-dependent manner (no effect in Drp1 knockout cells). DRP1i1 was successfully loaded into the cubosomes (entrapment efficiency of 99.3±0.1%, cumulative release of 28.3±0.1% in the first 8 hours in PBS, n=3). Nano-DRP1i1 has an average diameter of 171.3±0.4nm and a zeta potential of -31.6±1.6mV (n=3). In iPSC-cardiac organoids subjected to simulated IRI, treatment with Nano-DRP1i1 at reperfusion significantly reduced cardiac cell death, contractile dysfunction and mitochondrial superoxide. *In vivo*, following acute MIRI, DRP1i1 (intravenously at reperfusion) significantly reduced infarct size and serine-616 phosphorylation of Drp1, and restored cardiomyocyte mitochondrial size to that of sham group. Imaging by mass spectrometry revealed higher distribution and accumulation of

DRP1i1 in the heart tissue when delivered as Nano-DRP1i1. In chronic MIRI, DRP1i1 significantly reduced cardiomyocyte hypertrophy and interstitial fibrosis.

Conclusions: DRP1i1 is a promising tool compound to study Drp1-mediated mitochondrial fission and exhibits promising therapeutic potential for cardioprotection, especially when delivered using the cardiac-targeted cubosome nanoparticle drug delivery system.

HUMAN PLATELET MITOCHONDRIA IMPROVE THE MITOCHONDRIAL AND CARDIAC FUNCTION OF DONOR HEART

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Better preservation of the donor hearts is essential for the success of the heart transplantation. Here, we hypothesized that the preservation of the donor heart with human platelet-derived mitochondria (pl-MT) could improve mitochondrial and cardiac function. pl-MT supplementation resulted in their transportation into primary cardiomyocytes and the enhancement of ATP production *in vitro*. Incubation of rat whole hearts with pl-MT for 9 hours clearly demonstrated pl-MT transfusion into the myocardium and isolated myocardial mitochondria (with pl-MT) showed showed improved mitochondrial membrane potential, greater ATP synthase activity and citrate synthase activity without affecting reactive oxygen species production. The heartbeat and the volume of coronary circulation perfusate were significantly increased in the Langendorff perfusion system and the viability of cardiomyocytes were increased from pl-MT hearts. The pl-MT incubation improves maintains cardiac function of rat hearts by improving mitochondrial activity. We suggest that provides the proof of principle for pl-MT application as an enhancer of the donor heart.

MITOCHONDRIAL DJ-1 AS A THERAPEUTIC TARGET FOR CARDIOPROTECTION

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Background: Translating cardioprotection to clinical practice has been difficult, and it remains a challenge between the bench and the bedside. Nanotechnology has shown significant improvements in the settings of myocardial ischemia-reperfusion (I/R) injury. Given the cytoprotective effect observed with the protein DJ-1, we designed nanoparticles (NPs) loaded with ND-13, a highly conserved peptide from the DJ-1, to achieve cardioprotective outcomes against I/R injury.

Methods: We tested the efficacy of our nanoparticles loaded with ND-13 (ND-13NPs) in the attenuation of myocardial I/R injury in the *ex vivo* and *in vivo* I/R-murine model.

Results: ND-13 (20μM) perfused continuously for the first 15 minutes of reperfusion significantly improved LV pressure (non-treated, 16 ± 7 mmHg vs treated, 74 ± 8 mmHg) and systolic function following 45 min of global ischemia and 120 min of reperfusion. Afterwards, using the AMI *in vivo* model, 60 mg/Kg of ND-13 have injected intravenously 5 minutes before reperfusion. ND-13 reduced 35% of the infarct size (non-treated, 49 ± 6.4% *vs*. treated, 32 ± 5 %; $p<0.05$). Fluorescently loaded NPs were intravenously injected into AMI mice to assess the distribution of NPs in cardiac tissue using the IVIS *in vivo* imaging system. The NPs were abundantly detected in the infarct border and minimally detected in the remote myocardium. ND-13NPs (20mg/Kg) reduced 45% the infarct size by compared with 60mg/Kg and 20mg/Kg naked ND-13 (27 ± 6% vs. 32 ± 5% and 44 ± 8%, respectively). Demonstrating that NPs improve the delivery and efficacy of ND-13 in the ischemic heart following AMI. The activation of the myocardial reperfusion injury salvage kinase (RISK) and the survivor activating factor enhancement (SAFE) pathway at reperfusion protects the mitochondria against IRI. Therefore, we addressed whether ND-13 impacts mitochondrial function and decreases ROS production. We observed a strong antioxidative effect when the peptide treated the infarcted heart (90 ± 1.5% vs. 39 ± 9%, *respectively*). Can ND-13 protect beyond a cardioprotective pathway, or has the threshold of protection already been achieved by activating a direct effect on mitochondria?

Conclusion: Intravenously injected ND-13NPs selectively accumulated in the infarct area, protects the myocardium from IRI via antioxidative-mitochondria effects. This new drug may have the potential to bridge the gaps between basic and clinical research.

COLLAGEN SYNTHESIS INVOLVES DISTINCT FUNCTIONS OF VITAMIN C

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Aim. Extracellular collagen remodeling is one of the central mechanism responsible for the structural and compositional coherence of myocardium in patients undergoing myocardial infarction. Vitamin C deficiency is partially responsible for synthesizing abundantly unstable collagen (fibrotic tissue) over the injured area. This stiff fibrotic tissue affects the optimal pump activity of heart. Herein, we investigate the role of vitamin C on remodeling of fibrillar collagen in an *in vitro* ischemic replica model of heart healing after myocardial infarction.

Methods and results. Mouse cardiac fibroblasts (wild-type C57BL/6 mice) were cultured under normal and profibrotic settings on fibronectin, fibronectin-collagen assembly, and collagen coatings mimicking the extracellular matrix conditions of inflammation, proliferation, and maturation post-myocardial infarction. The expressions of fibrotic genes, protein, and immunofluorescence of markers-related cell differentiation, and fibrillar collagen types were assessed in time in the presence of vitamin C. In profibrotic conditions, at the gene expression level vitamin C seemed to stabilize col1a1 mRNA at steady values on the fibronectin coatings and stimulate col5a1 mRNA gene transcription independent of coating composition. These findings may indicate that vitamin C in the ischemic heart regions may modulate rapidly the gene transcription levels for an optimum *de novo* collagen synthesis. In the same line, at the protein level vitamin C seemed to inhibit the synthesis of collagen I precursor but stimulated the expression of collagen I. The stimulation of the synthesis of collagen V by vitamin C presents a co-dependence of fibronectin coatings. These findings may indicate the modulating role of vitamin C for the correct structural assembles of collagen molecules.

Conclusions. Our study evidenced the unraveling pleiotropic function of vitamin C on modulating the synthesis of *de novo* collagen fibrils. Under inflammatory-to-proliferative mimicking settings the equilibrium functionality of gene transcription stabilization of vitamin C was temporarily complemented by its modulating role of the turnover of *de novo* collagen fibrils formation. These findings are particularly interesting as these variations may partially explain the temporal interplay of various subtypes of cardiac collagen during inflammatory-to-proliferative-to-maturation phases after myocardial infarction.

THE EFFECT OF VITAMIN C ON OXIDATIVE STRESS IN ISCHEMIC CONDITIONS

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Reactive oxygen is a small, unstable molecule that contains oxygen and readily signal transmission with other molecules in the cell, also known as free radicals. One of the central mechanisms leading to ventricular remodeling in patients is the accumulation of reactive oxygen species (ROS) during myocardial infarction. The activated fibroblasts following myocardial infarction are investigated in recent years to establish anti-oxidation therapies to improve ventricular remodeling. However, data regarding anti- oxidation mechanisms in the myocardial infarction are scarce and incomplete. In this study, we aimed to assess systematically and comprehensively vitamin C functions as a potential therapeutic agent in an in vitro model mimicking heart healing after myocardial infarction. Mouse cardiac fibroblasts were isolated from hearts of wild-type C57BL/6 mice and cultured under normal and profibrotic settings on freshly prepared coatings mimicking extracellular matrix remodeling undergoing inflammation, proliferation, and maturation post-myocardial infarction. Surprisingly, vitamin C enables only at lower levels the increase of the respiration profile of mitochondria, but not at higher levels. Further, Vitamin C reduces total intracellular ROS and mitochondrial ROS accumulation in mouse cardiac myofibroblasts cultures on fibronectin, the main component of provisional early extracellular matrix, while in later phases in the present of collagen vitamin C was able to regulate only the total ROS. Overall, our study demonstrates that low doses of vitamin C have a positive effect on the function of cardiac fibroblasts and not higher doses. Its ROS scavenging ability is primarily in the early, proliferation phase during myocardial infarction.

TARGETING MITOCHONDRIA TO PREVENT VASCULAR RESTENOSIS

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Background: New treatments are needed to prevent post-angioplasty and stent restenosis in coronary artery disease (CAD) and peripheral arterial disease (PAD) patients. Accumulated findings have shown that mitochondrial dysfunction is involved in the pathophysiology mechanisms underlying several cardiovascular conditions, however, in the scenario of vascular remodelling after injury, the role of mitochondrial dynamics is not completely understood.

Purpose: In this study, we investigate the effect of mdivi-1 on neointimal hyperplasia and plaque development in a wire-induced endothelial injury animal model.

Methods: Using ApoE-/- mice on a background of hyperlipidaemia, we investigated weather in vivo administration of mdivi-1 (1.2mg/kg/d) could reduce atherosclerotic plaque volume, plaque complexity and inflammation in a carotid-wire injury model of neointimal hyperplasia. Also, THP-1 monocytes (T-M) and THP-1-derived Macrophages (T-DM) were stimulated in vitro with LPS + IFN-gamma to induce M1 polarization and then subjected to gene expression and protein analysis in the presence or absence of mdivi-1 (50μM). Transmigration and 3D chemotaxis assays were performed in T-M cells and Human peripheral blood monocytes, to evaluate the effect of mdivi-1 on cell migration in response to M-CSF and MCP-1. Finally, we investigated the effects of Mdivi-1 on mitochondrial respiration using Seahorse assay in T-M and T-DM.

Results: In vivo treatment with mdivi-1 reduced neointimal hyperplasia by 37% when compared to control, and was associated with a significant decrease of vascular smooth muscle cell and macrophage cell numbers, as well as reduction of the pro-inflammatory mediators TNF-α and ICAM-1 in the plaque. In vitro M1 polarization of T-M and T-DM induced both up-regulation and production of pro-inflammatory mediators and mdivi-1 significantly suppressed this effects. In addition, monocyte chemotaxis response to M-CSF and MCP-1 was significantly reduced in the presence of mdivi-1. Lastly, mdivi-1 reduced oxygen consumption rate in T-M and T-DM, and prevented M1-cell polarization.

Conclusions: We show that mdivi-1 significantly reduces vessel wall thickness, neointimal hyperplasia, monocyte recruitment, and inflammation after vascular endothelial injury, positioning Mdivi-1 as a potential pharmacological strategy to reduce restenosis following angioplasty and stenting in CAD and PAD patients.

Session 6

Session Chair – Anca Hermenean & Laura Cristina Ceafalan

THE INHIBITION OF GALECTIN-1: NEW INSIGHTS FOR TREATMENT OF DIABETIC COMPLICATIONS

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Chronic hyperglycemia is a risk factor for the development of retinopathy and cardiac fibrosis, two important complications of diabetic conditions underlined by marked fibrotic processes. These are mainly mediated by Transforming Growth Factor beta (TGFβ), responsible for the both the development of retinal fibrovascular tissue, which impairs the function of retina during diabetic progression, and the activation of cardiac fibroblasts, contributing to diastolic dysfunction and arrhythmogenesis.

Interestingly, the β-galactoside-binding lectin Galectin-1 (Gal-1), was found to be upregulated in retinal Müller glial cells of patients with diabetic retinopathy, as well as in cardiomyocytes from patients suffering of cardiovascular pathologies. However, Gal-1 role in diabetes-induced fibrosis leading to diabetic retinopathy and cardiac dysfunction still needs to be investigated, in order to evaluate the possibility of its selective inhibition as therapeutic tool to alleviate diabetic retinopathy and cardiomyopathy.

To this regard, we first set an in vitro model of diabetic retinopathy and of diabetic cardiomyopathy by exposing respectively the human retinal pigment cells (ARPE-19) and the rat cardiomyocytes H9C2 to normal and high glucose conditions. After assessing the expression of Gal-1 in hyperglycemic conditions by immunocytochemistry or Western Blotting, we treated both cells in normal or high conditions with different doses of OTX008, a selective Gal-1 inhibitor. Then we assessed cell viability, content of radical oxygen species, protein and mRNA levels (by ELISA, Wester Blotting, Immunocytochemistry and Real Time qRT-PCR).

Our results showed a significant up-regulation of galectin-1 expression in both cells exposed to high glucose, with a reduction of cell viability. Gal-1 inhibition by OTX008 improved the morphology and the vitality of both cell types, also reducing NF-kB and ROS content. Also TGF-β pathway was down-regulated by OTX008, leading to a reduction of cardiac fibrosis in H9c2 cells and particularly, to decreased epithelial-mesenchymal transition (EMT) markers in ARPE-19 cells.

Therefore, Gal-1 inhibition by OTX008 could be considered a possible novel therapeutic target to prevent and counteract the complications mediated by fibrosis in diabetic retina and heart.

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ROLE OF PRO-RESOLVING MEDIATORS IN RETINAL DISEASES

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The main acquired retinopathies like diabetic retinopathy, age-related macular degeneration and uveitis that collectively represent the main causes of visual impairment in Western countries, are underlined by oxidative stress or aging-induced retinal inflammation, which contributes to vision impairing or loss.

For example, in diabetic retinopathy, it is demonstrated that the prolonged hyperglycemia causes an increase in the flow of the polyol pathway, an increased formation of advanced glycation end products, an abnormal activation of the various signaling cascades such as activation of the protein kinase C (PKC) pathway and an increase in flow of the exosamine pathway and reactive oxygen species (ROS).

All these changes lead to an increase in oxidative stress with systemic and local hyper-production of numerous pro-inflammatory cytokines and chemokines and microglial activation. These multiple alterations ultimately determine an alteration of the vessel wall and of blood –retinal barrier with consequent hyperpermeability or vascular obstruction, that results in an overproduction of Vascular endothelial growth factor (VEGF).

Similarly, also in age-related macular degeneration, which represents the main cause of legal blindness in industrialized countries in the population over 65 years old, and in uveitis that is a chronic inflammatory intraocular disease, it has been shown that inflammation plays a crucial role, with local and systemic hyperproduction of pro-inflammatory mediators and cytokines such as IFN-γ, TNF-α, IL- 6, IL-8.

The therapies used in the management of these three retinal diseases often involve the use of drugs not free from local and systemic complications such as steroid in uveitis and anti-VEGF in diabetic retinopathy and in age-related macular degeneration. Therefore, new anti-inflammatory therapies could potentially prevent or change the course and severity of retinal disorders without causing major side effects.

Resolution of inflammation is emerging as a critical phase able to counteract the inflammatory process leading to the progression of retinal damage. Particularly, pro-resolving mediators (PMs) play a key role in the modulation of inflammatory exudates and could be considered a new target to be investigated in different inflammatory-autoimmune pathologies.

PMs are local mediators of a lipidic nature, derived from omega-6 and omega-3 polyunsaturated fatty acids (PUFAs). They are converted into potent PMs through the action of specific enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), and are aimed at resolution of the inflammation in a tissue.

Here, we highlight the most recent studies concerning the role of the main PMs like lipoxins, resolvins, protectins, maresins and annexins, in retinal inflammation, in order to collect the best evidence in the field of inflammatory retinal damage resolution and to propose novel pharmacological approaches in the management of the most common retinal diseases.

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INVESTIGATION OF THE BIOLOGICAL EFFECTS OF β-CYCLODEXTRINS

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Cyclodextrins are widely used excipients for increasing water solubility and bioavailability of lipophilic drugs. Cyclodextrins are applied mainly as excipients in drug formulations, however they have several well-known biological effects related to cholesterol complexation. Cyclodextrin derivatives were intensively studied in the treatment

of cholesterol-related neurological disorders and vascular disorders in recent years. To understand the mechanism of action of cyclodextrins in the above-mentioned diseases cellular mechanisms and *in vivo* behaviour should be revealed. We showed previously, that cyclodextrins are able to enter cells by endocytosis, in different cell types and can accumulate *in vivo* in tumors. Cyclodextrins must be labelled by fluorescent dyes or radioisotopes for *in vitro* and *in vivo* imaging techniques. In the presentation the main findings of the biological effects of cyclodextrins related to the potential therapeutical applications and their behaviour in experimental cellular and animal models will be summarized.

INVESTIGATING THE CHEMOPREVENTIVE POTENTIAL OF NATURAL PRODUCTS ON BREAST CANCER IN VITRO MODELS

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Epidemiological studies have demonstrated the close relationship between a diet rich in natural compounds and a lower risk of chronic disease development, including cancer. The complex mechanism of nutrients bioactivity in humans is far from being completely understood. Red Clover is a plant used for century in traditional medicine to alleviate symptoms of asthma, psoriasis, arthritis and menopause. Currently, it is considerate a promising functional food with a high nutritional content, due to the presence of a large amount of isoflavones, phenolic acids, vitamins and minerals. The aim of this study was to explore the chemopreventive potential of an aqueous and ethanolic extracts of Red Clover using in vitro breast cancer model: MCF-7 (estrogen-dependent), MDA-MB-468 (estrogen-independent) and non-tumorigenic (MCF12A) human cell lines. A pro-oxidant effect of ethanolic extract at high-doses was observed by videomicroscopy on all cell lines, followed by cellular death. At low-doses, both extracts had a cytoprotective activity against generation of reactive oxygen species (ROS). Additionally, the plant extracts had a different impact on antioxidant enzymes activity depending on cells type, based on colorimetric and fluorimetry measurements. Aqueous extract showed a stronger estrogenic activity (E-screen test) in comparison with ethanolic one after prolong treatment on estrogen dependent cell line. Both extracts had a significant anti-proliferative effect on estrogen-independent tumor cells. Moreover, on normal cells no cytotoxicity and a pro-proliferative activity was observed at the same tested concentrations. Our study had demonstrated that Red Clover extracts might have a chemopreventive potential by affecting the cellular viability and reducing proliferation rate of breast tumor cells and by inducing apoptosis via oxidative

stress. Additionally, the plant extracts have a significant estrogenic and antioxidant activity and can be recommended as food supplements.

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GENERATION OF A REPORTER CELL LINE TO STUDY IL-1β-MEDIATED INFLAMMATION

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Cytokines and chemokines are primary mediators of systemic inflammation and neuroinflammation; amongst these interleukin (IL)-1β is the most potent pro-inflammatory cytokine which is produced as response to tissue damage or infections by cells of the immune system, predominantly by macrophages. The cytokine is synthesized as inactive form (*pro*IL-1β) which is cleaved by activated caspase-1 to its mature form (*m*IL-1β) and is rapidly secreted to the extracellular space without entering the classical endoplasmic reticulum-Golgi pathway. Generating an IL-1β reporter cell line is challenging due to the complex process of generating the biologically active form of the protein and any overexpression system would induce a constitutive inflammatory response which does not maintain the physiological conditions. Therefore, we generated a reporter cell line by endogenously tagging IL-1β using CRISPR/Cas9 technology, which recapitulates the physiological response to classical inflammatory stimuli and can be used for quantitative assessment of IL-1β secretion associated with inflammation.

Keynote Lecture

EXTRACELLULAR VESICLES IN CARDIOVASCULAR THERANOSTICS

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Session 7

Session Chairs – Mihaela Gherghiceanu & Gener Ismail

ADVANCES IN UNDERSTANDING OF NEPHROTIC LESIONS

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https://www.northwell.edu/find-care/find-a-doctor/anatomic-clinical-pathology/dr-vanesa-bijol-md-11377544

AN UNEXPECTED KIDNEY BIOPSY FINDING IN A PATIENT WITH ACUTE KIDNEY DISEASE

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Is presented the case of a 59-year-old woman with recently diagnosed non-keratinizing squmous cell carcinoma of the lung was reffered to our hospital for acute kidney disease.

Three days prior to the hospitalization, standard blood tests before the initiation of chemotherapy showed a increase in serum creatinine (4.4 mg/dL), which delayed the start of the oncologic treatment.

At our nephrology unit the patient presented with macroscopic hematuria. Laboratory testing revealed AKD, with a further elevation of serum creatinine (5.7 mg/dL).

Kidney ultrasound evaluation showed bilateral diffuse architectural alteration with absence of the structural definition of the corticomedullary complex, compatible with a diffuse infiltrating process, with no evidence of kidney mases or calculi.

Due to AKD of unknown origin, a percutaneous ultrasound-guided left kidney biopsy was performed. The histopathologic diagnosis was infiltrative kidney metastasis due to nonkeratinizing squamous cell carcinoma.

Metastases to the kidney are relatively rare and generally detected late in the course of a primary malignancy. Although metastases are usually multifocal or solitary

large masses, rarely they can have an infiltrative pattern and pose a diagnostic challenge. Therefore, the differential of a kidney infiltrative process involves a wide spectrum of conditions including hematological, inflammatory, and autoimmune diseases.

A high index of suspicion and a careful investigation by all involved physicians is necessary in the patients with a non-renal malignancy who presents with acute kidney disease and renal imaging abnormalities.

URINARY SOLUBLE CD163 IN ANCA ASSOCIATED VASCULITIS

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For the moment, the kidney biopsy remains the gold standard in diagnosing glomerulonephritis (GN). Despite refined diagnostic and treatment options, most renal diseases invariably progress to ESKD in about 10-20% of cases. Furthermore, most GNs are known to frequently relapse even after adequate treatment. This has led to an ongoing search for ways in order to diagnose the relapses at the right moment. Repeat biopsies have been used by some institutions with some degree of success. The problem is that the kidney biopsy is not always available and moreover, it is an invasive procedure that comes with some risks.

Urine has served as a very good place in which to search for noninvasive markers and, in recent years, urinary CD163 (usCD163) has received a fair amount of attention. In order for the marker to reach the urine, the glomerular basement membrane must have leaks that are caused by fibrinoid necrosis and subsequent cellular crescent formation. This makes usCD163 a perfect marker for diagnosing active glomerulonephritis, such as ANCA associated vasculitis or proliferative lupus nephritis. For the moment, there are relatively few studies that examine the evolution of usCD163 in the setting of ANCA associated disease. The cut off value for usCD163 for determining if there is a relapse ranges from 30 ng/ml to 300 ng/ml in these studies. The sensitivity and specificity of usCD163 for detecting relapses ranges from 72.2% to 96.8% and from 67.3% to 97.5%.

There is a lot of promise in the development of noninvasive markers of active GN. usCD163 seems to be a very sensible and specific marker that, in certain situations, can replace the kidney biopsy and traditional noninvasive markers in the search for the perfect moment in which to treat relapses.

A NOVEL ACTIVITY AND CHRONICITY INDEX FOR HISTOLOGIC ASSESSMENT OF RENAL BIOPSIES IN ANCA-ASSOCIATED VASCULITIS

Bogdan Obrișcă[1,2], Alexandru Procop[3], George Terinte Balcan[4], Mihaela Gherghiceanu[4], Roxana Jurubita[1], Alexandra Vornicu[1,2], Vlad Herlea[3], Andreea Andronesi[1,2], Gener Ismail[1,2]

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Introduction. Prediction of renal outcome in ANCA-associated vasculitis (AAV) remains a major challenge. We aimed to evaluate a novel score for histologic assessment of renal biopsies in AAV.

Material and Methods. A semiquantitative activity and chronicity score were assessed in relation to achievement of remission of ESRD. The activity index consisted of the percentage of normal glomeruli, cellular/fibrocellular crescents, fibrinoid necrosis, neutrophil infiltration, the severity of interstitial inflammation, the presence of tertiary lymphoid organs, arteritis and thrombotic microangiopathy. The chronicity index consisted of a total glomerulosclerosis score, the percentage of glomeruli with fibrous crescents, the severity of IFTA and the presence of arteriosclerosis.

Results. Twenty-seven patients with AAV were included in the study. Their mean age was 60 ± 10 years, 70% were females and 74% had a pANCA-associated vasculitis. The baseline eGFR was 21 ± 16, while 70.4% of patients achieved a remission and 22.5% progressed to ESRD. The baseline eGFR significantly correlated with total activity score (r=-0.53;p=0.005), total chronicity score (r=-0.37;p=0.05) and individual components, cellular/fibrocellular crescent score (r=-0.44;p=0.02), interstitial inflammation score (r=-0.5;p=0.009) and tubular atrophy score (r=-0.38;p=0.04). Baseline C-reactive protein significantly correlated with total activity score (r=0.53;p=0.005) and the individual scores of cellular/fibrocellular crescents (r=0.45;p=0.02) and interstitial inflammation (r=0.44;p=0.02). Non-responders had higher median total activity score [11.5 (IQR:6.5-16.25)], total chronicity score [8.5 (IQR:5.7-10)] and cellular/fibrocellular crescent score [6 (IQR:2.5-6)] compared to responders [8 (IQR:5-12); 6 (IQR:5-8) and 2 (IQR:2-4), respectively] (Figure). Patients that progressed to ESRD had higher total activity score [11 (IQR:6-13.2)] and fibrinoid necrosis score [3 (IQR:1.5-4.5)] compared to non-progressors [8 (IQR:5.5-14) and 0 (IQR:0-4), respectively].

Conclusion. This novel histologic score correlated with the baseline severity of renal involvement in AAV and may be useful for predicting the renal outcome.

NEFRITA LUPUS-LIKE: O COMPLICATIE AUTOIMUNA RARA A INFECTIEI CU VIRUSUL HEPATITEI C

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Session 8

Session Chair – Bogdan O. Popescu

NEURO … PERSPECTIVES

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Neuroscience is an extremely powerful science. Its ultimate goal is to understand how the brain "works", that is how it integrates and responds to the huge array of information that surrounds us, from the basic sensory information (external, but also internal) to the social and cultural influences. In assuming this task of investigating the most complex structure in the Universe, neurosciences use a vast array of techniques, technologies and experimental models. Many of the successes of neuroscience in understanding brain processes come from applying various forms of reductionism. However, implicit in this approach is the danger of over-reaching: attempting to explain large-scale phenomena by reference to facts and processes that play, at best,only a small role. A better understanding of the limits of reductionism should allow for a better and stronger neuroscience effort to explain the emergent properties of the brain.

PAIN, FROM ARISTOTLE TO THE GATE THEORY, VIA DESCARTES

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Pain is a complex perception, comprising sensory, affective and cognitive dimensions. While humanity's aim to alleviate pain is as old as our species, our attempts to understand pain started with the contribution of Aristotle, who considered pain to be a passion of the soul, thus emphasizing its emotional and affective components. During Renaissance, the French philosopher Rene Descartes conceived a purely physiological view of pain, according to which signals traveling along fixed nerve fibres acted upon a postulated unique pain centre in the brain to trigger motor reflexes aiming to protect the body from damage. This relatively simple model predicts a straightforward correlation between the amount of tissue damage and pain intensity. However, a number of observations contradict this relatively simple understanding of pain: one the one hand, there is severe injury without pain, while on the other, many people complain of pain in the absence of any detectable physical lesion. To explain these occurrences, Patrick Wall and Ronald Melzack proposed in the 1960' the famous Gate Theory of pain, paving the way to the modern understanding of this puzzling phenomenon. Finally, the 2021 Nobel prize for Medicine and Physiology was awarded, in part, for the discovery of key molecules in pain signalling. The long and winding road from Aristotle's insight to the pain molecules of today will be summarized at the meeting.

NEUROETHICS, NEUROMODULATION AND THE NEUROLOGIC CATASTROPHE OF OUR ERA

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Session 9

Session Chair – Gabriel Becheanu & Adelina Maria Cohn

DIAGNOSTIC CHALLENGES IN PRIMARY RHABDOID CUTANEOUS MELANOMAS - A CASE SERIES

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Objective. Rhabdoid melanoma is a rare subtype, most often diagnosed in metastatic lesions. Primary rhabdoid melanomas are exceedingly rare and represent a diagnostic challenge due to their unusual histopathological features.

Material and methods. We present three cases of male patients with primary ulcerated cutaneous tumours which were surgically removed. The tissue samples were fixed in formalin and paraffin-embedded. We performed classical histopathological as well as immunohistochemical analysis for PRAME, HMB-45, SOX10, vimentin, desmin and myoglobin in order to establish the diagnosis.

Results. In all three cases, histopathological analysis revealed ulcerated nodular proliferations composed of mostly poligonal cells with abundant, eosinophilic cytoplasm and large, eccentric nuclei with prominent nucleoli, reminiscent of rhabdoid differentiation. Few scattered pigmented cells were noted, thus raising the suspicion of cutaneous melanomas. All three cases showed positive immunorseaction for vimentin, desmin and myoglobin. HMB-45 was negative in one case and focally positive in the other two, particularly in areas with less obvious rhabdoid features. SOX10 and PRAME were diffusely positive in all three cases.

Conclusion. Based on histopathological and immunohistochemical features, the diagnosis of primary cutaneous melanoma with rhabdoid differentiation was established in all cases. Thorough histopathological examination is crucial in order to raise the possibility of a melanocytic origin which was later confirmed by immunohistochemistry. Multiple markers should be used for this purpose. PRAME was particularly useful as HMB45 may be negative and SOX10 is also positive in malignant peripheral nerve sheet tumours with rhabdomyosarcomatous elements.

MESONEPHRIC-LIKE ADENOCARCINOMA OF THE FEMALE GENITAL TRACT-AN UNDERDIAGNOSED ENTITY

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Background: Mesonephric adenocarcinoma is a rare type of aggressive tumor which can affect the uterine cervix and/or the uterine corpus. In the cervix, mesonephric adenocarcinoma develops from mesonephric remnants, which may be present deep in the cervical wall. Upon microscopic examination, the tumor is characterized by a well-differentiated glandular proliferation featuring eosinophilic intraluminal secretions.

Methods: A 56 years old patient presented to the Gynecological Department complaining of abdominal pain. Ultrasound examination revealed a 12/8,5/7 cm solid left adnexal tumor with variable echogenic areas. The patient underwent surgery and the intraoperative examination revealed a grey-tan solid tumor with multiple areas of necrosis, diagnosed as well-differentiated adenocarcinoma NOS. Paraffin-embedded sections evaluated in usual H&E staining were supplemented by additional immunohistochemical stains and the final diagnosis of mesonephric-like adenocarcinoma was established.

Results: Histopathological examination revealed a solid proliferation of well-differentiated glandular structures with elongated nuclei and irregular lumens. The tumor was diffusely positive for CK7, showed "wild-type" pattern of staining for p53 and was negative for hormone receptors as well as WT1 and p16. Additional stains showed diffuse positivity for PAX8, GATA3 and TTF1. CD10 showed apical membranous positivity in most tumor cells and Ki67 proliferation index was 80% within hot spots.

Conclusion: Mesonephric-like adenocarcinoma is an extremely occurrence in the ovary (<1% of all ovarian adenocarcinomas), and should always be considered when dealing with a well-differentiated adenocarcinoma, which is negative for ER and PR. Additionally, clinical and imaging correlations are required in order to exclude a metastasis from the uterus, cervix or even other organs.

ANALYSIS OF THE TUMOR MICROENVIRONMENT IN GASTRIC ADENOCARCINOMA WITH THE EMPHASIS ON LAG-3

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Background: The tumor microenvironment is the one that provides support and nutrition to the cancer cells. It is composed of stroma cells, endothelial cells and immune cells. T lymphocytes play an important role in antitumor immunity and their function is regulated by the cells in the tumoral microenvironment.

Methods: We present a series of 63 cases of gastric carcinomas diagnosed in Fundeni Clinical Institute and their immuno-profile. To assess tumor microenvironment and the interaction of the carcinoma with the immune cells, we studied the expression of PD-L1 (programmed cell death ligand 1) and PD-1 (programmed death-1), LAG-3, CTLA4, CD4 and CD8 in gastric carcinomas.

Results: Our cohort contained 47 male patients and 16 female patients, with an average age of 62,15. Tumor dimension ranged between 0,2 cm and 15 cm, and the average dimension was 5,6 cm. We studied the histopathological parameters, lymphovascular and perineural invasion and the immunohistochemical profile using: Ki67, mismatch repair protein expression (MLH1, PMS2, MSH2, MSH6), e-cadherin, MUC2, MUC5AC, MUC6 and CDX2 in all cases. 53 cases were positive for PD-L1 and 10 cases were negative. LAG-3 was expressed in 49 cases, CTLA-4 was positive in 4 cases and a CD4/CD8 equal to one in 23 case, 35 cases had sub-unitary report and the remaining 4 cases were supra-unitary.

Conclusion: Targeted cancer immunotherapy is a developing field and the immunohistochemical evaluation of the tumor microenvironment helps to understand its interaction with tumor cells and to explain the results of the treatment. The anti-LAG3 antibodies that are under development are aiming to decrease the immune surveillance escape of the tumor cells and to enhance anti-tumor immunity. Even if further research is required, immunotherapy is beginning already to play an important role in cancer therapy.

VEIN INVASION, A RARE MORPHOLOGICAL FEATURE OF BREAST CANCER

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Introduction. The morphology of vein invasion in cancer is well recognised at certain anatomical sites (colorectum, pancreas, kidney) however it has not been closely evaluated and distinguished from invasion of lymphatic vessels in breast cancer. In our experience, in breast cancer, vein invasion is a separate event from lymphatic vessel invasion and is predominantly seen in invasive ductal carcinoma. There are two distinct anatomical sites where we observe vein invasion, one in the primary tumour bed and the other in the perinodal tissue of the axilla. We observe two types of vein invasion, one where tumour emboli are seen within the lumen of small veins and another where the wall of the vein has been infiltrated and sometimes replaced by carcinoma. The clinical significance of vein invasion in breast cancer has not been defined. To raise awareness, we present a small series of breast carcinoma displaying vein invasion.

Methods. In our department, we screen for and routinely report presence of vein invasion in invasive breast carcinoma. All cases positive or suspicious for vein invasion on morphological grounds are confirmed with IHC for desmin. In order to establish prevalence, we reviewed the surgical excision of 93 consecutive cases of invasive ductal carcinoma treated at a single institution between June and November 2022 and including both screening and symptomatic patients.

Results. A total of 8 cases with vein invasion were identified (8.6% of all cases). These were mostly in elderly women (>80 years old) with larger and higher grade carcinomas that were also lymph node positive. All cases were receptor positive (1 case Her-2 positive, 7 cases ER positive). Of these 8 cases, 4 had vein invasion only at the primary site, 1 within the perinodal tissues only and 3 in both sites.

Conclusion. Vein invasion is a underrecognized morphological parameter in breast cancer. We have identified desmin as a robust IHC tool to assess vein invasion in breast tissue. The terminology of the current guidelines (lymphovascular invasion) is equivocal and should be refined to distinguish lymphatic vessel invasion (LVI) from blood vessel invasion (BVI). Our series indicates that this is mostly encountered in elderly women however we have seen cases also in middle aged women. Vein invasion at other tumour sites is associated with worse prognosis and higher risk of systemic metastases and further studies are warranted to establish any links with systemic disease in breast cancer.

DIFFERENTIAL DIAGNOSIS OF BONE SARCOMAS USING IMMUNOHISTOCHEMISTRY

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Primary bone sarcomas represent less than 0,2% of malignancies in general population. Paediatric population comprises more than 90% of these cases, and main groups are either osteosarcomas (50%) or Ewing sarcomas (41%). In the current practice, different subtypes are defined by a combination of classic imaging appearance, histopathologic criteria, immunohistochemistry and particular genetic alterations. Most of these sarcomas benefit from neoadjuvant radio and chemotherapy, therefore a tumour biopsy is mandatory for accurate therapy. Given the tumour location and surgical approach, pathologists receive tissue samples extracted by core needle biopsy or incisional biopsy. Immunohistochemistry panel could be of value in selected cases, where routine histology depicts uncommon findings. It is commonly composed of SATB2, CD99, FLI1, S100 and assists in differentiating between osteosarcoma, Ewing sarcoma and chondrosarcoma. SATB2 positivity is detected in 90.4% of osteosarcomas and serves as a specific marker for this entity. CD99 showed a positivity of 92.7% in the Ewing sarcoma cases, rendering a complete membranous staining pattern. FLI1, a transcription factor involved in the EWS-FLI1 translocation, is reported in 85-90% of Ewing sarcomas. S100 is mainly used to identify a chondroid differentiation. Nevertheless, there are difficult cases where the gathered data is deceitful and this basic approach is insufficient to render a diagnosis. We selected a series of particular cases (4 osteosarcomas, 6 Ewing sarcomas and one mesenchymal chondrosarcoma) to highlight the necessity of a multifactorial strategy for a precise diagnosis. In this series, one case of osteosarcoma was negative for SATB2 and positive for CD99 and Fli1 and the mesenchymal chondrosarcoma was misdiagnosed as an Ewing sarcoma on a biopsy, based on its CD99 staining. The rest of cases showed peculiar histology or imaging findings, but the IHC results were conclusive to provide the final diagnosis.

A RARE CAUSE OF RECTAL MUCOSAL ULCERATION

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Background. Rectal mucosal ulcers are non-specific lesions that may be associated with various conditions such as inflammatory bowel disease, infection, solitary rectal ulcer syndrome, rectal prolapse, diversion colitis, ischemia, drug-induced colitis, and benign and malignant tumors.

Case report. We present the case of a 38-year-old female patient, who underwent an endoscopic examination for rectal bleeding. Colonoscopy revealed a rectal ulcer with erythema and edema of the surrounding mucosa. Endoscopic biopsies were taken. Histopathological assessment of the tissue samples showed a glandular proliferation and a moderately cellular stroma within the lamina propria. The glands had irregular contours and were lined by a single layer of cuboidal cells. There was no evidence of cellular or nuclear atypia. The overlying mucosa was ulcerated. The immunohistochemical evaluation showed that the glandular structures were positive for CK7 and negative for CK20 and CDX2. The stroma was highlighted by the nuclear positivity for ER. The morphological features and the immunohistochemical profile support the diagnosis of rectal endometriosis.

Conclusion. Rectal endometriosis is a rare condition and it may lead to diagnostic pitfalls. At low-power magnification, intestinal endometriosis may be mistaken for adenocarcinoma, especially when the typical stroma is not prominent. As the differential diagnosis of rectal ulcers encompasses both inflammatory and neoplastic lesions, a careful medical history of the patient and a thorough histological examination are needed in order to avoid overtreatment. Unfortunately, currently, there are no non-invasive biomarkers that could be used in clinical practice to detect endometriosis.

A DIFFICULT CASE IN NEPHROPATHOLOGY

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Background: Anti-glomerular basement membrane (anti-GBM) disease is an extremely rare glomerulonephritis that has been described in association with several other entities, including membranous nephropathy and ANCA associated disease. Fabry's disease is also a rare illness that has been diagnosed on the kidney biopsy along with lupus nephritis, IgA nephropathy, membranous nephropathy and also thin glomerular basement membrane nephropathy.

Case presentation: A 29-year-old male presents with abdominal and right flank pain. Laboratory results show a severe acute kidney injury (serum creatinine 5.6 mg/dL) and massive hematuria. His serum albumin was 3 g/dL. A kidney biopsy was performed and showed cellular crescent formation in over 80% of the glomeruli. There was also marked cytoplasmic vacuolization of the tubules and of the podocytes. Focal red blood cell casts were seen. There was mild interstitial fibrosis and tubular atrophy. Immunofluorescence microscopy showed linear IgG, kappa and lambda staining along the capillary wall of the glomeruli. Fibrin highlighted the glomerular crescents. Semithin sections of plastic embedded tissue showed 2 glomeruli with circumferential cellular crescents and numerous toluidine blue granules in podocytes, tubular epithelial cells and endothelial cells. Electron microscopy showed dense, lamellar intracytoplasmic bodies that were located in parietal epithelial cells, podocytes and tubular epithelial cells. There were no electron dense deposits in the glomerulus. Based on all the kidney biopsy findings, a diagnosis of diffuse necrotizing and crescentic anti-GBM glomerulonephritis associated with Fabry's disease was made. Further genetic testing confirmed the diagnosis of Fabry's disease. The patient remained dialysis dependent and was started on enzyme replacement therapy.

Conclusion: This case highlights the importance of carefully examining the kidney biopsy for additional findings, even when there is already one obvious diagnosis that can explain the clinical scenario.

Session 10

Session Chair – Laura Cristina Ceafalan

MUSCLE SYSTEMIC SIGNALING VIA MYOKINES

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Skeletal muscle health and function are important determinants of systemic metabolic homeostasis and organism-wide responses, including disease outcome. While it is well known that exercise protects the central nervous system (CNS) from aging and disease, only recently this has been found to depend on the endocrine capacity of skeletal muscle. In my talk, I will discuss some examples of how muscle-secreted growth factors and cytokines (myokines) and metabolites (myometabolites) can mediate muscle-brain and muscle-retina communication and neuroprotection in response to exercise and associated processes, such as the muscle unfolded protein response and metabolic stress. In addition to impacting brain proteostasis, muscle-brain signaling influences complex brain-dependent behaviors, such as feeding behavior and the biosynthesis of neurotransmitters. We propose that tailoring muscle-to-CNS signaling by modulating myokines and myometabolites may combat age-related neurodegeneration and brain diseases that are influenced by systemic signals.

DIAGNOSTIC PROBLEMS IN PEDIATRIC MUSCLE PATHOLOGY

Magdalena Budișteanu[1,2,3]

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Centronuclear myopathies represent a group of congenital muscle diseases with different pattern of inheritance: autosomal dominant, autosomal recessive, and X-linked. The common features of these conditions include muscle weakness, hypotonia, feeding and respiratory problems (in severe cases). The severity of symptoms can vary from mild to severe. For autosomal centronuclear myopathies, *DNM2*, *BIN1* and *RYR1* genes were

identified, and for X-linked form, *MTM1* gene. *MYF6* gene is a member of human gene family of muscle determination factors with a possible role in centronuclear myopathy.

In this paper we report on the case of a patient who presented an atypical association of two mutations of these two genes.

Method and Results: The poband is a 3 year-old boy with autism spectrum disorder (ASD), speech delay, and mild intellectual disability who was referred for neurogenetic evaluation due to a constant elevated CK level. Neurological examination showed no motor deficits. The muscle biopsy revealed a pattern suggestive for centronuclear myopathy. The whole exome sequencing analysis revealed an association of two heterozygous missense mutations of *MYF6* and *CAPN3* genes. Both are variants of unknown significance, but the first one correlated with the pattern of muscle biopsy.

Conclusion: This is the first report on a patient with ASD and intellectual disability associating mutations in *MYF6* and. *CAPN3* genes. The role of these mutations in the cognitive development is not clear. A plan for the follow-up of the motor development of the boy was established.

CHARCOT-MARIE-TOOTH NEUROPATHY AND NEUROMYOTONIA – A RARE ASSOCIATION

Diana Anamaria Epure[1], Florinela Gisela Rotaru[1,2], Mihaela Ioghen[1,2], Alexandra Bastian[2,3], Raluca Ioana Teleanu[1,3]

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Objective: The recessive mutation of the gene encoding HINT1 (histidine triad nucleotide binding protein 1) was recently identified to be associated with axonal, motor-predominant Charcot-Marie-Tooth neuropathy and neuromyotonia. We aimed to establish the importance of HINT1 mutations as a cause of hereditary neuropathy.

Methods: We report a case of a 15-year-old boy which presented into our clinic with pes cavus deformation and gait impairment that worsened over time. A neurological examination, blood work-up, electrophysiological studies of peripheral nerves, genetic testing, and nerve and muscle biopsy were performed.

Results: Genetic testing for neuropathies revealed a homozygous mutation in the HINT1 gene at p.Arg37Pro. The electroneurography and needle electromyography results were compatible with the diagnostic of axonal peripheral neuropathy with neuromyotonia. The nerve biopsy showed demyelinating sensory neuropathy and the muscle biopsy revealed important muscle damage which supports the diagnosis of axonal motor neuropathy.

Conclusions: With the increase in accessibility to genetic testing we managed to raise awareness on axonal motor neuropathy and describe neuromyotonia as a hallmark sign of this disease. Our case report contributes to the establishment of a common phenotype of HINT1 mutations, which is insufficiently explored.

THE TWO DIFFERENT FACES OF INTERLEUKIN-6, AS A MYOKINE AND AS A CYTOKINE

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Interleukin-6 (IL-6) acts in a pro-inflammatory way as a cytokine, and in an anti-inflammatory way as a myokine. It is encoded by *IL6* gene. As myokine, it exerts extensive anti-inflammatory effects in other organs via an inflammatory independent pathway (reducing TNF-alpha concentration or NFkB activation). It was the first identified myokine and it is the most studied myokine which is produced and released by skeletal muscle. It was found significantly elevated with exercise, and precedes the appearance of other cytokines in the circulation. During exercise, IL-6 myokine mobilizes extracellular substrates and/or augment substrate delivery. In a classic signaling, IL-6 stimulates target cells through a membrane bound interleukin-6 receptor (IL-6R), which upon ligand binding associates with the signaling receptor protein sgp130 (soluble glycoprotein130), triggering the activation of the JAK/STAT signaling pathway. As a pro-inflammatory cytokine, IL-6 is produced by monocytes or macrophages in response to specific pathogen molecules. In cells that express only sgp130 but not IL-6R, IL-6 binds to the soluble IL-6R and this complex binds to sgp130 triggering the activation of an intracellular signalling pathway, known as trans signalling. Here we would like to discuss the role of IL-6, especially as myokine that appears in muscle tissue and in the circulation during exercise at levels up to one hundred times basal rates and is seen as having a beneficial impact on health and bodily functioning. IL-6, among an increasing number of other recently identified myokines, remains an important subject in research, as its mechanism of action it is not fully known.

CHALLENGES IN MUSCLE BIOPSY EVALUATION

Alexandra Bastian[1,2], Emilia Manole[1,3]

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2. University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania
3. „Victor Babes" National Institute of Pathology, Bucharest, Romania

Muscle biopsy continues to be an extremely useful investigation for the diagnosis of a variety of neuromuscular disorders, even after the revolutionary advent and implementation of the molecular genetic studies in this field. In the daily practice, there are numerous challenges related to the appropriate selection of muscle to be biopsied, to the chosen type of biopsy (needle or open biopsy), to the optimal application of specific sampling, handling and processing techniques to prevent artifact and especially to the correct interpretation of the meaningful morphological changes to get the full benefit of this procedure and establish the diagnosis. In myopathology, very few features are, by themselves, specific for a certain disorder, no single abnormality should be evaluated in isolation, there are many overlaps of aspects in completely different diseases, some pathologies are patchy and even immunohistochemical reactions require a cautious interpretation. Because of the strong connections between the various structural proteins located in different muscle compartments, a reduced immunolabeling may be indicative for a primary or a secondary protein deficiency. In this presentation we describe and exemplify different challenges encountered in processing and evaluation of muscle biopsies diagnosed in the Pathology Department of Colentina Clinical Hospital. Most of the difficulties can be overcome through detailed clinico-pathological correlations.

DAY 3 – SATURDAY, NOVEMBER 19

Course: Stem cell-based models

Lecture: Intracellular signaling through ITAM-motif receptors

Session 11: Short communication - young researchers

Best Presentation Award

Communication (K)now

Course

HUMAN INDUCED PLURIPOTENT STEM CELL-BASED MODELS FOR DEVELOPMENT AND DISEASE

Irina Roxana Deleanu[1]

1. Department of Anatomy, Histology and Embryology, Medical University of Innsbruck, Austria

https://www.i-med.ac.at/neuroanatomy/irina-roxana-deleanu.html

Lecture

ENDOSOMAL SIGNALING OF ITAM-COUPLED IMMUNE RECEPTORS

Loredana Săveanu[1]

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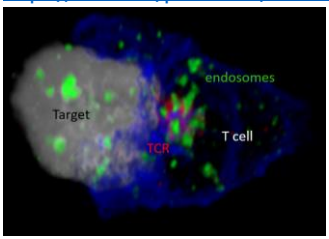
https://cri1149.fr/personnel/saveanu-loredana/



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Session 11

Session Chairs – Ana-Maria Enciu & Tudor Emanuel Fertig

MULTIMODAL MICRO-CT IMAGING FOR 3D PATHOLOGY

Victor Gabriel Ungureanu[1,2], Antoanela Tanca[1], Daniel Anghel[1], Elisa Anamaria Liehn[1,3], Octavian Bucur[1]

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2. Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
3. University of Southern Denmark, Odense, Denmark

Aims and Background. Human tissue investigation with the standard approach only allows a two-dimension (2D) interpretation of the underlying pathological process. However, this planar representation fails to render the complete spatial configuration of pathological specimens. We propose an improved version of micro-CT scans, an algorithm which is similar to classical CT scans and which allows three-dimensional (3D) reconstruction from sequential microscopy slides. Our mathematical representation allows us to concatenate multiple descriptors, such as colour and fluorescent marker intensities in a compact and easy to understand representation.

Methods. Whole tissue sections images were acquired using the Leica Aperio LV1 scanner, resulting in twenty carotid artery sections cut 50 µm apart and over one hundred consecutive colon 2 µm tissue sections, hematoxylin and eosin (H&E) stained. Twenty additional carotid artery tissue sections had been stained with trichrome-orcein, and immunohistochemistry was performed for SMA and Mac2 detection (smooth muscle actin and macrophage markers) respectively. Their colour spaces were converted from RGB (Red Green Blue) to HSV (Hue Saturation Value). Noise filtering was applied by Gaussian blurring. Over-segmentation was performed using SLIC (Simple Linear Iterative Clustering), producing an intermediate model which was refined using a region adjacency graph with a custom metric. An autoencoder was also compiled and trained on the same images. The final 3D model was built with the marching cubes algorithm.

Results. AI and parametric procedures yielded similar segmentation results. Compared to RGB, the HSV colour space is able to detect more subtle colour changes and results in a finer 3D reconstruction. We modelled a colon polyp and noticed that the mucosal and connective tissues were automatically segmented. We also modelled an atherosclerotic carotid artery. Our protocol was able to detect and classify the endothelium, arterial laminae, inflammatory tissue and fatty plaque from H&E and immunohistochemical stains in 3D.

Conclusion. Classical algorithms, as well as AI techniques were applied to modelling the specimens. Our protocol allows for multiple channels of data to be combined into one single representation. The final models can be used as input for more advanced machine learning techniques, such as deep learning, for advanced classification and

analysis of 3D tissue specimens. Examining the 3D structure of human specimens can yield valuable information that can significantly impact clinical investigation, diagnosis and treatment of a wide variety of pathologies, including cancer and cardiovascular diseases, laying the foundation for 3D investigation in pathology.

TUMOR CELLS UNDER IRRADIATION

Elena-Mihaela Dragnea[1], Ana-Maria Enciu[1], Ionela Victoria Neagoe[1], Maria Dobre[1]

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Background. Cancer represents one of the most widespread pathologies globally. Although this condition has been studied for a long time, the therapeutic strategies have limitations in efficacy and safety. Radiotherapy is the gold standard in cancer therapy and remains the most effective therapeutic approach for a multitude of cancer types. However, it has several limitations, such as intrinsic and acquired radioresistance following repeated radiation exposure, as well as the different radiosensitivity of cells in the tumor niche. In this context, it is necessary to identify some co-therapies that allow increasing the efficiency of radiotherapy by modulating the radiosensitivity/radioresistance of tumor cells, as well as for the protection of normal cells.

Aim. The study has as main objective to identify molecular networks involved in tumor and normal cellular responses to ionizing nuclear radiation, with the purpose of identifying new therapeutic targets for modulating cellular radiosensitivity.

Method. Human colon carcinoma cells HCT116 were exposed to different doses of γ radiation. Proliferation and adhesion were evaluated using the xCELLigence platform. Cellular viability was assessed by the MTS reduction test, which informs on the number of metabolically active cells in culture, complemented by the lactate dehydrogenase release (LDH) assay, which gives information on membrane integrity alterations that are specific for necrotic cells. The expression profile of 84 stress genes was evaluated by qRT-PCR using the RT² Profiler™ PCR Array Human Stress & Toxicity PathwayFinder array (Qiagen).

Results. Experimental data showed significant changes occurring in the time interval between 48 and 72 hours post-irradiation. The real-time measurement of cellular impedance generated by the xCELLigence platform showed that after 48h, irradiated cells enter a plateau phase indicating that the proliferation is affected, unlike control cells that continue to have an ascending trend. MTS reduction test showed a decline in cellular metabolic processes after 48h which correlates with a significant increase of LDH levels suggesting that cells undergo necroptosis. The expression profile of stress genes highlighted that irradiated cells attempt to counteract the damaging effects of γ radiation by activating repair mechanisms, but also cell death. Genes with modified expression are

known to be involved in anti-oxidant response, hypoxic response, and inflammation as well as DNA damage and repair and cell death.

Conclusion. Although irradiated cells appear to evolve better that non-irradiated controls on term of adhesion and proliferation, repair and death mechanisms were activated after 48 hours post-irradiation. Part of the repair mechanisms were acting less or were even suppressed later on, at 72 hours post-irradiation, when cellular adhesion/proliferation decreases. The gene expression study is a valuable method for investigating in vitro the dose-dependent biological effects exerted by γ radiation, that can be further used to identify counteracting measures.

Acknowledgement. Work was financed by the Ministry of Research, Innovation and Digitization under the grant ELI-09/2020 and PN19.29.02.02.

TUMOR SPHEROIDS AS MODELS FOR IN VITRO TESTING

Iulia Costache[1], Lucian Albulescu[2], Ionela Daniela Popescu[2], Elena Codrici[2], Maria Dudau[1,2], Ana-Maria Enciu[1,2], Cristiana Tănase [2,3]

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2. „Victor Babeș" National Institute of Pathology, Bucharest, Romania
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Background. Tumor spheroids are currently emerging as the novel standard for cytotoxicity testing as well as *in vitro* molecular studies. Our aim was to generate a 3D model of tumor spheroids, useful to test the response to cancer drugs.

Material and method. We used three breast tumor cell lines (MCF-7, MDA-MB-231, MDA-MB-361) and one glioblastoma cell line (U87), to generate spheroid formation in a dextran based hydrogel. Viability was tested using dual fluorescein diacetate/propidium iodide stain. Spheroids were measured for diameter, perimeter and fluorescence intensity.

Results. Different cell concentrations and hydrogel recipes were used to generate tumor spheroids, including ultralow adherence plates. The best option was a dextran-based hydrogel, which yielded spheroids as soon as 5 days of incubation. Different sizes of viable spheroids were obtained for all tested cell lines, but the intensity of fluorescence was not correlated to size, hence it was not a good parameter to quantify viability.

Conclusion. We have established a 3D system to generate spheroid tumors as *in vitro* models for drug testing.

Acknowledgement: This work has been supported by the project PN 19.29.01.04.

APPLICATION OF CRISPR/CAS-9-MEDIATED GENOME EDITING – FIRST STEPS TO GENERATE A K.O. CELL LINE

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The CRISPR/Cas9 system is a powerful genome-editing tool and has been applied in several fields, to generate new cell lines and animal models. Standard CRISPR/Cas9 consists of single guide RNA to direct the Cas9 endonuclease activity against a specific genome sequence, where it introduces a double strand break. The DNA damage is then repaired by non-homologous end joining (NHEJ) or homology (HDR) direct repair process, allowing to generate a gene knock out or introducing a specific mutation within a gene target. In the present work, the objective was genome manipulation with CRISPR/Cas9 technology of MG63 (human osteosarcoma cell line) to generated new in vitro cell model – knock-out of Fbxw11 (F-Box and WD Repeat Domain Containing 11) involved in phosphorylation-dependent ubiquitination. Fbxw11 codes for a protein involved in the formation of E3 ubiquitin ligase complex of the ubiquitin-proteasome system (UPS). It binds to the phosphorylated forms of IkB and b-catenin. Fbxw11 regulates cell cycle, differentiation, development, and metabolism. Its impairment is involved in carcinogenesis. This gene has been selected as a target to be knocked-out with CRISPR/Cas9 in an osteosarcoma cell line (MG63) for further studies. The CRISPR/Cas9 pros and cons are analysed for a full technique understanding, regarding its potential limits, such as off-targets, delivery systems, and editing screening.

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TELE-ENCOUNTERS – BEYOND THE HUMAN

Marina Hanganu
Artistic Director TELE-ENCOUNTERS (Creative Europe cooperation project)

GAME DESIGN INTRODUCTION

Alexandru Baltă
University Lecturer at National University of Theatre and Film I.L. Caragiale Bucharest

INDEX

F

G

H

I

J

K

L

M

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KEYNOTE SPEAKERS

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Alexandru Babeș	University of Bucharest, Romania
Anita Rauch	Institute of Medical Genetics, Zürich, Switzerland
Bogdan O. Popescu	University of Medicine and Pharmacy Carol Davila, Bucharest, Romania
Donato Zipeto	University of Verona, Italy
Emil C. Toescu	University of Birmingham, United Kingdom
Fabio Demontis	St. Jude Graduate School of Biomedical Sciences, Memphis, USA
Giuseppe Bardi	Istituto Italiano di Tecnologia, Genoa, Liguria, Italy
Irina Roxana Deleanu	Medical University of Innsbruck, Austria
Konstantinos Nikolouzakis	School of Medicine, University of Crete, Heraklion, Greece
Liron Pantanowitz	University of Michigan, USA
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Lucio Barile	Università della Svizzera Italiana, Lugano, Switzerland
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Nurhan Özlü	Koç University, Istanbul, Turkey
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Rajan Dewar	McLaren Greater Lansing, Michigan State University, USA
Sauri Hernandez-Resendiz	National Heart Centre Singapore
Shiang (Max) Lim	St. Vincent's Institute Medical Research, Australia
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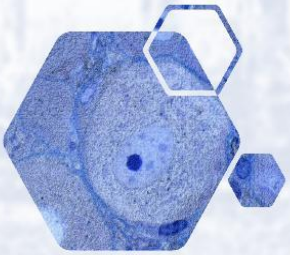
Vanesa Bijol	School of Medicine at Hofstra/Northwell, New York, USA
Xu Yichen	Institute for Molecular Medicine, University of Southern Denmark
Yin Hua Zhang	Seoul National University College of Medicine, Korea



Topics: cellular and molecular pathology, histopathology, omics in pathology, nephropathology, neurosciences, cardiovascular pathology, and more

Young investigator award, CMR 18 CME credits

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