

**Joint International Scientific Conference
and International Network of Young
Scientists Conference**



Smolenice Castle, Slovakia

April 24 – 27, 2023

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Book of Abstracts

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and International Network of Young Scientists Conference (JISC&INYSC VISION)

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PROGRAM

Monday, April 24, 2023

14:30 – 14:40 **Welcome**

Chairpersons: **Agapi Katakis and Lucia Kucerova**

14:40 – 15:15 *Nuria Malats*
A germline whole genome methylation score to predict pancreatic cancer risk

15:15 – 15:45 *Laura Garcia Bermejo*
EATRIS: a key European distributed Infrastructure for Translational Medicine

15:45 – 16:15 **Coffee break**

16:15 – 16:45 *Bruno Sainz*
Advancements in the understanding and targeting of pancreatic CSCs: from bench to pre-clinical model

16:45 – 17:15 *Bozena Smolkova*
Epigenetic inhibitors in the treatment of solid tumors

17:15 – 17:45 *Marina Cihova*
Experimental gene therapy mediated by extracellular vesicles in the treatment of PDAC

17:45 – 18:00 *Verona Buocikova*
Nanocarrier-mediated multimodal anticancer therapy

18:00 – 18:15 *Maria Urbanova*
Development of orthotopic pancreatic PDX models

18:15 – 19:15 **Dinner**

19:30 Social program *Vcelovina* (Mead and honey craft excursion)



Tuesday, April 25, 2023

Chairpersons: Julie Earl and Bozena Smolkova

8:30 – 9:00

Silvia Pastorekova

Role of hypoxia and acidosis in cancer progression

9:00 – 9:30

Agapi Katakaki

Liquid biopsy modalities in pancreatic cancer metastatic potential

9:30 – 9:45

Emma Barreto

Comprehensive analysis of Familial Pancreatic Cancer (FPC) suggests a different molecular subtype of patients with PDAC

9:45 – 10:00

Lenka Trnkova

Advanced in vitro models of pancreatic cancer

10:00 – 10:15

Ioanna Angelioudaki

Mutational and Circulating Biomarkers Landscape in pNENs

10:15 – 10:45

Coffee break

10:45 – 11:15

Lucia Kucerova

Circumventing chemoresistance in refractory tumors

11:15 – 11:45

Miroslava Matuskova

Combinational therapy mediated by polyamidoamine dendrimers is efficient in colorectal cancer treatment

11:45 – 12:00

Alexandros-Georgios Tzingounis

Immunoprofiling of pancreatic tissue microenvironment in cancer patients

12:00 – 12:15

Jorge Villalon Lopez

PANGENFAM: The Spanish Familial Pancreatic Cancer Registry

12:15 – 13:30

Lunch

Chairpersons: Nuria Malats and Andrew Collins



- 13:30 – 13:50 ***Silvia Tyciakova***
The role of mitochondria in colorectal cancer cells – overexpression of mitofusin-2 changing mitochondrial dynamics
- 13:50 – 14:10 ***Viera Horvathova Kajabova***
DNA methylation changes in tumor progression
- 14:10 – 14:30 ***Zuzana Kozovska***
Gene editing of selective genes in gastrointestinal cell lines by CRISPR/Cas9 method
- 14:30 – 14:50 ***Martina Poturnajova***
Genetic editing of ALDH1 stem cell marker isoforms is linked to distinct cellular and molecular properties in colorectal cell lines
- 14:50 – 15:10 ***Efthymios Koniaris***
Pancreatic lesions: The spectrum of syndecan-1 expression
- 15:10 – 15:25 ***Peter Dubovan***
Surgical viewpoint of neoadjuvant therapy in pancreatic ductal adenocarcinoma
- 15:25 – 15:55 **Coffee break**
- 15:55 – 16:25 ***Andrea Babelova***
Periostin as a marker of chronic kidney disease progression
- 16:25 – 16:55 ***Elise Runden-Pran***
Glutamate oxaloacetate transaminase nanoparticles: A promising novel therapy for Ischemic stroke
- 16:55 – 17:10 ***Katarina Gercakova***
Animal models for gastrointestinal cancer
- 17:10 – 17:25 ***Nikoleta Mojzesova***
Effect of epigallocatechin gallate on the response of CRC-derived cells to chemotherapy
- 17:25 – 18:30 **Dinner**
- 19:00 – 22:30 Social program ***Majolika*** (Ceramics manufacture excursion), bus departure at 19:00



Wednesday, April 26, 2023

Chairpersons: Bruno Sainz and Miroslava Matuskova

8:30 – 9:05

Stefano Bonassi

A roadmap for translating early effects biomarker results into clinical practice: From observational studies to randomized controlled trials

9:05 – 9:35

Maria Dusinska

Recent updates in risk assessment of nanomaterials

9:35 – 10:00

Coffee break

10:00 – 11:30

VISION final meeting

11:30 – 12:20

Lunch

Social program:

1. Whole-day trip to *Small Carpathia*, "lunch-to-go" will be provided, departure at ~10.0 am
2. *Driny cave*, departure at 12:30

16:00 – 16:30

Refreshment

19:00

Conference dinner



Thursday, April 27, 2023

Chairpersons: Maria Dusinska and Stefano Bonassi

9:00 – 9:35

Andrew Collins

DNA damage and repair; possible predictors of cancer risk

9:35 – 9:55

Eleonora Longhin

Hazard assessment of nanomaterials - meeting requirements for risk assessment

9:55 – 10:10

Michelle Muller

Human-induced pluripotent stem cell-derived alveolar epithelial cells as a promising cell source for in vitro lung models

10:10 – 10:25

Lucia Balintova

Biological safety of innovative nanocomposites with therapeutic potential

10:25 – 10:55

Coffee break

10:55 – 11:25

Thorsten Knoll

Microfluidic platform for in vitro toxicity testing

11:25 – 11:45

Elisabeth Elje

Genotoxicity assessment of nanomaterials in advanced lung models

11:45 – 12:00

Radka Macova

Analysis of tissue structural and functional changes following biodistribution and accumulation of 10 nm gold nanoparticles with BSA coating in mouse

12:00 – 13:45

Lunch

Chairpersons:

Elise Runden-Pran and Thorsten Knoll

13:45 – 14:05

Karin Danz

Developing advanced blood-brain barrier models for pharmacological research

14:05 – 14:25

Tanima SenGupta

C. elegans as a model system for assessing neurotoxicity



- 14:25 – 14:45 ***Barbora Svitkova***
Plate reader spectroscopy as an alternative to atomic absorption spectroscopy for the assessment of nanoparticle cellular uptake
- 14:45 – 15:00 ***Michaela Blazickova***
Comparison of the biological activity of thymol derivatives on 2D and 3D intestinal models
- 15:00 – 15:20 ***Michal Selc***
Anti-fibrotic effects of silymarin and silymarin-coated gold nanoparticles against hepatic fibrosis in mouse
- 15:20 – 15:30 **Closing the conference**
- 15:30 – 16:00 **Refreshment**
- 16:30 **Bus departure**

Abstracts

A Germline Whole Genome Methylation Score to Predict Pancreatic Cancer Risk

Evangelina Lopez de Maturana, Lola Alonso, and Nuria Malats

on behalf of the PanGenEU Study Investigators

Genetic and Molecular Epidemiology Group, CNIO, and CIBERONC, Madrid, Spain

To implement screening interventions to earlier diagnose sporadic pancreatic cancer (PC) it is crucial to define high-risk populations. The well-established PC risk factors and genetic susceptibility loci do not account for the whole complexity of PC etiology. DNA methylation (DNAm) in peripheral blood has been barely explored. Here we show the potential of whole leukocyte-DNA methylome risk score (WMRS) to predict PC. We followed a two-phase epidemiological design using population (N = 900 subjects) and resources from the PanGenEU Study. We determined CpG 5mC levels by Infinium MethylationEPIC array from leukocyte-DNA. We applied a single marker and a Bayesian kernel-based regression (BKR) to train the model in the discovery phase and then, validate and evaluate the biomarkers and evaluate their predictive ability in the testing set. Both uni/multimarker approaches were adjusted for immune cell composition, age, gender, region of recruitment, diabetes, and smoking status. For the first time, we evaluated and estimated the variability explained by the main effect of leukocyte-DNA methylome, as well as that of its interactions with the main risk factors for PC. Moreover, we propose a 77 CpG signature showing a good performance (AUC = 0.73) to predict PC.

EATRIS: A Key European Distributed Infrastructure for Translational Medicine. Pancreas Cancer Studies

Elisa Conde, Edurne Ramos Munoz, Lorena Crespo, Macarena Rodriguez-Serrano, Patricia Alonso, Val Fernandez, Carolina de la Pinta, Mercedes Rodriguez-Garrote, Ana Garcia-Garcia de Paredes, Julie Earl, Bruno Sainz and Maria Laura Garcia Bermejo

Ramon y Cajal Health Research Institute, Madrid, Spain

EATRIS is a non-profit European Distributed Research Infrastructure Consortium for Translational Medicine. EATRIS brings together resources and services for research communities to translate scientific discoveries into benefits for patients. We provide access to a vast array of pre-clinical and clinical expertise and facilities that are available within 148 top-tier academic centers across 15 Europe countries. We focus on improving and optimizing the preclinical and early clinical development of drugs, vaccines, and diagnostics. Solutions are developed in the fields of advanced therapy medicinal products, imaging and tracing, small molecules, vaccines, and biomarkers.

Ramon y Cajal Health Research Institute (IRYCIS) belongs to EATRIS since the beginning (2013), becoming very active when Dr. García Bermejo was appointed as co-chair of Biomarker Platform in 2017. Moreover, since May 2022, IRYCIS hosted the Scientific Direction of EATRIS Spanish Node, in Dr. García Bermejo figure, leading 29 out of 34 accredited Health Research Institutes in Spain.

IRYCIS has an important role in EATRIS Flagship projects including EATRIS Plus and Remedi4all. EATRIS Plus is devoted to develop multiomic approaches to healthy individuals in order to establish multiomic data integration pipelines and reference values, among others. Relevant content generated in EATRIS Plus, including protocols for omics, data exploitation methodology, and quality control procedures are offered to the community in an open-access resource: the multiomic toolbox. Remedi4all is a platform devoted to establish appropriate procedures for an effective drug repurposing. For probing the effectiveness, 4 demonstrators have been proposed. One of them addresses the beneficial effect of valproic acid and simvastatin in chemotherapy-treated pancreas cancer. Clinical trial for proving the efficacy (VESPA Trial), biomarkers of response, and mechanisms underlying the effect will be identified. Finally, IRYCIS is leading a use case in the new application EATRIS Connect, aiming to implement the digital transformation in clinical practice, based on the pancreas cancer dataset.

EATRIS role and evolution as well as projects in the pancreas cancer context will be discussed.

Advancements in the Understanding and Targeting of Pancreatic CSCs: From Bench to Pre-Clinical Model

Bruno Sainz, Jr.^{1,2,3,4}

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²*Area 3 Cancer, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), 28049, Madrid, Spain*

³*Centro de Investigación Biomédica en Red, Área Cáncer, CIBERONC, ISCIII, Madrid, Spain*

⁴*Spanish National Research Council (CSIC)'s Cancer Hub*

Pancreatic ductal adenocarcinoma (PDAC), the most common type of pancreatic cancer, has a median overall survival of 6–12 months and a 5-year survival of less than 7%. While PDAC currently represents the 4th most frequent cause of death due to cancer worldwide, it is expected to become the second leading cause of cancer-related death by 2030. These alarming statistics are primarily due to both the inherent chemoresistant and metastatic nature of this tumor, and the existence of a subpopulation of highly plastic “stem”-like cells within the tumor, known as cancer stem cells (CSCs). Over the past decade, we have achieved considerable advancements in our understanding of PDAC at the molecular, cellular, and metabolic levels. These advancements have helped us to discover 1) specific targets that can be pharmacologically inhibited, such as the non-receptor protein tyrosine phosphatase SHP2 or 2) cellular processes, like mitochondrial respiration, that represent a targetable Achilles’ heel of pancreatic CSCs. Likewise, advancements in our understanding of the biology of pancreatic CSC and the identification of new markers to identify and isolate these cells have proved beneficial for patient subtyping studies and for developing personalized medicine platforms. Lastly, our understanding of the tumor microenvironment has revealed key processes that are essential for PDAC metastasis. Herein we will highlight these recent PDAC-related research advancements and discuss their applicability at the level of therapy and personalized medicine.

Epigenetic Inhibitors in the Treatment of Solid Tumors

Bozena Smolkova, Maria Urbanova, Verona Buocikova, Svetlana Miklikova, Marina Cihova, Lenka Trnkova, Alena Gabelova

Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Cancer-cell biology is dictated by alterations in the epigenetic regulation of gene expression. Epigenetic changes such as DNA methylation, histone deacetylation, and non-coding RNAs expression are capable of silencing the expression of tumor suppressor genes and inducing oncogenes, leading to clonal proliferation of tumor cells. Mechanisms underlying these regulators are being increasingly elucidated, and agents that alter their function emerge as epigenetic therapies.

Cancer epigenome therapeutics can be grouped into two categories: broad and narrowed spectrum reprogrammers. The first group that causes genome-wide gene expression alterations includes the inhibitors of DNA methyltransferases (DNMTs), histone deacetylases (HDACs), and bromodomain and extra-terminal motif proteins (BETs). Narrowed spectrum epigenetic modifiers such as lysine-specific histone demethylase 1 (LSD1), DOT1-like histone lysine methyltransferase (DOT1L), or enhancer of zeste homolog 2 (EZH2) allow specific inhibition of epigenetic regulatory proteins.

Except for tazemetostat, approved for the treatment of epithelioid sarcoma, FDA-approved epigenetic drugs are limited to hematologic malignancies. However, clinical trials are also ongoing in solid tumors. Due to the limited efficacy of epigenetic drugs in monotherapy and the complexity of epigenetic modification in cancer, many preclinical studies and clinical trials are investigating the effects of combination therapies. Recent clinical trials include combinations of various epigenetic agents or their combination with cytotoxic chemotherapy, hormonal therapies, and immune checkpoint inhibitors. Epigenetic drugs combined with chemotherapy demonstrated the ability to re-sensitize cancers to the standard cytotoxic agents.

Importantly, epigenetic agents have also exhibited a significant “reprogramming” activity in immune cell components. They have been shown to modulate the tumor microenvironment, and the combination of their “reprogramming” abilities with other approved or novel therapies is extensively investigated. However, it is essential to better understand the underlying mechanisms and translate these findings into clinical trials as well as optimize combinatorial approaches with the exploration of predictive biomarkers. Poor stability, toxicity, and potential broad-reprogramming abilities of epigenetic drugs require developing novel delivery strategies to overcome their limitations. Recent advances in nanomedicine offer several advantages, such as targeted drug delivery, sustained release, reduced systemic toxicity, and enhanced tumor penetration. This approach presents important perspectives on future research directions that can guide the rational design of novel and robust nanoscale platforms, particularly with respect to targeted therapies and immunotherapies.

This work was supported by grants APVV-21-0197, projects Transcan II NExT, RESCUER, and H2020 project VISION

The Potential of Extracellular Vesicles in the Treatment of PDAC

Marina Cihova¹, Verona Buocikova¹, Bozena Smolkova¹, Maria Urbanova¹, Ursula Altanerova², Jana Jakubechova², Cestmir Altaner²

¹*Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences*

²*Department of Stem Cell Preparation, St. Elisabeth Cancer Institute, Bratislava, Slovakia; Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia*

Extracellular vesicles (EVs) such as exosomes are released by all living cells and contain diverse bioactive molecules, including nucleic acids, proteins, lipids, and metabolites. The components of EVs reflect the characteristics of the originating cells. The potential for EV applications has been expanding quite rapidly over the past few years, particularly in the cancer-related field. Circulating EVs are considered not only an essential clinical target for use as cancer biomarkers but have promising potential as therapeutics, as they can act as carriers to deliver therapeutic agents. PDAC is particularly difficult to treat, because of an abundant desmoplastic stroma which acts as a physical barrier that hinders the effective delivery of chemotherapeutic agents to tumors.

Therefore, the development of ideal drug delivery systems (DDSs) for improving PDAC treatment is of great interest. Exosomes are emerging as a promising DDS as they generally have low levels of immunogenicity and cytotoxicity, stronger targeting specificity, deeper tissue permeability, longer circulating half-life, and can improve drug stability to reduce dosages and side effects. Based on these advantages, exosomes have been applied for engineering functional cargo loads, such as packaged nucleic acid, functional proteins, and other therapeutic molecules into exosomes.

Mesenchymal stromal cell (MSC)-derived exosomes have been recently transfected with functional RNAs to target cells, suggesting their potential as an alternative for cell-based therapy. The use of MSC-derived exosomes for the delivery of anticancer therapy was shown to hold more promise than the use of MSCs themselves.

In our work, we are focusing on the development and potential of MSC- or PDAC stromal cell-derived exosomes loaded with mRNA of yeast cytosine deaminase::uracil phosphoribosyl transferase fusion gene (yCD::UPRT), an enzyme converting non-toxic 5-fluorocytosine (5-FC) into cytotoxic 5-fluorouracil (5-FU). These exosomes similarly migrate to the tumor as MSCs, where they are internalized by the tumor cells. Subsequently, they kill the tumor cells by intracellular conversion of prodrug 5-FC to cytostatic drug 5-FU.

This work was supported by APVV-20-0143 and APVV-21-0197.

Nanocarrier-Mediated Multimodal Anticancer Therapy

Verona Buocikova¹, Svetlana Miklikova¹, Miroslava Matuskova¹, Marina Cihova¹, Martina Labudova², Lucia Csaderova², Aiva Plotniece³, Karlis Pajuste³, Martins Rucins³, Arkadij Sobolev³, Alena Gabelova¹, Bozena Smolkova¹

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New innovative multidisciplinary approaches can help resolve the current clinical challenges of cancer treatment. Progress in the field of nanotechnology has enabled the development of a wide range of biocompatible and low-immunogenic nanomaterials intended for the transport of antitumor drugs directly into tumor tissue. The encapsulation of therapeutic agents in nanocarriers (NCs) prevents their rapid clearance and degradation, which also increases their half-time in circulation, increases solubility or stability, and reduces toxicity. Epigenetic deregulation of gene expression demonstrably contributes to the progression of oncological diseases and the development of tumor cell resistance to standard treatment agents. Moreover, an increasing body of evidence demonstrates that combining epigenetic drugs with conventional therapeutic approaches can ultimately increase the effectiveness of treatment also in patients with a poor prognosis, including breast cancer (BC).

In our work, we examined the capacity of decitabine (DAC), an inhibitor of DNA methyltransferases (DNMTs), to increase the sensitivity of BC cells to anthracycline antibiotic doxorubicin (DOX). The ability of DAC to sensitize trastuzumab-resistant HER2-positive JIMT-1 cells to DOX and its commercial PEGylated liposomal formulation Caelyx® (CX) was studied *in vivo*. The sequential administration of DAC + DOX combination significantly reduced the tumor growth, global DNA methylation, and DNMT1 expression in xenograft tissues. The effectiveness of combination therapy was comparable to that of CX monotherapy, used exclusively to treat metastatic BC.

Furthermore, we tested the ability of folate-coated liposomal nano-prototypes for targeted delivery of DOX into BC cells. The coating of NCs with folate resulted in their specific uptake *in vitro*, particularly apparent in MDA-MB-231 cells. However, in the orthotopic MDA-MB-231 mouse model, we did not confirm folate-mediated uptake of liposomal NCs. In addition, the DOX-encapsulated nano-prototypes had lower efficacy in reducing tumor volume *in vivo* compared to the CX.

Our work demonstrates the potential of epigenetic drugs to modulate tumor cells' sensitivity to other forms of anticancer therapy, especially in resistant BC subtypes. However, further studies with innovative nanoformulations, which enable the active transport of various drug combinations, including new-generation epi-drugs, are needed to confirm this hypothesis.

This work was supported by the project INNOCENT (ERA-NET, EuroNanoMed II).

Development of Orthotopic Pancreatic PDX Models

Maria Urbanova¹, Verona Buocikova¹, Marina Cihova¹, Lucia Rojikova¹, Marianna Makova², Ladislav Baciak³, Julie Earl⁴, Laura Ruiz-Cañas⁵, Bruno Sainz⁵, Jörg Schrader⁶, Bozena Smolkova¹

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Xenografts are *in vivo* models commonly used in translational research of cancer tumorigenesis and metastatic potential and for testing novel therapeutic options. Typically, resuspended tumor cells are injected subcutaneously into immunodeficient mice for developing subcutaneous tumors. However, the development of orthotopic models by direct injection of tumor cells to tumor origin, as to pancreatic tissue for developing pancreatic cancer model, results in a more accurate *in vivo* model. Orthotopic tumors are capable of better mimicking real tumor stroma, and vascularisation, and more authentically predict the response to treatment.

Thanks to the VISION training program, we were able to learn and establish the protocol for the development of orthotopic *in vivo* models of pancreatic cancer in our institute. We successfully developed an orthotopic model of several cell lines of pancreatic ductal adenocarcinoma as well as the newly established neuroendocrine neoplasm line NT-38, which was further used for *in vivo* verification of targeted treatment. Also, orthotopic patient-derived xenografts (PDX) were established by implanting the tumor tissue into the mouse pancreas. The orthotopic tumor growth and treatment response were monitored over time by serial magnetic resonance imaging.

This work was supported by grants APVV-20-0143, APVV-21-0197, projects Transcan II NExT, and H2020 project VISION.

Role of Hypoxia and Acidosis in Cancer Progression

Silvia Pastorekova, Martina Takacova, Miriam Zatovicova, Ivana Kajanova and Eliska Svastova

Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovakia

During solid tumor growth, subpopulations of cancer cells are exposed to stresses caused by the irregular local and temporal supply of oxygen, nutrients, and signaling molecules by aberrant vasculature. These physiological stresses support the development of highly heterogeneous tumor tissue with a microenvironment characterized, among other features, by regions of hypoxia and/or acidosis. Hypoxia is a biologically and clinically important phenomenon associated with aggressive tumor phenotype, cancer progression, and therapy resistance. Reduced oxygen availability stimulates cellular adaptive processes that include a shift to oncogenic metabolism, slowed cell proliferation, diminished cell adhesion, increased invasiveness, angiogenesis, and other energy-saving and metastasis-enabling alterations. Oncogenic metabolism generates an excess of lactic acid, protons, and carbon dioxide. Intracellular accumulation of these acidic metabolites is incompatible with survival, and thus cells activate constituents of pH regulating machinery, including ion exchangers and transporters that mediate acid extrusion resulting in extracellular acidosis. Among these molecules, a key role is played by the carbonic anhydrase IX (CA IX), which is expressed in tumors in response to hypoxia. CA IX is functionally involved in the protection of tumor cells from acidosis (through its ability to regulate pH) as well as in the promotion of metastatic phenotype (through its ability to regulate adhesion-migration-invasion and contribute to immune suppression in tumor tissue). For these reasons, CA IX is used as an intrinsic biomarker of tumor hypoxia and an indicator of poor response to treatment and is evaluated as a target for therapy aimed against hypoxia- and acidosis-driven cancer progression.

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Biopsy Modalities in Pancreatic Cancer Metastatic Potential

Agapi Katakaki¹, I. Angelioudaki², A. Mitrousias², A.G. Tzingounis², L. Chardalias², L. Rentifis¹, E. Koniaris³, G. Zografos¹, M.M. Konstadoulakis²

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Liquid biopsy is a minimally invasive approach well tolerated by patients, allowing the real-time analysis of tumor cells (CTCs) or their products including exosomes and cell-free DNA (cfDNA) present in peripheral blood. Among its clinical applications are, tumor recurrence and metastasis. However, due to their low concentrations, their detection remains a task and their validated use in clinical practice as valuable biomarkers remains a challenge. The aim of this study was to assess the application of liquid biopsy in patients with pancreatic lesions.

Thirty-seven individuals (16 men and 21 women, mean age 61.3 ± 2.83 years) were enrolled in the study of CTCs analysis. The patients' cohort consisted of 30 patients diagnosed with pancreatic adenocarcinoma (PDAC: $n = 26$) or pancreatic neuroendocrine tumors (pNET $n = 4$) and 12 ml of blood was sampled within 24 h before surgery avoiding positive contamination from needle-cored epithelial cell entering the venesection needle lumen. Six normal individuals (3 men and 3 women, mean age 25 ± 1) were also included as negative controls. CTCs were isolated using either OncoQuick Density Gradient Centrifugation or Metacell technology according to the manufacturers' proposed protocol. Isolated CTCs were downstream stained with Cytokeratin (CK+) and Vimentin (Vim+) and analyzed by flow cytometry. Concerning exosomes detection and cfDNA our cohort was expanded to include 74 subjects (PDAC: 51, benign lesions $n = 4$, pNET $n = 13$, Neuroendocrine Carcinomas (pNEC) $n = 4$ and MANEC $n = 2$; mean age 65.8 ± 1.7). dsDNA and ssDNA were quantified in serum by Qubit 4 Fluorometer whereas, exosomes were quantified using CD9-based ELISA and verified by NTA analysis. Statistical analysis was performed using SPSS v28.

In our cohort, the percentage of CTCs per ml blood was increased in patients with PDAC compared to normal individuals and pNET but without reaching statistical significance. Still within the PDAC group, the presence of CTCs was increased in the presence of lymph node metastasis and was associated with poor overall survival but also without statistical significance ($p = 0.074$). As it concerns cfDNA levels and exosome concentrations, neither varied significantly among groups nor was related to overall survival. Nevertheless, in PDAC patients cfDNA was significantly increased in the presence of perineural invasion and inflammation ($p = 0.025$, $p = 0.05$ respectively).

The present study provides evidence supporting the clinical validity of liquid biopsy in pancreatic lesions, still, our feeling is that more research is required in the standardization of these challenging procedures before their introduction into everyday clinical practice.

Comprehensive Analysis of Familial Pancreatic Cancer (FPC) Suggests a Different Molecular Subtype of Patients with PDAC

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Up to 15% of a total pancreatic cancer cluster in families with a high incidence of disease. 80% of these families suffer familial pancreatic cancer syndrome (FPC), that does not fit in other hereditary syndrome criteria. The genetic basis of the disease is unknown in the majority of families.

Thus, our objective was to study the genetic background of FPC cases identifying germline variants that define the disease in this group and distinguish them from the sporadic cases (SPC).

Custom panel sequencing was performed in 140 patient cohort (54 FPC cases, 43 relatives of high-risk (HRI), and 43 sporadic PDAC cases) using Agilent SureDesign technology. Whole-exome sequencing (WES) was performed in 27 patients (9 FPC and 18 HRI) using SureSelect SSXTV6 8-10 Gb. Data were analyzed using the Varsome Clinical platform and tertiary analysis was performed according to custom filtering criteria.

In panel sequencing, the FPC group harbored more pathogenic variants when compared with SPC cases, as expected. The top mutated genes were *CFTR*, *ATM*, *BRCA2*, *MLH1*, and *MUTYH*. TP53 pathway, genome integrity, and cell cycle control pathways were more frequently altered in FPC cases.

In exome sequencing, we found novel genes that carried interesting variants in 80% of our cohort, such as *PRH1*, *CFTR*, *MUC4*, *MUC6*. Pathways more frequently affected were TP53, PI3K, RTK-RAS, Hippo, and genome integrity.

NGS highlights a wide heterogeneity of germline mutations especially in FPC cases, affecting mostly genes involved in DNA repair processes and genome stability. WES permitted the identification of novel genes that could be associated with the development of disease in these families and their different molecular biology disease, with a family-specific pattern.

In general terms, FPC patients show a different molecular profile based on their multiple germline variants and this could be used in the clinics for better profiling and the use of targeted therapies.

Advanced *In Vitro* Models of Pancreatic Cancer

Lenka Trnkova, Verona Buocikova, Maria Urbanova, Bozena Smolkova, Marina Cihova

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Cell cultures represent the most common pre-clinical *in vitro* model. Monolayer cultures of cancer cells have been used as the main model for cancer research for many years. However, such cultures have multiple limitations like possible phenotypic changes caused by prolonged *in vitro* culture, inadequate representation of patient population due to the use of a limited number of established cell lines, or their low reliability when compared to *in vivo* situation observed in the patient. Moreover, monolayer cultures can be hardly used to model some of the processes occurring in patient tumor like hypoxia gradient, changes in metabolic processes, or variable response to the therapy arising from the 3D organization of cancer cells within the tumor. Therefore, experimental models that better recapitulate conditions observed in the patient are becoming more popular in cancer research.

Recently, cancer organoids emerged as a novel 3D *in vitro* model of patient tumor tissue. They represent cellular clusters derived from individual patient embedded in the extracellular matrix and grown in specific culture conditions. Organoid cultures have been developed from multiple cancer types like breast, colorectal, lung, liver cancer, and organoids have been developed also from pancreatic tumors. Benefits of cancer organoids include maintenance of cancer cell heterogeneity, histological features, or genetic and mutational status, which are all factors that make cancer organoids highly significant *in vitro* experimental model with the potential for clinical applications. For example, cancer organoids have been used to assess response to the therapy in the settings of personalized medicine. Pancreatic tumors contain a large amount of tumor stroma and the implementation of stromal cells to organoid culture allows studying the impact of tumor microenvironment on cancer cell sensitivity or cancer progression. Furthermore, co-culture methods of cancer organoids with immune cells can be used to study the efficacy of immunotherapy.

In conclusion, we summarize current knowledge in cancer organoid methodology with an emphasis on cancer organoids derived from pancreatic tumors. We also summarize the possible experimental application of cancer organoids like co-culture methods to study tumor microenvironment or therapy response.

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Mutational and Circulating Biomarkers' Landscape in pNENs

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Pancreatic neuroendocrine neoplasms (pNENs) include distinct clinical entities which are classified as carcinomas (pNECs), and tumors (pNETs). Their emergence remains challenging to diagnose and treat on time due to the absence of specific symptoms. Understanding their carcinogenesis process starting with genetic mutations and communication pathways with their microenvironment is crucial for their clinical management. This study aims to explore the variability in their mutational profile and circulating biomarkers such as exosomes between pNETs and pNECs.

DNA was extracted from 10 FFPE tissues (6NETs:4NETs) and proceeded to Next Generation Sequencing (NGS) using TruSight Oncology 500 assay. Detected variants were filtered for a mutational allele frequency > 5% and identified based on ClinVar or Varsome platforms. Pathway enrichment analysis was performed using the Cytoscape plugin ClueGO/CluePedia search tool. Seven of the above samples with available serum were included in a larger study cohort consisting of 20 pNET (age: 59.05 ± 3.183) and 11 pNEC patients (age: 61.18 ± 3.185). Exosomes-associated biomarkers, CD63 and caveolin-1 were detected through immunohistochemistry. Stainings were histologically evaluated and results were statistically analyzed using IBM SPSS Statistics 28.0.

In pNETs 64 variants were identified vs. 52 in the pNECs group, with 53% of variants representing missense mutations and T/C substitutions being the most prevalent (22.5%). Exclusively in pNECs variants in HNF1A, CDKN2A, CTNNB1, KRAS, TP53, ERBB2 genes are identified as pathogenic. Pathway enrichment analysis indicated significant epigenetic modification involvement in histone H3-K4 methylation and H3K9 acetylation pathways. The Overall Survival of pNENs patients was negatively affected by higher cytoplasmic CD63 staining in the stroma (p = 0.039), and this is attributed primarily in pNECs (p = 0.07) vs pNETs (p = 0.224). Its significantly higher expression (p = 0.004) was observed in the presence of LN metastasis. In pNETs, CD63 membrane expression was higher than the cytoplasmic expression (p = 0.008), while in normal adjacent tissue, CD63 cytoplasmic expression was higher than in pNETs (p = 0.014). Finally, Caveolin-1 expression was not expressed pNETs epithelium where it was detected in 36% of pNECs lesion epithelium (0 vs. 6.09 ± 5, p = 0.011).

Combining the mutational profile analysis of pNENs pointing out the crucial role of epigenetics, with the increased expression of exosomes-associated molecules in more clinically aggressive entities, highlight molecular pathways which contribute to pNENs heterogeneity and cellular plasticity.

Circumventing Chemoresistance in Refractory Tumors

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Tumors that represent curable chemosensitive malignancies, even in their metastatic stage, have been described. However, some patients do not have a durable complete remission with initial standard chemotherapy. We established several *in vitro* and *in vivo* models for the experimental exploration of the underlying mechanisms including isogenic variants of the chemosensitive human cell lines. We identified potential biomarkers and therapeutic targets, such as fibrillin-1, PD-L1 protein, PARP protein, DNA repair proteins, aldehyde dehydrogenase (ALDH), microRNA - mir-371a-3p, carbonic anhydrase IX, and beta-catenin. ALDH3 A1 inhibition by napabucasin overcame cisplatin resistance in ovarian germ cell tumor-derived cell line by inhibiting cancer stemness *in vitro*. Our hypothesis was confirmed in the experiments using both cell lines *in vitro* and xenografts, however, decreasing ALDH activity using disulfiram in phase II clinical trial did not lead to clinical benefit in the patients. It remains challenging to develop novel agents that circumvent chemoresistance or may achieve a synergistic effect in combination with the approved treatment regimens in those tumors, which acquire chemoresistance during the treatment or exhibit inherent chemoresistance to standard therapy. Novel research approaches and technologies for better mimicking the clinical situation are needed; further exploration and development will bring benefit to the patient regarding their therapeutic management.

Combination Therapy Mediated by PAMAM Dendrimers Is Efficient in Colorectal Cancer Treatment

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. In many patients, the disease relapses, metastatic spread occurs, and their prognosis is unfavorable. Therefore, it is necessary to look for new therapeutic approaches. Multifunctional nanocarriers enable the targeted administration of several drugs simultaneously while preserving their physicochemical properties and biological functions. Dendrimers are highly branched polymer nanocarriers with a controllable structure, many terminal functional groups, low viscosity, and good solubility. These properties enable their use also in biomedicine. We focused on developing a multimodal nanotherapeutic system. We conjugated polyamidoamine-(PAMAM) based dendrimers with 5-fluorouracil (5-FU) and a plasmid carrying the gene for the TNF-related apoptosis-inducing ligand (TRAIL). Folate was used to improve the targeting, and silicon-rhodamine (SiR) was used for tracking nanoparticles (NPs). We prepared several variants of PAMAM dendrimers that differed in size and charge (the ratio of NH₂ and COOH functional groups). After analyzing the physicochemical properties, we tested the ability of the selected nanoparticles to interact with tumor cells. Using a luminescent cell viability assay, we showed that NPs do not cause a cytotoxic effect unless conjugated with therapeutic molecules; we observed a slight genotoxic effect. Only dendrimers with a high number of NH₂ groups, which are essential for interaction with plasmid DNA, showed some cytotoxicity. NPs entered tumour cells efficiently. We observed the cytotoxic effect of NPs conjugated with 5-FU and/or a TRAIL plasmid in cells expressing DR4 and DR5 death receptors. We confirmed the therapeutic efficacy *in vivo* on the model of subcutaneous xenografts induced by the HCT116 colorectal cancer cell line. Furthermore, using *in vivo* imaging, we demonstrated the accumulation of dendrimers in tumors, liver, and kidneys.

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Immunoprofiling of Pancreatic Tissue Microenvironment in Cancer Patients

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Pancreatic ductal adenocarcinoma (PDAC) is a rapidly progressing disease with poor patients' outcome. Highly desmoplastic stroma and extracellular vesicles (EVs) contributing to tumor cell proliferation, migration, and immune cell activation characterize these tumors. In this study we examined the relationship of pro-metastatic molecule CD44v6 expressed on pancreatic cancer stem-like cells (Pa-CSCs), with mesenchymal markers CD90 and CD73, exosomal marker CD63, and human monocyte differentiation antigen CD14 in the context of the disease's histopathological data.

Fifty PDAC patients (mean age 68.08 ± 1.24) were enrolled in the current study. Frozen PDAC tissue, and their corresponding normal adjacent tissue (NAT), when available ($n=33$), were analyzed cytometrically using CD44v6, CD90, CD73, CD63, and CD14 antibodies. CD44v6 and CD63 were also immunohistochemically evaluated using corresponding paraffin-embedded tissue sections. Demographic and clinical data retrieved from patients' medical files, were used during the analysis. The study was approved by the 'Hippocratio General Hospital of Athens. Statistics were performed using SPSS28.

Flow cytometry (FC) analysis showed that CD90 and CD73 expressions were higher in PDAC compared to the NAT (22.25 ± 2.53 vs. 13.3 ± 1.73 , $p < 0.001$, 19.93 ± 1.87 vs. 12.97 ± 1.82 , $p < 0.001$). Still, no significant relationship was detected while comparing it with the histopathological data. Increased CD63 positivity in PDAC (52.65 ± 3.24 vs. 44.13 ± 3.52 , $p = 0.009$) was also found. CD14 expression was also increased in PDAC compared to NAT (7.74 ± 0.7 vs. 10.29 ± 0.83 , $p = 0.004$), especially in those patients with no lymph node metastasis (12.91 ± 1.63 vs. 9.15 ± 0.87 , $p = 0.046$). Circulating monocytes were increased in patients presenting a low degree of desmoplasia (7.54 ± 0.35 vs. 6.33 ± 0.38 $p = 0.042$). CD44v6 expression was higher in PDAC compared to NAT (2.56 ± 0.23 vs. 1.49 ± 0.16 $p < 0.001$) and was similarly increased in these tumors (3.05 ± 0.38 vs. 2.18 ± 0.25 , $p = 0.047$). Additionally, they presented increased CD44v6 positive expression in their corresponding metastatic lymph node epithelium (78 ± 21.07 vs. 20.56 ± 10.22 , $p = 0.029$) as detected immunohistochemically.

Our results further support the pivotal role of stromal density in the PDAC tumors microenvironment, as in those cases where immune evasion is permitted, stem cell migration is promoted.

PANGENFAM: The Spanish Familial Pancreatic Cancer Registry

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer death in the EU and the US. One of the keys to improving patient prognosis is an early diagnosis. Familial pancreatic cancer (FPC) is a genetic syndrome that increases the frequency of PDAC among the members of the family. An estimated 4-10% of pancreatic cancers diagnosed have a familial or hereditary background.

The Spanish familial pancreatic cancer registry (PANGENFAM) was established in 2009 by our research group. The main objective of the study is the genetic and phenotypic characterization of families with pancreatic cancer and the screening of high-risk individuals. It offers a screening program for healthy high-risk relatives, consisting of an annual MRI scan, echo-endoscopy, and blood CA19-9 analysis, which is followed by a biopsy if a suspicious lesion is identified.

The registry currently includes over 150 families and 350 familial PDAC cases and high-risk individuals, with an associated biobank of biological samples and clinical data. The establishment and maintenance of our extensive registry and biobank are thanks to a stable collaboration with basic scientists and clinicians from the departments of Medical Oncology, General Surgery, Gastroenterology, Radiology and Pathology, who diagnose and treat patients. We have shown that FPC can be attributed to pathogenic germline mutations/variants in DNA repair genes and that at the somatic level, these tumors are mainly KRAS mutation-negative and harbor tumor-specific mutations that are potential treatment targets in the clinic. We have demonstrated the benefits of screening by detecting and successfully treating 4 malignant pancreatic tumors early (3 PDACs and 1 neuroendocrine) since the program began in 2009. Over the years we have developed extensive international collaborations and have identified and validated new biomarkers for the early detection of PDAC in collaboration with international consortiums and the pharmaceutical industry. Furthermore, we are establishing *in vitro* organoid models for use in translational research to test new therapies in this subgroup of patients.

Our results can help provide an individualized risk assessment of pancreatic cancer, as we are a reference center for FPC screening in Spain and collaborate with international registries to establish standard protocols for high-risk screening. Furthermore, we are working towards a personalized medicine approach in FPC that will prolong survival and quality of life.

The Role of Mitochondria in Colorectal Cancer – Overexpression of Mitofusin 2 Changes Mitochondrial Dynamics

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The mitochondria of cancer cells differ from the mitochondria of healthy cells. Cancer cells have reprogrammed mitochondrial bioenergetics towards aerobic glycolysis (Warburg effect), one of the most important hallmarks of cancer. Tumor cells usually display different mitochondrial morphology and shortened mitochondria. Cells respond to energy needs and stress stimuli by adapting mitochondrial function accompanied by continuously changing mitochondrial morphology as a result of fission and fusion. Fission is executed by cytosolic dynamin-related protein 1 (Drp1), often activated in cancer cells. Mitofusin 1 and 2 (Mfn1, Mfn2) are responsible for mitochondrial outer membrane fusion and usually are downregulated in cancer cells. Cristae-shaping protein Opa1 is responsible for inner mitochondrial membrane fusion.

Our study aimed to monitor mitochondrial morphology, bioenergetic function, cell proliferation, and invasiveness of the tumor cells with upregulated Mitofusin 2. After retroviral transduction of colorectal cancer cell lines HCT116 and HT29 with recombinant gammaretrovirus, stable overexpression of the human *MFN2* gene up to 15-times was achieved, but the overexpression was not followed by changes in expression of genes *MFN1*, *OPA1*, and *DRP1*. Cell proliferation was not significantly decreased, but the proportion of apoptotic cells increased. The mitochondrial mass measured by MitoTracker DeepRed and the amount of mitochondrial DNA were unchanged, but the changes in mitochondrial membrane potential detected by MitoTracker Orange increased. This finding is in accordance with the increased production of ATP, pyruvate, and ROS in cells overexpressing *MFN2*. To evaluate the chemotherapeutic drug sensitivity, the inhibitory concentrations (IC₅₀) for three chemotherapeutics indicated for treating colorectal carcinoma (5-FU, oxaliplatin, irinotecan) were measured. The cells overexpressing *MFN2* were more sensitive to the treatment, indicating, that chemotherapeutics induce the reorganization of mitochondrial functions and morphology in tumor cells.

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DNA Methylation Changes in Tumor Progression

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Epigenetic modifications are heritable chemical or physical changes in chromatin. Three different epigenetic mechanisms associated with gene expression have been identified such as DNA methylation, chromatin modifications, and non-coding RNAs.

The most well-studied epigenetic modification is DNA methylation, which involves the addition of a methyl group directly to a cytosine nucleotide within a cytosine-guanine sequence (CpG), which are often surrounded by other CpGs forming a CpG island. DNA methylation regulates gene expression and shows the uneven genome-wide distribution. In normal cells, most CpG sequences in the genome are methylated, but CpG islands and the nearby CpG island shores (the region within 2 kb of the islands) are exceptionally hypomethylated.

In addition to various environmental and genetic risk factors, epigenetic deregulations are also associated with tumorigenesis. From the genome-wide studies, it became clear that the dynamic regulation of DNA methylation is a critical epigenetic mechanism of cancer initiation, maintenance, and progression. Cancer cells are characterized by aberrant DNA methylation pattern, the most widely observed features are global hypomethylation and site-specific hypermethylation. Promoter hypermethylation is frequently observed as an inhibitory molecular mechanism in various genes associated with DNA repair, cell cycle regulation, and apoptosis. Hypomethylation can be an early event in tumorigenesis and is frequently detected in benign hyperplasia. Loss of methylation is more prominent with tumor progression, and metastatic lesions possess greater demethylation than primary tumors.

Changes in DNA methylation were suggested to be useful biomarkers for diagnosis, and for the determination of prognosis and treatment response. Aberrant promoter methylations are targetable and prepare novel therapeutic options for personalized medicine in cancer patients.

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Gene Editing of Colorectal Cancer-Derived Cell Lines by CRISPR/Cas9 Method

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The study aimed to prepare knockouts in genes associated with the aggressive phenotype of cancer cells. We selected the aldehyde dehydrogenase 1 (ALDH1) enzymes, promising therapeutic targets in several tumors, including colorectal cancer (CRC). Their overexpression and high activity have been demonstrated in cancer stem cells (CSCs) of various cancers, indicating that they are involved in maintaining CSCs, their proliferation, detoxification, differentiation, and drug resistance by the oxidation of cellular aldehydes. Therefore, ALDH1, especially ALDH1A1, and ALDH1A3, provide drug protection and resistance of tumor cells to treatment (Angius et al., 2021; Poturnajova et al., 2021).

We confirmed overexpression of ALDH1A1 or ALDH1A3 in several cancer cell lines, including the primary cell line derived from the patient's rectal cancer tissue. Subsequently, we prepared knockouts in ALDH1A1 or ALDH1A3 gene. We utilized CRISPR/Cas9 method based on lentivirus transduction. We used one plasmid system – Cas9 and gRNA were localized on one plasmid. Transduced cells were selected based on puromycin resistance, and single cell-derived clones were prepared by a limited dilution. These populations were deeply characterized on genomic, transcriptomic, and proteomic levels. The western blot and the functional Aldefluor^R assay confirmed the phenotype of knockouts.

ALDH-knock out cell lines underwent several functional *in vitro* and *in vivo* tests and molecular expression analyses to confirm the role of ALDH in colorectal cancer development. Changes in several key pathways associated with cancer behavior, such as apoptotic, cell division, and metastasis were detected.

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Genetic Editing of *ALDH1* Is Linked to Distinct Cellular and Molecular Properties in Colorectal Cancer

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Aldehyde dehydrogenase (ALDH) isoforms 1A1 and 1A3 belong to intracellular enzymes with a broad spectrum of functions linked with an advanced stage of solid tumors and the stemness of the neoplastic cells. Both share the ability to detoxicate intracellular aldehydes and regulate tumor initiation and progression by triggering retinoic acid (RA) signaling and are related to the main molecular pathways responsible for tumor cells proliferation, chemoresistance, and stem cell properties.

A chemoresistant derivative of HT-29/EGFP cells obtained from lung metastasis referred to as FURiv-met was used in this study. The upregulation of the *ALDH1A3* gene was detected in this cell line and correlated to increased resistance and metastatic ability. Genetic knockout of *ALDH1A3* in FURiv-met led to changes in chemoresistance, clonogenic potential, migration ability, and tumorigenicity. Molecular analysis by SurePrint G3 Human Gene Expression v3 microarray (Agilent) revealed the upregulation in stem cell markers *ALDH4A1* and *CD133*. Microarray showed significantly decreased expression of *ALCAM/CD166* in cells with *ALDH1A3* knockout, and subsequent flow cytometry analysis confirmed the downregulation of this adhesive protein, linked to poor CRC outcome and metastatic spreading.

The ability to form subcutaneous xenografts of cells with deleted *ALDH1A3* was increased compared to the parental FURiv-met cells. On the other hand, the capacity to develop spontaneous lung metastasis was proven in FURiv-met parental but not in the cells with knocked-out *ALDH1A3*.

We confirmed the critical role of *ALDH1A3* in colorectal cancer biology, resistance, and tumorigenesis. Therefore, we can assume that although this gene inactivation increased the tumorigenicity, the decline in metastatic ability, chemoresistance, and migration properties changed the disease phenotype to be more favorable for the patient.

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Pancreatic Lesions: The Spectrum of Syndecan-1 Expression

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Syndecan-1, a member of the proteoglycan subfamily, is involved in many processes including cell proliferation, migration, stromal interactions, and metabolic pathways. Recent evidence reports its cell surface localization to be important for disease progression as it is involved in essential cellular processes. Our aim was to compare the Syndecan-1 expression profile and its specific localization in various pancreatic lesions including pancreatic adenocarcinomas (PDAC), mixed adenocarcinomas with neuroendocrine carcinoma (MANEC), neuroendocrine carcinomas (NEC), neuroendocrine tumors (NET) and benign cases.

Syndecan-1 was studied immunohistochemically in FFPE pancreatic lesions from 146 patients, 87 men and 59 women with a mean age of 64.61 ± 0.86 , 75 histologically categorized as PDAC, 20 as NEC, 30 as NET, 7 as MANEC, and 13 as benign. The staining pattern of Syndecan-1 was evaluated in lesions' epithelial cells, its corresponding desmoplastic stromal cells and compared with the matching normal adjacent tissue (NAT) epithelium and stroma. Cases were scored according to the intensity and immunoreactivity of their expression into three separate groups: weak or no; intermediate; intense expression and data were statistically analyzed using SPSS 28.

In NAT epithelial expression of Syndecan-1 was detected in 39 cases (26.7%) with 20 being membranous, 11 cytoplasmic, and 8 presented expression in both. In the corresponding lesions, 54 (36.9%) were scored as positive; 23 in the cell membrane, 12 in the cytoplasm, and 19 in both. Membranous protein expression was enhanced in lesions' epithelial cells compared to NAT ($p < 0.001$). In between groups, PDAC and MANEC cancer epithelium presented increased Syndecan-1 expression compared to NET ($p < 0.001$; $p = 0.003$, respectively) and NEC ($p = 0.04$; $p = 0.04$, respectively). In NAT stroma Syndecan-1 expression was only found in the cell membrane of benign lesions, NET and NEC lesions whereas, in PDAC cases NAT stromal expression wasn't detected ($p = 0.026$; $p = 0.026$; $p = 0.032$, respectively). In contrast, the lesions' stroma presented only cytoplasmic expression. Still, no significant difference was found between the different groups. In all cases, patients presenting increased Syndecan-1 expression seems to have a shorter overall survival still only in the case of the NEC group of patients reached statistical significance ($p = 0.014$).

Our study revealed that Syndecan-1 expression is not restricted to the cell membrane and that is significantly increased not only in PDAC but also in other pancreatic lesions such as NET, NEC, and MANEC. Furthermore, it seems possible to have a prognostic value, as in the present study higher expression was associated with shorter overall survival.

Surgical Viewpoint of Neoadjuvant Therapy in Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma is one of the leading causes of cancer-related death worldwide with growing incidence predominantly in developed countries. Treatment options are often limited by late clinical manifestation further promoted by the absence of effective screening programs. However, thanks to the progress in the field of chemotherapeutic regimens, as well as advances in surgical techniques and perioperative care, patients that would once be deemed borderline resectable or unresectable, may be offered a chance to undergo surgical treatment as the only potentially curative option. Current evidence is suggesting the change in the therapeutic plan paradigm with upfront surgery followed by postoperative chemotherapy into a neoadjuvant setting in order to deliver the oncological treatment to a bigger number of patients as we know that pancreatic surgery is burdened with a high morbidity rate, which may prevent patients from reaching adjuvant therapy. Furthermore, we are able to test the biological behavior of the disease, achieve systemic control with the downsizing of the tumor mass, and offer a higher chance of R0 resection. This change however brings new questions into the spotlight as to what is the effect of the neoadjuvant treatment on the surgical approach and postoperative complication rate in a surgery that is already moribund for some.

Periostin as a Marker of Chronic Kidney Disease Progression

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The global estimation of chronic kidney disease (CKD) prevalence increased by almost 30% in the last decades [1]. A dramatic increase in mortality due to CKD is associated with the tight connection of the renal system to the cardiovascular system and impaired kidney function is an important factor of cardiovascular disease burden [2]. CKD may lead to complete loss of kidney function combined with cardiovascular complications resulting in premature mortality even before life-saving transplantation [3].

The onset of CKD is preceded by kidney fibrosis. In recent years, efforts have been made to identify novel targets participating in renal fibrosis development. Periostin has been identified as a candidate protein marker of kidney injury. Its overexpression has been linked to various CKD etiologies such as diabetic nephropathy and graft rejection. Potential exploitation of periostin as a renal injury marker is based on observations that expression of periostin is absent in healthy adult kidneys and only in case of injury expression of periostin is being activated.

To analyze and more precisely estimate the role of periostin in the renal fibrotic process, a murine model of unilateral ureteral obstruction (UUO) will be used, which serves as a well-characterized model of renal fibrogenesis reflecting human CKD. During 1-, 7-, 14-, and 21-day time-course of UUO, expression of periostin on mRNA and protein levels will be determined in both, ligated as well as non-ligated kidney. Histology and immunohistology will be used for the detection of periostin localization in the renal tissue as well as the estimation of the renal fibrosis grade.

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Glutamate Oxaloacetate Transaminase Nanoparticles: A Promising Novel Therapy for Ischemic Stroke

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Brain stroke is one of the world's leading causes of death and disability, and therapeutic treatment with enhanced efficacy is urgently needed. During a stroke, there is an increase in the extracellular concentration of glutamate, the main excitatory neurotransmitter in the brain. Excess stimulation of glutamate receptors leads to excitotoxicity, substantial neuronal death, brain damage, and loss of neurological functions. Administered glutamate-oxaloacetate transaminase (GOT) facilitates the degradation of glutamate in the blood and in turn an increased efflux of excess glutamate from the brain followed by a reduction of the ischemic lesions and improved recovery. One major shortcoming is that the effect of administered GOT is short-lived.

The aim of the EuroNanoMedIII project GOTTARG, Glutamate Oxaloacetate Transaminase Nanoparticles targeted to the Brain for Neuroprotection in Ischemic Stroke, was to develop, in a safe-by-design (SbD) approach, bio-conjugated nanoparticles of GOT, GOT-NP, with increased therapeutic half-life and a ligand for targeting GOT the blood side of the BBB, for enhanced neuroprotective efficacy.

The safety of GOT-NP was assessed *in vitro*, with the main emphasis on genotoxicity, which is a crucial endpoint and required for regulatory risk assessors. Toxicity testing showed that GOT-NP did not induce toxicity by cell death in neurons (human neuroblastoma SH-5YSY and mouse hippocampal HT-22 cells) or astrocytes (human 1321N1 cells), and did not induce genotoxicity, applying a test battery for detection of several endpoints: DNA strand breaks by the enzyme-modified version of the comet assay and γ -H2AX assay, mutations by the mammalian HPRT gene mutation test and chromosomal damage by the micronucleus assay. For mechanistic studies, an *in vitro* model of ischemic stroke, based on exposure of neurons and astrocytes to oxygen and glucose deprivation (OGD), was applied to test the neuroprotective effect of GOT and GOT-NP. GOT-NP showed enhanced efficacy compared with GOT and gave significant neuroprotection. The protective effect was also seen after exposure to glutamate to induce excitotoxicity.

GOT-NP showed enhanced and more sustained neuroprotective effect compared with GOT and turns out to be a promising candidate for new therapeutic treatment of stroke. Our data calls for further pre-clinical testing with the aim of generating new treatment strategies for brain stroke in humans. This work provides a new perspective on the treatment of brain injuries and offers new opportunities for using therapies based on polymer-modified proteins.

Animal Models for Gastrointestinal Cancer

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Colorectal cancer (CRC) is the second most common cancer in both sexes in Europe. Animal models represent one of the essential tools in preclinical cancer research. Since using carcinogen-induced rodent models in 1928, many animal models for CRC were established. An ideal animal model for CRC should recapitulate the changes from a precancerous adenoma to an invasive carcinoma with metastatic potential and imitate the genetic alterations. There is plenty of models to choose the best one. Carcinogen-induced model is easily administrated and can mimic carcinogenesis from early stages to metastasis growth. Allograft/xenograft models are often used for preclinical research of drugs and a better understanding of molecular mechanisms of cancer. The tumor cells can be engineered *in vitro* before administration. Allograft models require syngeneic cells and mouse strain, enabling the study of the tumor microenvironment and cancer-immune cell interactions. For the research performed on human cell lines or patient-derived xenografts (PDX), immunodeficient mice are necessary. PDX model better mimics the biology and heterogeneity of different cancers and recapitulates the tumor microenvironment. Recently, humanized mice have become more and more popular in preclinical research. This is because they enabled the study of human-derived cells in the context of the human immune system. Genetically engineered mice carry desired altered gene/s. These models can form tumors within an immunocompetent environment and are used to study specific molecular pathways in CRC. Each model has its limits and advantages. It is essential to know them to find the most suitable model for particular research. Improving *in vivo* models is critical for studying human disease etiology and drug development. Each animal model has some limitations, leading to the evolution of new, more efficient animal models.

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Role of Epigallocatechin-3-Gallate in Colorectal Cancer Chemoresistance

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The research was focused on testing the effects of the polyphenolic compound epigallocatechin-3-gallate (EGCG) on colorectal carcinoma (CRC) - derived cells including chemoresistant cell line HT-29/NLR/FUR. The EGCG is the major catechin in tea. Recently, it has been proven that EGCG carries the most significant biological activity mediated by green tea, including cancer chemoprevention.

The cells were cultivated in the presence of various concentrations of EGCG and 5-fluorouracil (5-FU) and their combination to evaluate proliferation via the IncuCyte® ZOOM live-cell imaging system. The cancer cells showed significant differences in the rate of cell division in a concentration-related manner of EGCG and 5-FU. By quantitative PCR (qPCR), we demonstrated significant downregulation of gene expression for the ATP binding cassette (ABC) transporter superfamily genes (MDR1, ABCC4, and ABCC5) in the presence of EGCG. This downregulation was present only in the chemoresistant HT-29/NLR/FUR cell line. Cultivation in the presence of EGCG also led to decreased expression of aldehyde dehydrogenase (ALDH) enzyme expressions (isoforms 1A1 and 1A3). We have shown that EGCG sensitizes chemoresistant cells to 5-FU, and induces apoptosis, as demonstrated by Annexin V assay.

The combined therapy of EGCG and 5-FU could be beneficial for patients suffering from refractory cancer in the future.

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Roadmap for Translating Early Effects Biomarkers Results into Clinical Practice: From Observational Studies to Randomized Controlled Trials

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According to the definition delivered by the WHO, a biomarker, independent from its role that may be indicative of exposure, response, or effect, is inevitably linked to a clinical outcome or to a disease. The presence of a continuum from early biological events to therapy, and the prognosis is the unifying mechanism that justifies this conclusion. Traditionally, the technical and inter-individual variability of the assays, together with the long duration between early pathogenetic events and the disease, prevented clinical applications to these biomarkers. These limitations became less important with the emergence of personalized preventive medicine because of the focus on disease prediction and prevention, and the recommended use of all data concerning measurable patient's features. Several papers have been published on the best validation procedures for translating biomarkers to real life. The history of cholesterol concentration is extensively discussed as a reliable example of a biomarker that - after a long and controversial validation process - is currently used in clinical practice.

The frequency of DNA damage associated with genomic instability is a reliable biomarker for the pathogenesis of cancer and other non-communicable diseases, and the link with clinical outcomes is substantiated by epidemiological evidence and a strong mechanistic basis. Available literature concerning the use of the micronucleus assay and the Comet assay in clinical studies is discussed, and a suitable three-levels road-map driving this biomarker toward clinical practice is presented. From the perspective of personalized medicine, the use of these assays can play a decisive role in addressing preventive and therapeutic strategies for chronic diseases. In many cases, they are either currently used in clinical practice or classified as adequate to consider translation into practice. The roadmap to clinical validation of early effects biomarkers finds inspiration from the history of biomarkers such as cholesterol, which clearly showed that the evidence from prospective studies or RCTs is critical to achieve the required level of trust from the healthcare profession.

Recent Updates in Risk Assessment of Nanomaterials

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There is a great increase in the use of engineered nanomaterials (ENMs) in virtually every area including health care and medicine. The safety of ENMs depends on an intimate knowledge of their physicochemical properties and their interactions with target biological systems. Though principles established for standard chemicals apply also to ENMs, nano-specific considerations must also be addressed: characterisation of pristine ENMs, dispersion method, characterization of ENMs in exposure medium, and demonstration of uptake to prove that cells are in contact with ENMs. The standard approach in risk assessment relies on animal experimentation which is time consuming, not economical or ethical- and extrapolation from animals to humans is challenging. Therefore, to facilitate fast and efficient hazard assessment and to understand the complexity of potential ENM risks there is a shift from traditional risk assessment to more complex and holistic approaches. However, specific OECD test guidelines (TGs) for hazard assessment of ENMs (or modification of existing TGs), and new advanced models, are missing.

The aim of this presentation is to give an overview of recent developments in risk assessment of ENMs with specific focus on development of TGs/DGs and new advanced methodologies (NAMs) through achievements of recent H2020 projects.

Several tools to better predict the impact of ENMs on human health and the environment have been developed for a more holistic ENM safety policy. Significant efforts towards standardization and validation processes for ENMs have been undertaken through the evaluation, optimization, and pre-validation of standard operating procedures (SOPs) or TGs, with a series of inter-laboratory studies. Sets of SOPs for physicochemical characterization, and human and environmental hazard assessment, have been adapted for testing ENMs. Additionally, new advanced skin, lung, liver and other models and high-throughput methods are being developed and standardised. RiskGONE and several other European projects have contributed to the development of standardised and validated methods for hazard assessment of ENMs and their implementation in the next generation of risk assessment (NGRA) without animal testing. The most promising methods will be proposed for OECD TGs/DGs.

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Studying DNA Damage and Repair: A Retrospective

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It is almost 50 years since I started to study DNA damage and repair. At first, I worked with cultured hamster or human cells, and measured DNA breaks by alkaline sucrose sedimentation, soon superseded by alkaline unwinding. This method involves incubation of cells in alkali, which causes DNA to denature; the strands start to unwind from the ends of the molecule, or from any breaks, so after a set time there is a mixture of single and double stranded DNA; the % of ssDNA reflects the break frequency. It is a laborious technique, but using it we were able to demonstrate – against the prevailing opinion – that nucleotide excision repair of UV-induced damage could be blocked with standard inhibitors of DNA replication, which caused incision events, normally transient, to accumulate. In the late 1980s, I was introduced to the comet assay, and realized that it could be applied to the study of DNA damage and repair in human blood cells. We modified it to measure not just DNA breaks, but also oxidized bases; in addition, we measured breaks introduced by hydrogen peroxide, as an indicator of antioxidant status. With those tools, we carried out the first of many human trials – testing the ability of dietary antioxidants to decrease DNA oxidation in lymphocytes. Later, we developed a comet-based *in vitro* repair assay, in which a cell extract was incubated with a substrate of nucleoids containing specific base damage. Individual DNA repair capacity varies widely; is this because of intrinsic (genetic) differences, or because – for instance – exposure to DNA-damaging agents can influence repair capacity? Nutritional status may be important; a trial with kiwifruit supplementation showed, as well as decreased DNA damage, enhanced base excision repair. I will discuss various issues relating to the significance of DNA damage and repair – and I shall stress the importance of making our findings known not just in the scientific community but also in the real world.

***In Vitro* Cyto- and Genotoxicity of CeO₂, TiO₂, and Ag Nanoforms: The Role of Physical Properties**

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The continuous search for improved or specific properties of materials results in a large production of nanoparticles (NPs) and nanomaterials (NMs) with altered and diverse characteristics. As a consequence, a high number of distinct nanoforms exist i.e., in this context, NMs from the same substance but with different physical features. It is known that the chemical composition is not the only property determining the toxicity of NMs, but size, agglomeration/aggregation state, z-potential, etc. play also an important role. A full understanding of the impact of NMs' physicochemical properties in mediating biological effects is still missing. This is partly due to the fact that distinct nanoforms are often referred to under the same NM identifier (e.g. TiO₂ NP), and/or diverse experimental conditions are used in different studies, making it difficult to compare the data reported in the literature.

In this study, we compared in the same experimental setting three distinct nanoforms for each of the extensively used metal oxide NMs, TiO₂, CeO₂, and Ag, for a total of nine nanoforms. The tested nanoforms (namely 10x10nm CeO₂ stamps, 50 nm CeO₂ agglomerates, 3.5 nm CeO₂ NPs, TiO₂ nanorods, 50 nm TiO₂ aggregates, 8 nm TiO₂ NPs, and 20 nm Ag NPs, 50 nm Ag NPs, Ag nanowires) were thoroughly characterized for their intrinsic physical properties i.e. shape, size distribution, hydrodynamic diameter, and surface charge. The *in vitro* toxicity was investigated on the human lung epithelial cell line A549. Cells-nanoforms interaction was assessed by TEM imaging of exposed cells. Cytotoxicity was assessed by applying three methods based on diverse principles and cell functions, the Alamar blue (AB) assay, the electric cell-substrate impedance sensing (ECSIS), and the colony forming efficiency (CFE). Genotoxicity was assessed by the standard alkaline comet assay and the Fpg-modified version, to detect strand breaks and oxidized base lesions respectively.

The results showed that distinct nanoforms have diverse potency in eliciting cytotoxicity, while only one nanoform resulted to be genotoxic. The three cytotoxicity methods gave consistent results, although a difference in the sensitivity of the assays was observed. This study highlights in a simple and direct way the role of the NMs' physicochemical properties in eliciting biological responses, and the importance of a thorough characterization of these properties when investigating and reporting toxicological data, in order to properly identify diverse nanoforms.

The Norwegian Research Council, NanoBioReal project (288768)

Human Induced Pluripotent Stem Cell-Derived Alveolar Epithelial Cells as Promising Cell Source for *in vitro* Lung Models

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Human induced pluripotent stem cell (hiPSC)-derived lung cells are a promising cell source for reliable *in vitro* models in drug development or toxicity screening. Since simple cell lines do not exactly represent lung physiology and primary cells are rare to get and limited to cultivate, alternative physiological cell sources are highly demanded [1].

To date, hiPSC can be differentiated into several lung cell types and organoids with varying outcomes [2–5]. The success of differentiating hiPSCs into lung cell types depends on the quality of mimicking (a) complex embryonic developmental and (b) cellular environment including interactions with extracellular matrices *in vitro*. Properties, which can rather be achieved by three-dimensional (3D) than two-dimensional (2D) culture conditions [6]. However, from existing protocols it remains unclear, if differentiation into alveolar epithelial cells (AECs) from hiPSC is limited to 3D conditions or whether 2D approaches are also suitable for the generation of AECs. Additionally, it is not known so far, if 2D- and 3D-differentiated AECs from hiPSC differ in their gene and protein expression pattern regarding specific lung markers during the embryonal lung development simulated under *in vitro* conditions.

A reproducible protocol suitable for both 2D- and 3D-differentiation of the predominate cells of the alveoli, type1 and type 2 AECs (AEC1 and AEC2), has been established to perform a comparative analysis in five different hiPS cell lines (from healthy and Cystic Fibrosis donors) within five developmental stages of the lung. A dynamic suspension bioreactor was used to differentiate AECs under shear stress and homogenous nutrient supply, whereas 2D-differentiated cells were generated under standard static conditions.

Significant differences between 2D- and 3D-conditions, especially on gene expression level during the first embryonal stages, but also in the final AEC stages could be determined. Moreover, strong differences in the gene expression of lung surfactant proteins could be revealed in comparison to the adult human lung.

Finally, the gained knowledge helped to identify the 3D-bioreactor-based differentiation approach as a promising method to generate high amounts of AECs for alveolar *in vitro* models for drug development and toxicity screening, but also revealed that even simple 2D-differentiation approaches are suitable to generate AEC types from healthy and disease hiPS cell lines.

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Biological Safety of Innovative Nanocomposites with Therapeutic Potential

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Recent development and advanced methods in regenerative medicine are trying to invent suitable materials that can restore and reproduce the favorable and natural environment needed for skin regeneration. The development of multifunctional materials for wound treatment with the ability to provide multiple functions at once is crucial for clinical application. The utilization of nanohydrogels in regenerative medicine provides an innovative way to treat skin injuries.

As it is important to evaluate the biosafety of nanohydrogels as a degradable biomaterial for use in the biomedical field, the aim of this study was to determine the toxicity of newly prepared nanocomposites. The model system represents different types of skin cells, keratinocytes (HaCaT), fibroblasts (HFF-1), and a 3D skin *in vitro* model (MatTek). The experiments were focused on determining the cytotoxic and genotoxic effects of nanocomposites and their individual components in *in vitro* conditions, also their effect on skin structure was determined. Three hydrogels (Alginate, Pluronic F127, and GelMA) with different chemical compositions and iron oxide nanoparticles were used for nanohydrogel build-up.

Results of toxicity after 24 h nanohydrogel exposure, measured by LDH assay, micronucleus test, and comet assay showed a significant increase in both cytotoxicity and genotoxicity in higher concentrations of GelMA nanohydrogel. We did not observe any toxic effect in the two other nanohydrogels. Subsequently, we used an Fpg-modified comet assay to determine if this DNA damage is caused by base oxidation based on the GelMA hydrogel polymerization procedure, however, we did not observe any. We assume that DNA damage is a result of single and/or double-strand breaks incurred as the attempted repair of UV radiation-induced base damage in DNA. From the results of the micronucleus test, we noticed a higher amount of apoptotic and necrotic cells after GelMA exposure, also the presence of micronuclei was significantly higher. Using histological staining we determined that nanohydrogels exposure did not cause any pathological changes in skin structure.

These results suggest that above mentioned hydrogels loaded with iron oxide nanoparticles could be promising candidates for wound dressings as they do not show any toxic effects, but further investigation is essential for their implementation in practice.

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Microfluidic Platform for *In Vitro* Toxicity Testing

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During the last years, microfluidic technology has gained more and more importance in the field of chemicals and drug testing. Since microfluidic devices and systems can be used for high-throughput screening applications and integrated with automated systems, this technology provides valuable tools for a broad variety of animal-free test approaches for cost-efficient and fast risk assessment of substances. These new tools are of special interest for the pharmaceutical industry, for the safety assessment of chemicals within the frame of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemical Substances) regulation and for the implementation of the safety-by-design-concept in the development of new materials.

We present a microfluidic platform for in vitro toxicity testing, which combines microfluidic modules for cell handling and cell cultivation with compact microscopes for optical analysis and electrodes for impedance measurements [1]. The platform uses two microfluidic modules, each of them comprising a silicon chip with a transparent membrane having a regular array of micro-holes. The chip is placed between two channels representing the apical and basolateral sides of the system. By applying gentle negative pressure in the basolateral channel, cells are positioned on the microholes on the apical side of the membrane. This set-up allows for the analysis of single cells as well as for long-term cultivation to obtain dense cell monolayers. Cell morphology and cell proliferation are monitored by compact microscopes with CMOS cameras underneath the fluidic modules. Integrated electrodes in the apical and basolateral channels of the fluidic modules are used for electrical measurements to assess changes in the single-cell array or in the cell layer. Test substances are transported to the cells on the membrane and the cytotoxic effects are investigated using impedance spectroscopy and live-cell imaging with bright field and fluorescence microscopy.

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Genotoxicity Assessment of Nanomaterials in Advanced Lung Models

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For the next-generation risk assessment (NGRA) of chemicals and nanomaterials, new approach methodologies (NAMs) are needed for hazard assessment in compliance with the 3Rs to reduce, replace and refine animal experiments. As part of this, the development of advanced *in vitro* models is needed for the genotoxicity assessment of nanomaterials.

The aim of this study was to establish and characterize an advanced respiratory model consisting of human epithelial bronchial BEAS-2B cells cultivated at the air–liquid interface (ALI), and to compare the model’s performance with a commonly used alveolar model consisting of A549 cells. The bronchial and alveolar models were prepared on permeable inserts both in monocultures and in co-cultures with human endothelial EA.hy926 cells. All four ALI models were exposed to an aerosol of nanosilver (NM-300K) in the VITROCELL® Cloud system, before further processing after 24 hours cultivation to measure cellular viability (alamarBlue assay), inflammatory response (enzyme-linked immunosorbent assay), DNA damage (enzyme-modified comet assay), and chromosomal damage (cytokinesis-block micronucleus assay).

We demonstrated that these advanced lung models are applicable for the genotoxicity assessment of NMs. Cytotoxicity and genotoxicity induced by NM-300K were dependent on both the cell types and model, where BEAS-2B in monocultures had the highest sensitivity in terms of cell viability and DNA strand breaks. This study brings important knowledge for the further development of advanced 3D respiratory *in vitro* models for the most reliable human hazard assessment based on NAMs.

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Analysis of Tissue Structural and Functional Changes Following Biodistribution and Accumulation of 10 nm Gold Nanoparticles with BSA Coating in Mice

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Nanotechnology enables the synthesis of a wide range of nanomaterials from which specifically gold nanoparticles are considered the most suitable material for biomedical research, medical and laboratory practice. In nanomedicine, they could play a fundamental role as carriers in targeted drug delivery systems that aim to increase the effectiveness of treatment while reducing the amount of drug administered and eliminating side effects. Despite the countless positive effects of gold nanoparticles resulting from their specific properties, their biological safety is still questionable, as they represent an exogenous material for the human body, and it is not well-known what consequences may occur when such material is accumulated in vital organs. The aim of this study was therefore to check whether there are any possible adverse effects of 10 nm gold nanoparticles coated with BSA on the structure and function of mouse tissues.

According to the results obtained from quantitative analysis at the mRNA level the tested nanoparticles are able to change expression patterns of genes related to the inflammatory process, oxidative damage and tissue fibrosis in the organ samples collected from mice exposed to a single intravenous injection of nanoparticles in 5% glucose solution at a dose of 1 mg (Au)/kg (mouse) both 1 and 30 days after treatment even though there are no visible structural changes in the cells and tissues of organs with a preferential accumulation of nanoparticles at these time points. Moreover, immunohistochemical analysis revealed a higher representation of fibronectin-positive cells in the spleen and kidney of mice exposed to nanoparticles for 30 days compared to control ones. The fact that this change can be captured just by immunohistochemistry *in situ*, but not quantitatively by western blotting, suggests that the pathological processes may appear later in time, and thus further research is needed, especially focusing on the effects of the tested nanoparticles over longer time periods.

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Developing Advanced Blood-Brain Barrier Models for Pharmacological Research

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The blood-brain barrier represents one of the hardest to overcome biological barriers for most substances, including pharmacological compounds. In order to develop and evaluate potential treatment substances used in a neurological context, reliable, robust, and advanced human blood-brain barrier models are an excellent tool for further pharmacological research. This is especially important in neurodegenerative diseases that can influence the blood-brain barrier directly, either in tightness or transporter expression.

The development of an *in vitro* blood-brain barrier model based on human induced pluripotent stem cells (hiPSCs) [1] opened the door to any number of advances in this context. Based on the renewable cell source of hiPSCs, *in vitro* models have been developed to include a number of improvements on the original protocol and to address specific needs. These improvements can focus on different areas and could be used in a congruent manner depending on the specific questions when it comes to the evaluation of potential pharmacological substances. For example, a better replication of the *in vivo* circumstances where exposure to serum proteins and cellular orientation is concerned [2] would be a good basis for the initial evaluation of any number of compounds in a pharmacological context and allow for high-throughput investigations. Disease-specific *in vitro* models are a useful tool for the targeted evaluation of compounds envisioned for the treatment of a specific neurodegenerative disease like Parkinson's or Alzheimer's disease. In contrast, a more advanced *in vitro* model incorporating microfluidic solutions to mimic blood flow [2] would be more appropriate for final preclinical evaluations as a precursor for clinical studies, as their more complicated structure makes high-throughput studies difficult. The advantages and limitations of these examples will be discussed in detail.

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***C. elegans* as a Model System for Assessing Neurotoxicity**

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Caenorhabditis elegans (*C. elegans*) is gaining favor as a new approach method (NAMs) in neurotoxicity research. Since its emergence as a genetic model organism, *C. elegans* has provided insight into aging and neurodegenerative processes. Neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease pose a serious healthcare challenge. The most critical risk element associated with neurodegeneration are aging and environmental factors that influence genomic instability, epigenetic alterations, mitochondrial dysfunction, and other hallmarks of aging. Understanding how these complex processes interact to affect aging and neurodegeneration is a major challenge. Simple model organisms such as *C. elegans* continue to be valuable tools for studying neurodegenerative diseases by providing new molecular pathways behind neurodevelopmental disorders and neurotoxins.

C. elegans has several advantages for studying neurodegenerative diseases, including the availability of transgenics containing genes encoding normal and disease-variant proteins at single- or multi-copy levels under neuron-specific promoters that limit expression to specific neurons. The anatomical transparency of *C. elegans* allows co-expressed fluorescent proteins to follow the course of age-related neurodegeneration in these animals. Moreover, a completely described neural connectome allows for more in-depth knowledge of the impact of neurodegeneration on organismal health and behavioral deficits.

Plate Reader Spectroscopy as a Fast and Low-Cost Alternative to Atomic Absorption Spectroscopy for Evaluating the Cellular Uptake of Nanoparticles

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The interest in utilizing nanoparticles (NPs) for biomedical applications increases the demand for simple methods enabling their reliable intracellular quantification. Knowledge of the intracellular concentration of NPs is especially important as most of the biological effects manifest in a dose-dependent manner.

The gold standard method for quantifying internalized NPs is considered ultrasensitive atomic absorption spectroscopy (AAS). Considering the limitation of metal-based NPs, AAS also requires lengthy sample preparation and handling, making it time and money-consuming. In this work, we report a reliable, fast, low-cost, and easy alternative method to AAS – plate reader spectroscopy (PRS), which offers an available option for everyday laboratory use without advanced instrumentation.

In this study were employed magnetite nanoparticles coated with sodium oleate and bovine serum albumin (BSA-SO-MNPs). Our results showed, that data of intracellular concentrations of BSA-SO-MNPs obtained with PRS are fully comparable to AAS results. Specifically, the intracellular concentration BSA-SO-MNPs in human alveolar A549 cells was determined by PRS and AAS in parallel, with a remarkable correlation coefficient of $R = 0.9914$.

Biological Activity of Newly Synthesized Thymol Derivatives on In Vitro Intestinal Model

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The incidence of colorectal cancer is increasing worldwide. The most effective treatment still remains the surgical removal of the affected part of the colon. However, research into natural substances is showing interesting results. One of the potential active substances is thymol. Thymol has a proven bioactive effect on colorectal cancer cells and reduces tumor growth and metastasis in mice. However, the disadvantages are its poor solubility in aqueous solutions, availability, absorption, etc. With the intention of reducing these negatives, new thymol derivatives (acetic acid thymol ester and thymol α -D- glucoside) have been synthesized.

Our study focused on the comparison of the bioactive effect of thymol and new derivatives on the HT-29 and HCT-116 cell lines - colorectal cancer cells - after 24 hours of exposure to the concentration scale. Antiproliferative activity (IncuCyte), cytotoxic (MTT) and genotoxic effect (Comet assay), and ROS formation (ROS-Glo H₂O₂ assay) were determined.

Results from IncuCyte ZOOM demonstrated that the application of higher concentrations of thymol and derivatives significantly slowed cell growth. In the case of determining the cytotoxic effect of MTT, acetic acid thymol ester was the most effective for both cell lines. However, the second of the tested derivatives was effective only at a higher concentration than the standard - thymol. Comet assays have also shown a significant increase in DNA damage for the newly synthesized derivatives even at non-cytotoxic concentrations. The HCT-116 cell line showed higher DNA damage values than HT-29. ROS analysis was performed with a commercially purchased bioluminescence kit. Thymol did not cause any increase in ROS in the studied cell lines. For the derivatives, we observed significant increases when concentrations were applied that induced both cytotoxic and genotoxic damage. No significant difference in the response to ROS generation was observed between the tumor cell lines.

Our results confirmed the theory that newly synthesized thymol derivatives may act more efficiently and at lower concentrations than thymol depending on the chemical structure. Acetic acid thymol ester has the potential to act more effectively on colorectal cancer cells at much lower concentrations than thymol. The pairing of two thymol derivatives demonstrated that acetic acid thymol ester induces both cytotoxic and genotoxic damage to colorectal cancer cells at very low concentrations.

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Anti-fibrotic Effects of Silymarin and Silymarin-Coated Gold Nanoparticles Against Hepatic Fibrosis in Mouse

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Worldwide, about two million deaths per year are caused by chronic liver disease making it the 11th leading cause of death. Together with liver cancer, the chronic liver disease accounts for 3.5% of all deaths. Liver cirrhosis is also the most common cause of death in Slovakia (age group 35-44) [1]. Chronic liver disease mostly progresses from liver fibrosis. Reduction of hepatic fibrogenesis is therefore one of the key approaches to the treatment of chronic liver disease. For this reason, research focusing on the development of new antifibrotic drugs is of great importance. One of the suitable candidates for the treatment of liver fibrosis is considered **silymarin** - an extract from Milk thistle plant (*Silybum marianum*).

Silymarin has been traditionally used for centuries for its medicinal properties, particularly for liver-related conditions. For its potential therapeutic effects on liver diseases such as hepatitis, cirrhosis, and non-alcoholic fatty liver disease, silymarin has been intensively studied. Hepatoprotective properties of silymarin in protecting liver cells from damage and promoting their regeneration have already been confirmed [2, 3], however, further research is needed to fully understand the mechanisms of silymarin anti-fibrotic activity and determine its optimal dosing as well as potential interactions with other medications or effect on other organs.

Gold nanoparticles represent a promising option for drug-delivery vehicles due to their unique physicochemical properties and biocompatibility. With their efficient delivery of drugs to the liver, gold nanoparticles are thought to improve silymarin efficacy.

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