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Deliverable 3.5: Training activities update

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VISION

Basic information

Project title	Strategies to strengthen scientific excellence and innoVation capacIty for early diagnoSIs of gastrOintestinal caNcers
Project acronym	VISION
Call	H2020-WIDESPREAD-2018-2020
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Project type	Coordination and Supporting Action (CSA)
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Nature	**R** (Document, report - excluding the periodic and final reports)
Dissemination level	**PU** (Public, fully open, e.g. web)

Executive summary

This deliverable D3.5 is an update on training activities performed within task 3.1 in the second period of the VISION project implementation. Short-term academic stays of early-stage researchers in partners' laboratories allowed the applicants to improve and acquire new scientific and technical skills in various cellular, molecular, and genetic techniques involved in basic and clinical research. Moreover, they had the opportunity to strengthen their personal and professional development in gastrointestinal cancer and nanobiotechnology. The involvement of early-stage researchers in such scientific events enhanced the reputation, credibility, attractiveness, and networking activities of BMC SAV at both international and national levels. Besides BMC SAV, also other VISION partners sent their early-stage scientists for short-term academic stays to partner laboratories.

The coronavirus pandemic delayed the performance of training activities in 2020. In order to overcome these objective limitations in implementing the planned actions, and if it was possible, the training activities were replaced by theoretical courses and e-lectures (D3.1 Training activities). After the travel restrictions were removed, the first short-term early-stage researchers exchanges started in 2021, and the last academic stay finished in June 2023. At the 2nd Partner Assembly meeting in September 2021, the partners agreed to fully open all in-person training activities since January 2022. In total (2020-2023), 22 in-person training activities have been realized, and 28 early-stage researchers have participated. Some applicants were interested in more than one training.

1 Description of work & main achievements

To apply for the training event(s), each early-stage researcher had to submit the following documents:

- a motivation letter on the VISION template
- a support letter from the home institution (with signature)
- a full CV
- the acceptance letter from the hosting organization/host supervisor

For training activities intended for medical students, it was necessary to provide an additional:

- a copy of the Immunization Form
- proof of enrolment in their Medical School or a copy of their Degree or their Professional Medical License
- a Malpractice insurance

The Steering Committee members assessed all applications. After the approval of the applications, each applicant was informed about the final decision by a Letter of approval.

After the return from the academic stay, each participant had to provide the following:

- a Final report on the VISION template
- Annotation with photo(s) from the academic stay to be published on the VISION website

In 2020 four training activities - #1 -4 - were realized in-person at BMC SAV. The participants in these training activities were new Ph.D. students, and early-stage researchers, from BMC SAV. More details about the number of participants and the specific day of the events are available in the deliverable D3.1 Training activities.

1.1 Planned in-person training activities – 2021-2023

1.1.1 Training 1 "Advanced in vitro models" (lead BMC SAV)

Cell lines are an irreplaceable tool in cancer research. Many studies have been performed on adherent (2D) in vitro cultures which possess many limitations, including affected cell-cell and cell-microenvironment interactions, changes in cell morphology and polarity, and the absence of nutrient and oxygen gradients. Three-dimensional (3D, spheroid) cultures have overcome some of the mentioned limitations, but they do not recapitulate the heterogeneity of the original tumour.

Tumour organoids recently represent the most relevant *in vitro* model for studying tumour biology and testing therapeutic regimens. These self-organizing and self-renewing microtissues recapitulate the characteristics of original tumour tissue, such as complex organization, tissue-specific functions, and phenotype.

The aim of the course was to acquaint the participants with the preparation of 3D multicellular aggregates and their use in the assessment of treatment efficacy. The second part focused on preparing organoids from colorectal cancer patient-derived xenografts (PDXs) and training the method of tissue dissociation, organoids plating and sub-culturing.

1.1.2 Training 2 "Epigenetic analyses – DNA methylation" (lead BMC SAV)

DNA methylation is one of the key epigenetic mechanisms which plays a significant role in the regulation of gene expression. It is associated with a number of key processes, including genomic imprinting, X-chromosome inactivation, and repression of transposable elements. Aberrant epigenomic landscapes have been implicated in ageing, autoimmune diseases, and carcinogenesis. Quantitative analysis of DNA CpG methylation helps to elucidate its role in clinical pathologies. Pyrosequencing is a method based on a sequencing-by-synthesis approach originally designed to quantify single-nucleotide polymorphisms. Bisulfite modification permits the utilization of this method for the measurement of DNA methylation locally and globally in real time. The methylation pattern of DNA has the potential to serve as an early detection marker and a potential drug target. The course "Training in epigenetic analyses – DNA methylation" allowed participants to understand and perform all steps of DNA methylation analysis, including DNA extraction, bisulfite modification, PCR amplification and pyrosequencing reaction. Importantly, bioinformatic analyses, primer design and data analysis were also presented and trained.

1.1.3 Training 3 "qPCR and gene expression" (lead BMC SAV)

The synthesis of mRNA or gene expression is a critical part of protein synthesis. It is an area of active research that aids in the understanding of numerous biological pathways and diseases. Quantitative PCR (qPCR) provides information about the amount of mRNA present in the sample. Moreover, qPCR can be used for a wide number of applications including microRNA and non-coding RNA analysis, genetic variation and mutation detection. Course "INTRODUCTION TO RT-PCR" aimed to provide participants with all the information necessary for the successful implementation of the qPCR method and subsequent evaluation of the results. An introduction was directed to users not yet fully familiar will qPCR who wish a deeper understanding of the fundamentals. The following steps focused on all aspects of sample preparation, reverse transcription and different quantification strategies, such as comparative quantification. The course enabled participants to understand the principles of

designing an efficient real-time PCR assay, interpreting real-time PCR data and troubleshooting real-time PCR assays.

1.1.4 Training 4 "Cellular stress response" (lead BMC SAV)

One of the important parameters for the evaluation of nanomaterial toxicity in biological samples is the determination of cellular actin cytoskeleton changes. Cytoskeleton actively reflects mechanical interactions of cells with other cells or with the cell environment. In certain cell types, the cell shape is closely linked to the cell function, and disturbances in cell shape may lead to the loss of this function. As ann example can serve renal podocytes – cells ensuring proper blood filtration in the kidneys. Even slight changes in their cytoskeleton may lead to non-functional podocytes and proteinuria. Actin is a highly conserved cytoskeletal protein, and the actin filament network visualized by phalloidin is commonly used to detect actin cytoskeleton remodeling. Immunocytochemistry from protocol design, preparation, and stimulation of cells with nanoparticles, staining with fluorescently-labeled phalloidin, and visualization of samples by fluorescence microscopy was the scope of this workshop.

1.1.5 Training 5 "Advanced co-culture intestine model" (lead FhG)

The intestine is an important barrier for humans to absorb essential substances. *In vitro* intestinal models are used to study the transport and biological effect of substances, chemicals and active agents across or at the barrier. In the organism, the intestinal tissue consists of several cell types with a three-dimensional structure. In this training, the participants learned to set up and cultivate a three-dimensional co-culture of an intestinal model and to test the change in cell viability (fluorescence-based assay for cell viability determination) and barrier functionality (determination of the transepithelial electrical resistance TEER) after exposure to chemicals. After the performance of the cultivation, exposure and assays, the participants were trained in data analysis, data presentation and interpretation.

1.1.6 Training 6 "*in vitro* 3D cell cultivation and stem cell differentiation" (lead FhG)

Induced pluripotent stem cells (ipSCs) are pluripotent stem cells created by the artificial reprogramming of non-pluripotent somatic cells. The transformation is triggered by externally stimulated expression of special genes (transcription factors) in the somatic cell, for which various techniques exist. iPSCs have a high medical potential, as research on them involves fewer ethical problems than research on embryonic stem cells. In addition, iPS cells specifically adapted to patients can be generated.
This training focused on the cultivation of iPSCs as well as the first differentiation steps in the direction of lung or endothelial cells.
Also, two cultivation techniques were compared (the adhesion model in plates and the 3D model in bioreactors). Culturing cells in bioreactors can affect the process of differentiation, depending on the source of ipSCs and the differentiation direction.
After being trained in the special cultivation techniques of ipSCs, the participants characterized the Embryoid bodies (EB). In contrast to monolayer cultures, the spheroid structures that are formed when ipSCs aggregate enable the non-adherent culture of EBs in suspension, making EB cultures inherently scalable, which is useful for bioprocessing approaches, whereby large yields of cells can be produced for potential clinical application.
In the last step of the training, the ipSC differentiation was started and characterized by immunohistochemistry.

1.1.7 Training 7 "Study of cellular biological processes using nanoparticle devices" (lead SERMAS/IRYCIS)

Ultrasounds (US) are non-ionizing mechanical waves with different biological effects in cells depending on the acoustic parameters, such as the wave intensity, frequency and treatment dose. External mechanical forces generated by Low-Intensity Ultrasounds (LIUS) could be an effective technology to prevent tumor progression, providing an alternative or complementary therapeutic strategy to improve cancer patient outcomes. During the training, students learned about the physical and biodynamic aspects of cellular displacement, migration, growth and wound healing under the effects of ultrasounds.

1.1.8 Training 8 "The use of the liquid biopsy in precision medicine" (lead SERMAS/IRYCIS)

The term liquid biopsy (LB) refers to molecules such as proteins, DNA, RNA, cells, or extracellular vesicles in blood and other bodily fluids that originating from the primary and/or metastatic tumor. LB has emerged as a mainstay in translational research and has started to become part of clinical oncology practice, providing a minimally invasive alternative to solid biopsy for real-time monitoring of a tumor via a minimally invasive sample extraction, such as blood. Sample collection, quality, and storage are crucial steps that determine their usefulness in downstream applications. The types of samples that are used for liquid biopsy studies include but are not limited to blood, urine, saliva, and stool samples. These fluids may contain different types of cancer-derived materials, including circulating tumor cells (CTCs), or fragments such as exosomes and cell-free circulating nucleic acids. During the training, the students extracted circulating free DNA and miRNA for downstream applications, such as mutation and methylation analysis with a statistical comparison of the results with clinical data.

1.1.9 Training 9 "Isolation and culture of tumor and stroma cells from primary tumors" (lead SERMAS/IRYCIS)

The growth of solid tumors is strongly influenced by their microenvironment, and some tumors are characterized by a large desmoplastic stroma that can make up more than 90% of the tumor mass. This stroma is a very complex tissue composed of infiltrating immune cells and an extracellular matrix (ECM) generated by cancer-associated fibroblasts (CAFs) that consists of collagen, desmin, fibronectin, and hyaluronic acid. Organoids are *ex-vivo* three-dimensional (3D) cultures that can be expanded *in vitro* to provide cancer models that recapitulate the characteristics of the primary tumor and help to overcome the limitation of the availability of primary tumor tissue. Although organoid propagation is technically challenging, it is very affordable and well-established using colorectal cancer tissue. In this course, students were shown the isolation of tumor cells and fibroblasts from primary tumors or normal tissues and the establishment and maintenance of primary cultures, both as 2D (monolayer) and 3D (spheroids or organoids).

1.1.10 Training 10 "The involvement of tumor microenvironment in cancer progression" (lead SERMAS/IRYCIS)

Several studies have attempted to inhibit the formation and maintenance of the stroma to increase tumor permeabilization. Targeting the stroma could represent an important advance in cancer treatment. Stromal cells in wounds and tumors, including fibroblasts and/or myofibroblasts, endothelial cells and inflammatory cells, are important regulators of the migration and proliferation of epithelial cells. The students are shown *in vitro* co-culture models and cellular techniques during the training to analyze the cross-talk among the tumor,

fibroblasts, endothelial and immune cells, and the extracellular matrix. Furthermore, they perform cell migration and proliferation studies in 2D and 3D co-cultures of primary tumor cells with stromal cells such as fibroblasts.

1.1.11 Training 11 "The use of mouse models in oncology research" (lead SERMAS/IRYCIS)

Genetically engineered and PDX mouse models are powerful tools in translational cancer research, particularly to assess response to therapy. A fragment of the primary tumor (either from a resected specimen or a biopsy) is engrafted into the flanks of an immunocompromised mouse or implanted orthotopically (in the same organ of origin) and allowed to grow. The primary tumor cells can be mixed with an extracellular matrix such as matrigel or implanted directly. During the training, students learned about the development of xenograft and orthotopic mouse models to study tumor development, treatment strategies and the metastatic process.

1.1.12 Training 12 "Flow cytometry analysis of tumor biomarkers" (lead SERMAS/IRYCIS)

Flow cytometry and cell sorting are important aspects of studying tumor heterogeneity and cell enumeration. Several membrane (live or fixed cells) and intracellular markers (fixed cells) can be applied to the same sample to study different cell populations within the tumor and the surrounding stroma. This inlcudes tumor cells, fibroblasts and immune cells. Furthermore, exosomes can also be studied based on surface markers with more sophisticated flow cytometers. During the training, the students analyze and characterize tumor and stroma cells, exosomes and immune cells from dissociated primary tumors and blood samples.

1.1.13 Training 13 "Treating patients with GI tumors" (lead NKUA)

During the training, the trainee followed multidisciplinary meetings between surgeons, medical oncologists, and pathologists, aiming to design the best approach to obtain the best treatment for patients with GI cancer. The patient's willingness, the proposed surgical treatment if applicable, and the necessity and type of therapeutic approach required are presented and discussed. In addition, the trainee is present in the routine ward rounds. Furthermore, the trainee attends related literature review presentations during which high-input clinical papers on surgical oncology and the personal experience of experts in the field of GI cancers are presented.

1.1.14 Training 14 "Enrolling patients with GI tumors in research protocols" (lead NKUA)

During the training, the trainee is familiarized with the procedure of patient enrolment in clinical research projects. The definition of what clinical research means, the different types and what should be the aim are initially discussed. Other issues that were analysed were the assessment of risk for the participants by the investigator as well as the length and complexity of a study, as they represent important prerequisites for a successful implementation. In addition, major ethical rules are presented concerning the recruitment and retention of participants. The determination of inclusion and exclusion criteria are also reviewed. Shaping the informed consent is discussed. Finally, the organization of data, including patients' demographic data is considered to safeguard anonymity by being in line with GDPR.

1.1.15 Training 15 "Surgical operational procedures aiming to treat patients with GI tumors" (lead NKUA)

During the training the trainee attended surgical procedures related to the different types of GI cancer depending on the patients treated in the 2nd Department of Surgery, Aretaieio Hospital during the training period. Some of the procedures were Whipple's procedure, Hepatectomies, Oesophageal-Gastric cancer surgery, and laparoscopic colectomies.

1.1.16 Training 16 "Initiation in Medical Genetics and Genetic Counselling" (lead NKUA)

Over the last few decades the increased knowledge in the field of genetics in addition to the discovery of new rare conditions, the availability and complexity of new genetic tests, and new legislation, have led to the development of clinical genetic services, including genetic counselling. Therefore, the trainee was attending following the approval of the individuals of interest, the health service that provides information and support to people who have or are at high risk of developing genetic disorders. During the session, the trainee followed the meeting in which, by combining the detailed family and individual medical history, trying to estimate the genetic risk, and help them make informed decisions, and coordinate testing. Genetic counsellors spend extended time with the person/family to explain those details concerning risks, diagnosis and the impact of genetic test in a person's life, and therefore the trainee is familiarized with the necessity of a more holistic approach. The specific training was focused on familial cancer cases, and the spectrum depends on the group of patients/families interested in genetic counselling.

1.1.17 Training 17 "Exosome isolation methodology" (lead NKUA)

Exosomes are extracellular vesicles of endosomal origin that are between 30 to 100 nanometers in size. All mammalian cells that have been studied so far exhibit exosome-based communication. Exosomal contents can include proteins such as enzymes, mRNAs and ncRNAs, and lipids. Extracellular vesicles can be found in different body fluids including serum, plasma, urine, CSF, saliva, breast milk. Still, due to their size, their verification and isolation are a challenge. In the present training, exosomes were isolated both from serum and plasma. Initially, samples undergone three serial centrifugations (10min at 300g, 20min at 12000g, 30min at 10000g, and at 4°C) to ensure the removal of residual cells, cell debris, apoptotic bodies, and nuclei. Then exosomes were isolated by commercially available reagents, which by tying up water molecules, the selected reagent forces less-soluble components such as vesicles out of the solution, allowing them to be collected by short, low-speed centrifugation. Quantification was performed by ELISA by tracing and quantifying CD9 or CD63 proteins. Moreover, sizing distribution was detected by Nanoparticle Tracking Analysis using NanoSight. Exosome content analysis is performed by ELISA or RT PCR.

1.1.18 Training 18 "Circulating tumor cells isolation methodology" (lead NKUA)

In the 2nd Department of Surgery Training in isolation and characterization of circulating tumors. Enrichment of CTCs was applied through either the Metacell or Oncoquick systems following the manufacturer's protocol. The initial cell population isolated after the membrane filtration was 1 to 3 million cells, half of which proceeded to flow cytometry while the rest was reserved for culture and analysis on Day7, as well as storage at -80°C. About 500-2000 cells are run in each flow cytometry experiment, and the percentages of Cytokeratin (CK) and Vimentin (Vim) positive cells were evaluated. The cells were stained with Pan-cytokeratin and vimentin

antibodies so that both epithelial and mesenchymal cell populations or those which undergo Epithelial-Mesenchymal Transition (EMT) could be captured.

1.1.19 Training 19 "Protein expression analysis" (lead NKUA)

During the training, protein expression analysis was performed by immunohistochemistry, ELISA and flow cytometry.

Immunohistochemistry was applied to lesions and normal adjacent tissue specimens aiming to detect and evaluate the expression of protein markers associated with autophagy (LC3B, p62), senescence (p21), exosomes (CD9, CD63), stem cells (CD44) as well as other markers of interest which have been associated with cancer aggressiveness such as caveloin-1, vimentin, and syndecan-1. During the training, the trainee was trained to prepare all the necessary reagents and perform the whole procedure using standard laboratory equipment. Furthermore, the evaluation of both lesions and normal adjacent (NA) tissue concerning the expression profile of the protein was demonstrated by an expert pathologist focusing on the distinction between epithelial and stromal regions and membrane-specific or cytoplasm-specific staining for each protein.

The ELISA method was performed for various target proteins in serum or plasma samples derived from patients with GI. In some cases, exosome content was also analysed. The trainee had the opportunity to perform the whole procedure, count the ELISA plates and analyse the results using specific software.

Protein expression using flow cytometry analysis was applied either in isolated PBMCs from GI patients' blood samples or whole blood and in frozen tissue excised during surgical treatment either from the lesion (CT) or the corresponding normal adjacent tissue. Protein expression analysis using flow cytometry resulted in the characterization of circulating cells as epithelial/mesenchymal cells, helping to draft the immunity profile of patients' systemic circulation and also in the tumor microenvironment. The trainee was immersed in the implementation of the technique and the analysis of the results.

1.1.20 Training 20 "High throughput genotoxicity testing training" (lead NILU)

SOP and study plan for HTP comet assay from different cell types, including air-liquid lung model and 3D liver microtissues (spheroids), exposure of cells to chemicals and nanomaterials, positive and negative controls for each endpoint, mini-gel preparation, detection of DNA oxidation, semi-automated and automated analysis and data evaluation, and interpretation.

1.1.21 Training 21 "Confocal microscopy" (lead NILU)

The course included the preparation of samples (cells/tissues) for confocal microscopy, transfection of cells with the HYPer probe, online system setting, images analysis, documentation of nanoparticle internalization, online oxidative stress evaluation, and data presentation.

1.1.22 Training 22 "Advanced 3D models: ALI co-cultures and liver spheroids - application of the comet assay" (lead NILU)

The course includes presentations about 3D liver and lung models and the comet and alamarBlue assay, hands-on training for preparation of ALI co-cultures and 3D liver spheroids, preparation of nanomaterial dispersions, Vitrocell cloud exposure of ALI co-cultures, exposure

of spheroids, and application of ALI co-cultures and 3D spheroids for cytotoxicity testing by AlamarBlue and genotoxicity testing by the comet assay, including scoring and data analysis.

Detailed information on training activities carried out during 2021-2023 is described below.

Training activities at BMC SAV:

Topic of the training	Duration	No. of trained early-stage scientists
# 1 Advanced *in vitro* models		
# 2 Epigenetic analyses – DNA methylation	8.11. – 3.12.2021	Emma Barreto Melian (SERMAS/IRYCIS)
# 3 qPCR and gene expression	8.11. – 3.12.2021	Emma Barreto Melian (SERMAS/IRYCIS)
# 4 Cellular stress response		

Training activities at FhG:

Topic of the training	Duration	No. of trained early-stage scientists
# 5 Advanced co-culture intestine model	26.9. – 26.10.2022 8.5 - 2.6.2023	Jesús Frutos Díaz-Alejo (SERMAS/IRYCIS), Kristina Jakic (BMC SAV) Emma Barreto Melian (SERMAS/IRYCIS
# *6 in vitro* 3D cell cultivation and stem cell differentiation	26.9. – 26.10.2022 8.5 - 2.6.2023	Jesús Frutos Díaz-Alejo (SERMAS/IRYCIS), Kristina Jakic (BMC SAV) Emma Barreto Melian (SERMAS/IRYCIS

Trainings at SERMAS/IRYCIS:

Topic of the training	Duration	No. of trained early-stage scientists
# 7 Study of cellular biological processes using nanoparticle devices		

# 8 The use of liquid biopsy in precision medicine	10.1. – 2.2.2022 7.11. – 3.12.2022	Ioanna Angelioudaki (NKUA) Lucia Balintova (BMC SAV)
# 9 Isolation and culture of tumor and stroma cells from primary tumors	10.1. – 2.2.2022	Ioanna Angelioudaki (NKUA)
# 10 The involvement of tumor microenvironment in cancer progression		
#11 The use of mouse models in oncology research	1.10. – 31.10.2021 7.11. – 3.12.2022	Lenka Trnkova (BMC SAV), Maria Urbanova (BMC SAV) Lucia Balintova (BMC SAV)
# 12 Flow cytometry analysis of tumor biomarkers		

Training activities at NKUA:

Topic of the training	**Duration**	**No. of trained early-stage scientists**
# 13 Treating patients with GI tumors		
# 14 Enrolling patients with GI tumors in research protocols		
# 15 Surgical operational procedures aiming to treat patients with GI tumors	21.8. – 19.9.2021	Peter Dubovan (BMC SAV)
# 16 Initiation in Medical Genetics and Genetic Counselling	16.1. – 17.2.2023	Emma Barreto Melian (SERMAS/IRYCIS)
# 17 Exosome isolation methodology	16.1. – 17.2.2023	Emma Barreto Melian (SERMAS/IRYCIS)
# 18 Circulating tumor cells isolation methodology	16.1. – 17.2.2023	Emma Barreto Melian (SERMAS/IRYCIS)
# 19 Protein expression analysis	16.1. – 17.2.2023 5.6. – 22. 6. 2023	Emma Barreto Melian (SERMAS/IRYCIS) Nikoleta Mojzesova (BMC SAV), Maria Urbanova (BMC SAV)

Training activities at NILU:

Topic of the training	Duration	No. of trained early-tage scientists
# 20 High throughput genotoxicity testing training	3.6. – 25.6.2023	Radka Macova (BMC SAV), Michaela Blazickova (BMC SAV)
# 21 Confocal microscopy (characterisation, uptake of nanomaterials and in situ oxidative stress on live cells)	31.8. – 2.10.2022 3.6. – 25.6.2023	Lenka Trnkova (BMC SAV), Maria Urbanova (BMC SAV) Radka Macova (BMC SAV), Michaela Blazickova (BMC SAV)
# 22 Advanced 3D models: ALI co-cultures and liver spheroids - application of the comet assay		

1.2 Extra scientific events organized in 2021-2023

Several extra courses have been organized since the last update of this education activity on September 2022 (deliverable D3.6 Courses for early stage researchers update). All courses, except course 1.2.3, were organized as open-call events for all interested students, not limited to students at BMC SAV in Slovakia. As usual, interested persons could register for the course on the project website. A certificate of attendance, signed by the coordinator, was handed out to each registered participant. The Table below contains information on the number of participants, the name of the organizer, and the event date.

Overview of courses organized by VISION from October 2022 to June 2023

Date	Topic of the course	Course given by	No. of attendees
19.10.2022	Online course on Gastrointestinal Stromal Tumours (GIST)	**NKUA**	32
28.11.-29.11.2022	Course in Good Laboratory Practice (GLP) for *in vitro* nano and genotoxicology, best practices with nanomaterial handling and preparation for testing	**NILU**	118
2.5.-4.5.2023	3D Surgical Course: Getting Involved in Surgical Research	**NKUA**	3
21.6.2023	Online course on esophageal cancer	**NKUA**	28
29.6.2023	Online course on gut flora	**NKUA**	29

1.2.1 Online course on Gastrointestinal Stromal Tumours (GIST)

The cycle of three lectures focused on 1) Gastrointestinal stromal tumours: Histology and genetics, 2) Surgical challenges of GIST tumors, and 3) Targeted therapy for GIST. This online course was designed for medical and biology students and was held on October 19, 2022.

- **Gastrointestinal stromal tumours: Histology and genetics**.

George Agrogiannis, Histopathologist, Assoc. Professor, 1st Department of Pathology, School of Medicine, National and Kapodistrian University of Athens

GISTs are the most common clinically significant mesenchymal neoplasm of the GI tract. Population-based studies estimate the annual incidence at 10 cases per million. They are found along the entire digestive tract length but are most common in the stomach. No etiologic factors related to GIST have been identified, and although the vast majority of GISTs occur as sporadic tumors with somatic mutations, GISTs also occur rarely in various tumor syndromes. The lesions vary in their cellularity, ranging from low, to intermediate, to high. Regarding the immunoreactivity in gastrointestinal stromal tumors, cytoplasmic patterns for KIT B, dot-like or membranous patterns are all common. For DOG1 expression, the membranous staining pattern is typical.
Understanding underlying mutations in GISTs is critical for diagnosis and patient care, and these are tumors with quite well-defined molecular alterations which reflect the targeted therapy. Imatinib is the most used drug agent for the therapy of GISTs. Four different regions of KIT, namely exon 9, exon 11, exon 13, and exon 17, are most often mutated in sporadic GISTs. Exon 18, exon 12, and exon 14 are the 3 PDGFRA regions that are mutated in GISTs. Fifteen (15) percent of the tumors do not harbor either the KIT or PDGFRA mutations, and they are designated as Wild Type GISTs, while a small subset harbors SDH mutations. A subset of GISTs lack mutations in the KIT/PDGFRA or RAS pathways and yet retain an intact SDH complex. It was proposed that these tumors could be designated as quadruple wild-type (WT) GIST.
GISTs demonstrate diverse clinical profiles in that they can arise in any part of the GI tract and can present as tumors ranging from incidental microscopic tumors to those that follow a malignant and life–threatening course by metastasis. This diversity is also reflected in the oncogenic events that lead to their development.

- **Surgical challenges of GIST tumours**.

Nikolaos Memos, MD, PhD, MSc, Surgical Oncologist, B Department of Surgery, Aretaieio University Hospital, National and Kapodistrian University of Athens, Athens, Greece

Gastrointestinal stroma tumours are rare mesenchymal neoplasms that are mainly located in the gastrointestinal tract. Most commonly involve the stomach, with the second most common location being the small bowel, but they are also seen in the esophagus, rectum, duodenum etc. The treatment cornerstone of GIST is surgical removal with negative margins (R0 resection). GISTs are diagnosed as soft tissue tumour either as incidental findings in imaging scans or as the cause of GI symptoms such as early satiety, bloating, ileus, or GI bleeding. The prognosis of GIST depends on the size of the tumour, which ranges from a couple of cm to >10 cm, the mitotic index, and the GI location. In addition, the risk of tumour rupture either spontaneously or iatrogenically is an independent malprognostic factor. The surgical challenges for the GISTs mainly involve the location of the tumour and the extent of the resection. Large tumours located at the stomach, the duodenum or at the rectum may require multi-visceral resection increasing mortality and morbidity. In the imatinib era, the neoadjuvant treatment and the advances in surgical techniques, the R0 resections can now be more applicable. However, the response seen following treatment is mainly partial, making the multi-

visceral resection still the primary treatment modality for curing the patient. In the present lecture, the challenges that a surgical oncologist faces when asked to operate on the GIST tumours in the post-imatinib area was discussed. In addition, the input of minimally invasive surgical techniques for treating GIST was discussed.

- **Targeted therapy for GIST tumours**.

Jose Duran-Moreno, MD, MSc, Medical Oncologist, B Department of Surgery, Aretaieio University Hospital, National and Kapodistrian University of Athens, Athens, Greece

Gastrointestinal stroma tumours are rare mesenchymal neoplasms that are mainly located in the gastrointestinal tract. Carcinogenesis of GISTs is driven in about 85% of cases by a punctual mutation of gene c-kit or pdgfra. This knowledge permitted the successful development of several therapies that, targeting these mutations, improve the survival and quality of life of patients with unresectable GIST, turning this tumor into the pioneer and paradigm of targeted therapy in solid tumors. In the present lecture, we will discuss the achievements, limitations, and challenges of target therapy in GIST.

1.2.2 Course in Good Laboratory Practice (GLP) for *in vitro* nano and genotoxicology, best practices with nanomaterial handling and preparation for testing

This course was organized on November 28 - 29, 2022. Participants attending were from various institutions in different countries (Slovakia, Poland, Croatia, Spain, Finland, South Africa, Portugal, Italy, Germany, United Kingdom, Bosnia and Herzegovina) and were in different positions in their institutes (eg.- BSc., MSc, or Ph.D. students, postdocs, head of QA, technicians, specialists in the tox department, laboratory manager, associate professor, team leader, principal investigator, scientific officer, researcher, professor, …).

Presenters: *Alexandra Misci Hudecova, Naouale El Yamani, Maria Dusinska, Elise Rundén-Pran (NILU)*

Target groups:

- Those who want to apply for GLP accreditation,
- are already working under GLP and wants to make their work more efficient,
- needs thorough overview on practices with nanomaterials handling and preparation for testing in *in vitro* nano- and genotoxicology

The first day of the online course was dedicated to introducing NILU's Health Effects Laboratory and an in-depth explanation of Good Laboratory Practice (GLP). Participants got familiar with the basic aspects of the GLP, including history, the definition of terms, GLP roles, and responsibilities. Further, the focus on Quality assurance (QA) gave attendees a better understanding of the credibility of the GLP system. Participants were shown practical ways how to write Standard Operating Procedures (SOPs) with real-life examples. Guidance on how to establish, manage and perform under GLP was provided. The step-by-step process of performing a GLP study was explained, including most of the important aspects and practical tips (planning, writing of reports, raw data handling, archiving, …).

The second day was concentrated on nanotoxicology and nanosafety with a focus not only on nanomaterial (NM) theory (history, the definition of terms, characteristics, exposure, and mechanism of action, …) but also on how to practically handle NMs in the GLP laboratory. Participants were explained general guidelines for working with NMs to support nanosafety, how to correctly prepare and handle samples, as well as monitoring and risk assessments of the NMs. Attendees were shown different testing strategies, advanced models, and exposure

methods. A step-by-step procedure was provided upon receipt, identification/registration, storage, and disposal.

Each major topic was followed by a Mentimeter quiz with questions regarding the subject presented. At the end of the second day, a practical exercise focused on quality assurance was conducted and discussed with the participants involved.

1.2.3 3D Surgical Course: Getting Involved in Surgical Research

This course was organized in Greece on May 2-4, 2023, and was designed for medical students.

Agenda of the course

- An Introduction to Ethics in Clinical Research
- Being familiar with Basic Laboratory Equipment
- Blood Processing: Serum/Plasma separation - Red Blood Cell Lysis
- Frequently used Techniques: Gel Electrophoresis, Immunohistochemistry, ELISA, RT PCR, Flow Cytometry (Demonstration)
- Results analysis and Dissemination.

1.2.4 Online course on esophageal cancer

The cycle of three lectures focused on 1) Multimodal treatment strategies for esophageal carcinoma, 2) The extent of lymph node dissection in esophagectomy in the modern era, and 3) Immunotherapy for esophageal cancer. This online course was designed for medical and biology students and was held on June 21, 2023.

- **Multimodal treatment strategies for esophageal carcinoma. Minimally invasive and robotic surgical treatment for esophageal cancer. Current techniques and data**

Tania Triantafyllou, Surgeon, 1st Department of Propaedeutic Surgery, National & Kapodistrian University of Athens

A radical esophageal resection is the mainstay of the treatment of esophageal malignancy. Minimally invasive techniques, including laparoscopy, thoracoscopy, and robot-assisted approaches, are evolving to accelerate postoperative rehabilitation and decrease morbidity and mortality rates. It is believed that minimally invasive surgery tends to reduce surgical trauma and results in faster recovery and better quality of life. Furthermore, the various hybrid techniques have been associated with less blood loss and postoperative pain, whereas the length of stay in the intensive care unit and total hospital length is comparable to the standard surgical approach. Whether it leads to better oncological outcomes remains to be seen.

- **The extent of lymph node dissection in esophagectomy in the modern era**

Dimitrios Theodorou, Prof of Surgery, 1st Department of Propaedeutic Surgery, National & Kapodistrian University of Athens

- **Immunotherapy for esophageal cancer**

Maria Theoxari, MD, Oncologist 1st Department of Pathology, "Laiko" General Hospital of Athens

1.2.5 Online course on gut flora: How is healthy gut flora defined? Can it be modified?

The last course organized within the VISION project was focused on the human microbiome and its role-specific functions. This online course was designed for medical and biology students and was held on June 29, 2023.

- **How is healthy gut flora defined? Can it be modified?**

Apostolos Papalois PhD, KGJ, AMACS, 2nd Department of Surgery National and Kapodistrian University of Athens

Each individual is provided with a unique gut microbiota profile that plays many specific functions in host nutrient metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens. Gut microbiota is composed of different bacteria species taxonomically classified by genus, family, order, and phyla. Each human's gut microbiota are shaped in early life as their composition depends on infant transitions (birth gestational date, type of delivery, methods of milk feeding, weaning period) and external factors such as antibiotic use. These personal and healthy core native microbiota remain relatively stable in adulthood but differ between individuals due to enterotypes, body mass index (BMI) level, exercise frequency, lifestyle, and cultural and dietary habits. Accordingly, there is no unique optimal gut microbiota composition since it is different for each individual. However, a healthy host–microorganism balance must be respected in order to optimally perform metabolic and immune functions and prevent disease development. Understanding the cause or consequence of these gut microbiota balances in health and disease and how to maintain or restore a healthy gut microbiota composition should be useful in developing promising therapeutic interventions.

2 Deviation from the workplan

Despite the travel restrictions caused by the coronavirus pandemic at the beginning of the VISION project, there were no large deviations in the planned activities. After easing security measures, early-stage researchers and medical students took full advantage of the opportunity to work in renowned workplaces abroad. Thanks to the willingness and friendliness of the partners, all applications were approved. The last academic stays were finished close to the end of VISION in June 2023.

3 Conclusion

The academic stays allowed the early-stage researchers to acquire new skills and experiences that will impact their personal and professional growth in the future. Despite the objective obstacles at the beginning of the VISION project that hindered the early-stage researchers from visiting and working in the international environment in the partners' laboratories, 22 in-person training activities have been realized, and 28 early-stage researchers have participated in them. Moreover, much more educational courses than planned initially were organized. The partners in the project offered six courses, and 17 courses were performed. In addition, the main advantage of the online form was that more participants than expected initially, including students from abroad, could participate.