# ABSTRACT BOOK

BARCELONA FINAL MEETING FOR COST ACTION CA17118
(TRANSCOLONCAN)

01-03/06/2022

TRANS COLON CAN

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

**The impact of COVID 19 pandemic on CRC screening- what are the facts and what can be done?**

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COVID 19 pandemic and the mitigation steps that were taken to contain the viral spread affected many aspects of life and medicine since early 2020. One of the concerns is the impact that these steps and the public perception have had on colorectal cancer (CRC) screening and its subsequent effect on cancer diagnosis. Several studies from different countries report that a significant reduction in referrals and performing screening colonoscopies even in high risk individuals, and an expected effect on these delays on subsequent CRC numbers and stage at diagnosis in an adverse manner. This presentation summarizes some of the previous reported relevant findings, and offers some suggestions in order to devise a scheme for the next pandemic based on the conclusions drawn from the current one.

**Functional characterization of miR-93-5p in colorectal cancer by in vitro assays and in vivo chorioallantoic membrane assay**

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Background: MicroRNAs (miRNAs) have been implicated in the growth, progression, and metastasis of different tumors, including colorectal cancer (CRC). MiR-93-5p has been reported to have oncogenic and tumor-suppressive roles in different tumor kinds. The functional role of miR-93-5p in CRC has yet to be clarified. The aim of this study was to analyze the role of miR-93-5p in CRC cell proliferation, cycle and death, migration, angiogenesis, and tumor growth.
Material and Methods: Primary CRC cell line HCT116 and a metastatic SW620 CRC cell line were treated with miR-93-5p mimic or inhibitor, in order to increase or decrease miR-93-5p expression, respectively. Proliferation, cell cycle, anoikis, migration, and angiogenesis were analyzed by crystal violet staining, propidium iodide staining and flow cytometry, detachment assay, wound-healing assay, and tube formation assay, respectively. Tumor growth was analyzed by measuring the total ovograft volume in the in vivo chick embryo chorioallantoic membrane (CAM) assay.
Results: Increased or decreased miR-93-5p expression did not have an effect on HCT116 and SW620 cell proliferation, cell cycle distribution, anoikis and migration in vitro, or tumor growth in vivo. Treatment with miR-93-5p mimic induced tube formation in both HCT116 and SW620 cells.
Conclusions: MiR-93-5p does not mediate CRC cell proliferation, cell cycle, death, migration, and tumor growth which was confirmed by CAM assay in vivo. MiR-93-5p promoted CRC angiogenesis in vitro. Since CAM assay is well established model system for analysis of angiogenesis, CAM assay could be also used in further experiments to confirm the role of miR-93-5p in angiogenesis.

**Evaluation of m6a methylation of miR-93 direct targets in digestive cancer cellular models**

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MiR-93 has been reported to be overexpressed in a number of types of cancers including gastric cancer and pancreatic ductal adenocarcinoma (PDAC), acting as an oncomiR. N-6 methyladenosine is known to be the most abundant type of RNA methylation, and has been reported to appear in mRNAs during the miRNA-mediated mRNA silencing. The aim of the scientific stay is to evaluate the effect of miR-93-5p alteration on the N-6 methyl adenosine (m6a) methylation profile of its candidate target mRNAs in cellular models of PDAC depleted of miR-93. This objective will be accomplished by performing RNA m6a immunoprecipitation experiments. Global levels of m6a will be evaluated. 3'UTR methylation of proteins selected as potential targets of miR-93 in previous studies (CRMP2, YES1, MAPRE1), will be analysed and discussed.

**Profiling the response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer using data independent acquisition mass spectrometry (DIA-MS)**

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Introduction Neoadjuvant chemoradiotherapy (nCRT) has been widely used to improve the local control rate and survival rate in patients with locally advanced rectal cancer (LARC). Response to treatment varies among patients and it is still unknown who will have the best benefit from nCRT. The aim of this study was to discover the specific tissue molecular features that might lead to different responses to treatment. Research groups Greece, the Netherlands, Serbia and Spain collaborated on this project through two bilateral STSMs supported by the COST CA17118 TRANSCOLONCAN, and through one Twinning (HORIZON-WIDERA-2021-ACCESS-03) grant application.
Material and method During the period 2018-2019, pretreatment FFPE tissue samples from 97 patients diagnosed with LARC (stage II/III) were gathered at the Institute for Oncology and Radiology of Serbia. All patients were treated with neoadjuvant chemoradiotherapy (5-FU/Leucovorin) and their response was evaluated using a tumor regression grade (TRG) system. Depending on TRG the cohort was divided into responders/non-responders (TRG1/2 vs. TRG 3-5, Mandard scale). Data independent acquisition mass spectrometry (DIA-MS) was performed on a carefully selected cohort with the aim of analyzing patients who had the most distinctly different response to therapy. Bearing this in mind, we selected a group of 20 patients (11 non-responders and 9 responders) and analyzed their proteomic profiles. DIA-NN was used for searching the spectra and the Perseus software were used for the statistical analysis of data, while enrichment pathway analysis was performed using Metascape.
Results and discussion In total 4849 proteins were identified in 20 rectal cancer FFPE samples. Principal Component Analysis (PCA) indicates that responders compared to non-responders have a significantly different proteomics profile. Statistical analysis using a two-sided t-test resulted in the identification of 871 differentially expressed proteins ($p<0.05$) (235 overexpressed in responders and 636 overexpressed in non-responders). Fundamental cell processes (RNA processing, DNA processing, translation, and extension of telomeres), as well as neutrophil degranulation, were identified as the key characteristics of the responder's group by pathway enrichment analysis. The non-responder group, on the other hand, is characterized by signaling pathways related to intracellular/extracellular transport, and biomolecule metabolism (lipids, vitamins, cofactors, carbohydrates, nucleosides).
Conclusion The DIA-MS approach offered unprecedented proteome coverage for FFPE samples. The differentially expressed proteins and biological processes constitute interesting findings that hold the potential for improving LARC patient management.

Acknowledgements: This study is based upon work from COST Action CA17118, supported by COST (European Cooperation in Science and Technology); www.cost.eu. We acknowledge support of this work by the project "The Greek Research Infrastructure for Personalised Medicine (pMED-GR)" (MIS 5002802) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund). This study was supported by the Ministry of Education and Science of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200043). MC is supported by the Science Fund of the Republic of Serbia (PROMIS TRACEPIGEN project No. 6060876).

**Proteomic profiling of locally advanced rectal cancer before and after neoadjuvant chemoradiotherapy**

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In search for candidate predictive biomarkers to evaluate response to neoadjuvant chemoradiotherapy (nCRT) in rectal cancer, only a few studies report proteomic profiles of tumor tissue before and after nCRT. The aim of our study was to profile the proteome of rectal cancer prior to nCRT and after the treatment and to determine differentially expressed proteins between responders and non-responders in order to identify a set of candidate molecules for prediction and follow up of response to nCRT.
The study has included ten pairs of formalin-fixed paraffin-embedded (FFPE) rectal tissue samples from patients with locally advanced rectal cancer. Samples from tumor and gut mucosa of each subject were taken before and after neoadjuvant chemoradiotherapy. Proteins extracted from tissue samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis followed by a set of bioinformatics analyses.
Proteomics analysis provided a mean of approximately 1,050 protein identifications per sample. Comparison of proteomic profiles between responders and non-responders has identified 18 proteins with significantly altered expression between these two samples sets. Pathway analysis demonstrated high metabolic activity in non-responders' tumors before nCRT, indicating the presence of intrinsic chemoradioresistance in these subjects. Two proteins associated with poor prognosis in colorectal cancer, disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), were identified as candidate predictive biomarkers as they were present in non-responders only, with decreased expression after nCRT. Shortlisted proteins from our study should be further validated as candidate biomarkers for response to routinely applied nCRT protocols.
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**Microbiome analysis for colorectal cancer prediction**

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Recent studies have shown significant associations between gut microbiota and colorectal cancer where colorectal cancer core microbiome as well as cancer-stage specific microbial signatures have been detected from stool samples of colorectal cancer (CRC) patients. We have started a project in Estonia to identify microbiome based biomarkers for CRC detection and prediction and to define a risk assessment model by combining microbiome and metabolome data with genetic risk scores. We collect microbiome profiles from different body sites (oral, stool, mucosal tissue and blood) from individuals who participate national colorectal cancer screening program. We have shown that Fecal Immunohistochemical Test (FIT) tubes (QuikRead iFOBT tube) used in Estonian CRC screening programs are also suitable for microbiome analysis. Importantly, the microbiome community structure remained stable after 4 and 7 days in FIT tubes, indicating the potential to improve the eary detection of CRC with additional microbiome-based biomarkers. Our pilot study shows that both oral and intestinal microbiome communities in healthy people are different from those in people with polyps or diagnosed with cancer. Using population-based data from the Estonian Biobank and gut metagenomic data from 2,500 individuals from the Estonian microbiome (EstMB) cohort, we identify individuals at high risk for CRC based on genetic risk scores and intestinal metagenomic data. Our ultimate goal is to increase the effectiveness of CRC prognosis and screening by developing combined risk models for colorectal cancer, combining conventional risk factors with genetic, metabolic, and metagenomic data sets.

**Lifelong exposure to caloric restriction maintains or restores the microbiota profile of aging mice**

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Introduction

Microbiota founding in the gastrointestinal tract has a crucial role in various metabolic pathways, involving immune response, oxidative stress, and intestinal cell cycle regulation. The alterations of the microbiota composition through aging, unhealthy diets, or sedentary lifestyles may lead to various pathophysiological conditions including obesity, diabetes, and many types of cancer, such as colorectal cancer. Caloric restriction (CR) is known to be an effective intervention to increase a healthy lifespan although the effects of long term exposure are not fully studied. The aim of this study is to investigate the impact of two different types of CR including chronic and intermittent CR and identify the potentially beneficial influence on colonic health.

Materials and Methods

In this study, 10 weeks old female C57BL/6 mice were enrolled into three different dietary groups: ad libitum (AL), chronic caloric restriction (CCR), and intermittent caloric restriction (ICR). Mice in CCR group were applied to 15 % of restriction compared to AL group,while mice in ICR groups were applied to 60% of restriction for one week (ICR-R) followed by AL diet for three weeks (ICR-RF) in a cyclic manner from 10 weeks of age until 81/82 weeks of mouse age. The stool samples from the cecum region of the gastrointestinal tract of the mice were collected at four different time points: week 10 (baseline), week 17/18, week 49/50, and week 81/82 (n=5). Stool samples were collected from the caecum tissue of each mouse and homogenized. The microbiota composition of mice was examined using 16S rRNA amplicon sequencing and metagenomic analysis. Total DNA was extracted from the stool samples and bacterial 16S rRNA V3-V4 gene regions were amplified. The sequencing was carried out using the iSeq100 system (Illumina) pair-end read type and two reads of 151 bp read length. The sequencing data were analyzed by 16S Metagenomics software to determine the taxonomic distribution of bacterial communities from the kingdom to the species level.

Results

At the genus level, Barnesilla, Clostridium, and Eisenbergiella were the most abundant species in all the dietary groups. In the AL group, Clostridium species in the microbiota composition was increased by ~14% in cecum stool samples at week 82 compared to week 10 (baseline). In the CCR group, compared to the baseline, the increase of Clostridium species by 12 % at week 50 of mouse age was restored at week 80. In the ICR-R group, Eisenbergiella species were decreased by 14% with aging in week 80 compared to baseline level, while the levels of Clostridium species did not change. Barnesilla species was increased by about 17% in the ICR-RF group at week 17 compared to week 10, but it was then decreased to the baseline level at week 80. Also, Eisenbergiella species decreased by nearly 9% in week 17 of the ICR-RF group, but it is returned to the baseline level in the later ages. In between the diet groups, Eisenbergiealla species were decreased in all groups except the ICR-RF group. Barnesilla species were relatively increased in the composition in the AL and the CCR groups, while it is maintained, and decreased in ICR-RF and ICR-R groups respectively. The increase in the level of Clostridium species were only observed in the later ages of the AL group, when comparing all the groups.

Conclusion

The present study proves that CR, especially ICR, is significantly effective in maintaining or restoring a healthy microbiota profile against age-related changes. In addition, the present study suggest that changes in microbiota profile in the preventive effects of CR on cancer development depends on the type of calorie consumption.

**Can Selenium prevent Cancer? May depend on where not just whom you ask**

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The micronutrient selenium (Se) may help prevent cancer development at several organ sites. Furthermore, genetic variations in selenoprotein genes may impact the role of several encoded Se-incorporated proteins in countering oxidative and inflammatory processes implicated in carcinogenesis. We previously reported in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort that a higher Se status (as assessed by prediagnostic circulating levels of Se and its major transport protein, Selenoprotein P; SELENOP) was associated with lower risks of colorectal cancer (Hughes et al. 2015; PMID: 25042282) and hepatocellular cancer (Hughes et al. 2016; PMID: 27357089). We then showed that several common SNPs in selenoprotein and Se metabolic pathway genes may affect colorectal cancer development (Fedirko et al. 2019; PMID: 31027226). We are currently investigating the potential role of SELENOP autoantibodies to cause Se physiological deficiency even in subjects of adequate Se intake and how this may further contribute to colorectal cancer risk.

We have recently concluded similar EPIC studies of Se status, assessed by prediagnostic plasma measures of Se, SELENOP, and Glutathione peroxidase 3 (GPX3), SNP variants, and risk of breast and gastric cancer (2,208 and 702 case-control pairs, respectively). Genotypes were either derived by allelic discrimination assays or extracted from GWAS data. Multivariable logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval (CI) of the association between Se status markers, SNPs, and cancer risk. Higher Se levels (but not SELENOP or GPX3) were associated with a lower gastric cancer risk: OR for top quartile 4 versus the reference = 0.56, 95% CI: 0.39, 0.80, ptrend = 0.002. There were no significant association of Se status with breast cancer risk, and while several SNPs were associated with disease risk, none retained significance after multiple testing adjustment.

These findings highlight a possible important role for Se and selenoproteins in the prevention of gastrointestinal cancers, but not for hormonal-related cancers (as we also previously observed no association with prostate cancer risk; Outzen et al, 2021; PMID: 32838475). The results align with the hypothesis of a Se status in many European subjects that is insufficient for robust Se-mediated cancer prevention. However, they require replication in other settings, specifically in areas of suboptimal Se availability.

Conflict of interest Disclosure: LS is founder of selenOmed GmbH, a company involved in improving Se diagnostics. The other authors declare no conflict of interests.

**Shot-gun gut microbiome MWAS meta-analysis and CRC**

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Background: The gut microbiome diversity and composition has been related to CRC. It is a potential diagnostic and prognostic biomarker, and modifiable risk factor. Several studies have proposed microbial signatures for CRC risk, identified by microbiome-wide association studies (MWAS). Here we re-analyzed eight publicly shotgun sequencing data studies and conducted a MWAS meta-analysis. We used cross-validated LASSO predictive models identify consistent microorganisms linked to CRC and/or precancerous lesions, compared to healthy controls. These models were validated in a new study, COLSCREEN, that included 156 participants recruited in a CRC screening context, selected with balanced diagnosis of CRC, high risk colonic lesion (adenoma or polyps) and a control group with normal colon.

Results: The MWAS meta-analysis identified 95 species statistically significantly associated to CRC (FDR <0.05). The LASSO CRC predictive model reduced these MWAS species to 54 species (29 control-enriched and 25 CRC-enriched), with an area under the receiver operating characteristic curve (aROC) value of 0.82 (95% CI: 0.80–0.85) for the training model and an aROC value of 0.77 (95% CI: 0.67–0.86) for the validation in the COLSCREEN cohort. This CRC-trained model was not useful to predict the presence of polyp lesions (aROC= 0.53, 95% CI: 0.42-0.65). The functional analysis, based on orthologous groups showed that translation and amino acid metabolism and transport were the most relevant categories.

Conclusions: The identified signature of 54 species has good predictive accuracy to diagnose CRC, but not polyp lesions. These results suggest that the species of the signature are a consequence of the tumor.

**Identification of novel germline markers that allow us to predict ADR development after CRC chemotherapy**

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Chemotherapeutic treatment for colorectal cancer (CRC) allows for increased patient overall survival. However, current therapeutic regimens are often associated with the development of adverse drug reactions (ADRs), which represent a morbidity, mortality, and economic issue. We propose to identify novel germline markers that allow us to predict ADR development after CRC chemotherapy.
Exomes from 163 CRC patients with severe ADRs (CTCAE grades 3-4) and 52 controls were exome sequenced. We performed data analysis focusing on rare variants (MAF<=1%) that were present in >=4 cases but were absent from the controls. We then validated the candidate variants on an additional 74 cases and 16 controls. We have identified 3 novel candidate genes associated with hematological and digestive toxicity.
We also performed a case-control association study on common variants, including the comparison of different ADRs (N>25). After multiple test correction, there were no statistically significant associations. In order to increase the power of our study, we performed a TWAS and MWAS.

We describe different approaches for novel variant/gene identification in Pharmacogenomics, which have successfully identified novel candidates mediating ADR development. We now intend to validate such variants/genes found in our study in order to better characterize them and determine their pathogenicity. We hope that by identifying these genes as candidates for chemotherapy-mediated toxicity, treatment can be priorly adjusted accordingly to avoid life-threatening toxicities, and ultimately improve the quality of life of CRC patients.

**Taxonomic microbial profiles of Colorectal Adenomas**

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Identifying risk factors for precancerous colorectal adenoma (CRA) development is essential for improving colorectal cancer (CRC) screening and prevention. Some adenomas can present a mucous cap that may offer a niche for bacterial growth that could contribute to dysplastic progression. Detailed investigation is required to define microbiota patterns during the CRA to CRC transition.
We assessed the adenoma microbial profiles in colorectal neoplasia (n=14, grouping samples obtained from tissue and colonic brushes) vs control (n=9, normal mucosa at a distinct site). Furthermore, we also investigated taxonomic differences between the mucosal surface of the adenoma (n=9) and the tissue (n=5). Sequencing of V3-V4 16S rRNA region was performed to determine the bacterial composition.
Shannon index (H) was used to calculate the alpha diversity within the samples. Although the two pathology groups did not show substantial differences, disease samples from colonic brushes of the surface of the adenoma presented a lower H compared to the fresh frozen tissues (Hmean_brushes_disease=1.27, Hmean__tissue_disease=2.47). Differential abundance analysis showed that Bacteroides intestinalis and Bacteroides fragilis were depleted in disease compared to the controls (Log2 fold changes= -1.86, = -2.49, respectively) while Prevotella (species na), Bacteroides vulgatus, Bacteroides uniformis and Alistipes massilensis were enriched in colorectal neoplasia samples (Log2 fold changes= 2.51, =2.06, =1.73, =1.39, = 1.71, =1.35, = 1.41, respectively). Eighteen species were statistically different between the mucosal surface of the adenoma and the tissue. All of them were depleted in tissue compared to the adenoma surface. Bacteroides thetaiotaomicron, Bacteroides vulgatus, Bacteroides fragilis and Alistipes putredinis presented the highest log2 fold changes (Log2 fold changes= -5.88, = -3.70, =-4.84, respectively).
These results indicate that the gut microbiome present on the surface of the adenoma and the microbiome in the tissue may be compositionally different. In future, a functional characterization of the microbiome combining metatranscriptomic and metagenomic in colorectal adenomas will be performed to clarify whether adenomas progression is influenced by microbial dysbiosis.

**Consumption of fruits, vegetables and fiber and risk of colorectal cancer. A gene environment interaction analysis**

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Introduction: Consumption of fruits, vegetables, and fiber has been associated with lower colorectal cancer (CRC) risk. However, it is unclear if genetic variants can modify these associations.

Methods: Our sample included up to 69,734 participants (29,896 cases / 39,838 controls) of European descent, derived from three genetic CRC consortia (CCFR, CORECT, GECCO). Standard logistic regression as well as more efficient 2-step and joint tests were implemented to identify novel and biologically plausible gene-environment interactions between common genetic variants (allele frequency >1%) and the consumption of fruits, vegetables, and fiber in relation to CRC risk. Interactions between aggregated rare variant sets (allele frequency <1%) at the gene level and each of the three exposures were also explored.

Results: Fruits, vegetables, and fiber consumption were each inversely associated with CRC with odds ratios per an interquartile increase of 0.79 (95% confidence interval [CI]: 0.72, 0.86), 0.82 (95% CI: 0.73, 0.93) and 0.79 (95% CI: 0.74, 0.85), respectively. The 3DF joint test that considers the combined effects of G and GxE as well as GE correlation identified, three loci not previously identified by GWAS, including variants in or near the genes NEGR1 (rs1620977), MCC (rs192389100), and SLC26A3 (rs4730274) for consumption of fruits, vegetables, and fiber, respectively. The 2-step approach identified an additional suggestive G x fruit interactions with variants in EIF3H gene.

Conclusion: This study identified statistically significant interactions between several genetic variants and the consumption of fruits, vegetables, and fiber with CRC risk. Further research is needed to elucidate the exact mechanisms through which these interactions might influence CRC risk.

**Microbiome profiling from Fecal Immunochemical Test reveals microbial signatures with potential for Colorectal Cancer screening**

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer deaths worldwide. Early diagnosis of CRC, which saves lives and enables better outcomes, is generally implemented through a two-step population screening approach based on the use of Fecal Immunochemical Test (FIT) followed by colonoscopy if the test is positive. However, the FIT step has a high false positive rate, and there is a need for new predictive biomarkers to better prioritize cases for colonoscopy. In our study we used 16S rRNA metabarcoding from FIT positive samples to uncover microbial taxa, taxon co-occurrence and metabolic features significantly associated with different colonoscopy outcomes, underscoring a predictive potential and revealing changes along the path from healthy tissue to carcinoma. Finally, we used machine learning to develop a two-phase classifier which reduces the current false positive rate while maximizing the inclusion of CRC and clinically relevant samples.

**miRNA profiling in stool identifies fecal miRNAs able to accurately distinguish colorectal cancer and adenomas from healthy controls and related to cancer progression**

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Fecal miRNAs are becoming more and more investigated in relation to CRC and other gastrointestinal diseases. In this respect, our lab has explored for the first time the whole fecal miRNome in relation to cancer diagnosis (Pardini et al., in preparation). However, there are limited evidence on the potential correlation of miRNA profiling in stool with cancer progression. In light of future applications of fecal miRNAs as prognostic biomarkers, we investigated the relationship among microRNA expression profiles and CRC staging using both surrogate (stool) and primary cancer tissues. We considered three CRC independent cohorts within the Oncobiome EU project (from Torino, Prague, and Brno) globally consisting of 117 healthy subjects, 54 adenoma, and 269 CRC cases.
In a preliminary analysis, we identified 44 fecal differentially expressed microRNAs (DEmiRNAs) considering all possible comparisons among the patients stratified by the stage. Thirty-four out 44 DEmiRNAs were associated with a significant ($p<0.05$) increasing/decreasing expression trend from healthy subjects, to patients with colorectal adenoma, and from early to advanced CRC stages.
To identify clinically important CRC subtypes, such as the mesenchymal-like subgroup with high stromal infiltration, poor patient prognosis, and poor response to standard treatments, the gene expression profile of tumor tissues could be a promising data type. From about 4,000 primary tumors, an expert consortium proposed a classification scheme grouping CRCs into four biologically distinct subtypes called consensus molecular subtypes (CMS) (ref). In this respect, we analyzed the RNA-Seq

data of tissue samples collected in the Torino cohort. The most represented CMS were CMS3 (metabolic reprogramming, 37%) and CMS4 (mesenchymal-like cancers, with high stromal infiltration and poor patient prognosis, 29%). The CMS2 (oncogene amplification and high WNT and MYC signalling) and CMS1 (microsatellite instability and infiltration of activated immune cells) groups were represented by the 25% and 7% of CRC patients, respectively. Then, fecal miRNAs profiling was explored in patients stratified based on their specific CMS. Interestingly, 19 out of 44 DEmiRNAs dysregulated according to CRC stage were also differentially expressed among the four CMS.

These data suggest that fecal miRNA levels not only reflect the presence of a tumor but can be explored as novel biomarkers for monitoring disease progression.

**Bowel preparation for colonoscopy, pre-analytical potential flaw for diagnosis?**

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About 50% of prescribed colonoscopies report no pathological findings. A secondary screening test, after FIT+, based on thermal liquid biopsy (TLB), is proposed. The aim of this work is to study the possible effect of colonoscopy bowel preparation when discriminating between FIT+ patients with normal (CN) and pathological (CP) colonoscopy findings. Three groups were studied: 1) 514 patients enrolled in a CRC screening program (CN and CP with normal and pathological colonoscopy, respectively), with blood samples obtained just before colonoscopy and after bowel preparation; 2) 55 patients from the C N group in whom a second blood sample was drawn after 8-10 hour fasting (CNR) but with no bowel preparation; and 3) 55 blood donors considered as healthy reference set (BD). Samples were analyzed according to TLB. The results showed that from 514 patients undergoing CRC screening colonoscopy, 247 had normal colonic mucosa (CN) and 267 pathological findings (CP). TLB parameters were similar between CN and CP patients but different from BD. When avoiding bowel preparation, the re-sampled CNR patients had similar TLB parameters to BD. TLB parameters together with other serum indicators confirmed the statistically significant differences between CN and CNR, revealing the distorting effect of bowel preparation on biomarker determination. In conclusion, harsh conditions associated with bowel preparation affected TLB patient profiles and serum biomarkers, potentially affecting the diagnostic capability of methods based on liquid biopsy. Blood extraction during colonoscopy procedure (i.e., after bowel preparation) may lead to invalid conclusions in blood-based diagnostic tests.

**Colorectal cancer screening improvement by using miRNA biomarkers**

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New non-invasive approaches that can complement and improve on current strategies for colorectal cancer (CRC) screening are urgently needed. A growing number of studies have documented that components of tumors, which are shed into the circulation, can be detected in the form of liquid biopsies and can be used to detect CRC at early stages in a minimally invasive way. The analysis of circulating tumor-derived molecules such as circulating microRNA (miRNA) in blood or other body fluids, have a great potential to improve CRC screening. MiRNAs are more stable and resistant to storage and handling than other molecules, making them very attractive as novel liquid biopsy biomarkers for this aim. However, they also present some limitations. During the last years, our group has been optimizing methods and analyzing circulating miRNAs in different biofluids such as plasma or faeces in the context of early detection of CRC by different approaches. Moreover, we are now starting a prospective, multicenter, comparative, parallel study to validate one of our miRNA-based signatures for colorectal cancer screening.

**Integrative omic analysis of faecal samples shows novel miRNA-mediated host-microbiota interactions in colorectal cancer**

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Dysbiosis of the gut microbiota have been linked to colorectal cancer (CRC). Human microRNAs are also implicated in CRC, and recent findings support a crosstalk between miRNA release in the gut lumen and the gut microbiota. miRNAs and microbial species can be profiled from faecal samples representing a reliable biospecimen to explore the host-microbial relationships. In a pilot study, we previously demonstrated that the combination of fecal miRNA and microbial species levels can classify more accurately CRC patients from healthy controls than using a single omic information (Tarallo et al., 2019. mSystems. 4(5):e00289-19). Subsequently, in a further study, we observed that the combination of these data was able to accurately classify subjects adopting a specific dietary habit (Tarallo et al., 2021. Gut. gutjnl-2021-325168).
To further evaluate the candidate host-microbial interactions mediated by miRNAs in CRC, we performed shotgun metagenomic sequencing on stool samples from healthy subjects and patients with precancerous lesions or CRC from two independent European cohorts. The same samples were previously profiled for stool miRNome identifying 20 miRNAs significantly dysregulated in CRC patients in both populations. The levels of these miRNAs were evaluated in relation to the microbial abundances observing significant findings, including a correlation between miR-1276-3p and Fusobacterium nucleatum levels both increasing in CRC patients, and an anticorrelation between miR-149-5p and Bacteroides fragilis. These results support further host-microbial interactions in CRC and are consistent with previous evidence showing that the exosomal release of these miRNAs by CRC cell lines is modulated by the same bacteria.

Our data suggest that a specific microbial composition may mirror the expression and release of miRNAs in the gut lumen, with their subsequent detection in stool, highlighting novel cross-kingdom molecular interactions. These combinations of miRNAs and microbial species detectable in stool samples can improve an early CRC diagnosis by designing novel and non-invasive combination of different biomarkers.

**Novel liquid biopsy approaches to improve screening strategies for colorectal polyps and cancer**

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Colorectal adenocarcinoma (CRC) is the type of malignant tumour with the highest incidence in Spain, and it presents one of the highest mortalities by cancer. Prevention strategies in the health system are mainly focused in the assays implemented by the Bowel Cancer Screening Programs (BCSP), which rely on the faecal occult blood immunochemical test. Although screening programs have undeniably been proven efficient in detecting CRC earlier and are cost-effective, the specificity of the test is low, and up to half of the patients that receive a screening colonoscopy turn out to be healthy. Additionally, screening programs aim to detect cancer earlier and, ultimately, prevent it. For the former purpose, several biomarkers have been proposed in the last few years that show great promise. Prevention however, relies on the detection and removal of colorectal cancer precursors, polyps. Nevertheless, the studies published so far have shown low sensitivities and specificities. In this project, we propose to use novel liquid biopsy techniques, particularly circulating tumour DNA sequencing, as a proxy for the presence of bowel polyps. We will evaluate the positive predictive values given by these analyses and the correlation with the mutational profile of the polyps themselves, in an attempt to develop new strategies that can help optimize the resources and approaches in bowel cancer screening. Overall, the main purpose of the project is to advance in the development of personalized medicine by being able to provide with simple and efficient biomarkers that could be used to modulate current CRC risk prediction. The development of such biomarkers could help direct the health resources to target those individuals at higher risk of developing the disease.

**Identification and expression analysis of novel non-coding RNAs (ncRNAs) in cancer cells using a high-throughput sequencing approach: Two putative new cancer biomarkers in colorectal adenocarcinoma**

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Colorectal adenocarcinoma is one of the most common malignant tumors of the gastrointestinal tract and the second leading cause of cancer-related deaths among adults in Western countries. Non-coding RNAs are characterized as RNA molecules, which lack the ability to encode protein structures and appear to include a level of internal signals. Moreover, they are crucial regulators of gene expression, thus controllers of cell physiology and development. In this study, we implemented a high-throughput sequencing approach based on the primary semi-conductor technology to identity novel small non-coding RNAs in cancer cells. Furthermore, we examined the potential diagnostic and prognostic utility of two novel RNA molecules - MT815876 and MT815889 - in colorectal adenocarcinoma.
Bioinformatics analysis of NGS data was performed to reveal the existence of certain classes of ncRNAs using the miRDeep* and CPSS 2.0 software. To examine the existence of the predicted non-coding RNA sequences in cDNA pools of cell lines, a developed qPCR-based assay was implemented. Moreover, total RNA was extracted from colorectal adenocarcinoma specimens and non-cancerous colorectal mucosae. After polyadenylation of 2 μg total RNA by poly(A) polymerase and subsequent reverse transcription with an oligo-dT adapter primer, MT815876 and MT815889 expression were determined using an in-house developed reverse transcription quantitative real-time PCR method, based on SYBR Green chemistry. SNORD43 (RNU43) and SNORD48 (RNU48) were used as reference genes. Next, we performed extensive biostatistical analysis.
Our results support the existence of twenty (20) putative new small ncRNAs, ten (10) of which have had their expression experimentally validated and presented differential profiles in cancerous and normal cells. Analysis of NGS data revealed the existence of two 28S Ribosomal RNA fragments (28S-rRFs) using the miRDeep* and CPSS 2.0 software. Expression analysis showed differential expression between cancer and corresponding normal tissues, leading to the conclusion that the two new ncRNA molecules could potentially be used as biomarkers for colorectal cancer. Both of novel molecules were shown to be significantly downregulated in colorectal adenocarcinoma specimens compared to non-cancerous colorectal mucosae, suggesting its potential exploitation for diagnostic purposes.
Identification and expression analysis showed differential expression between cancer and corresponding normal tissues, leading to the conclusion that the two new ncRNA molecules could potentially be used as biomarkers for colorectal cancer. A deeper comprehension of the ncRNAs interactive network and its role in cancer can therefore be translated into a wide range of clinical applications. Despite this progress, further scientific research from different perspectives and in different fields is needed, so that the riddle of the human transcriptome can be solved.

**Latest Updates on 'Peptide Nucleic Acid based Diagnosis of CRC via Smartphone Sensing' Project**

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Colorectal carcinoma (CRC) is one of the most common cancers of the gastrointestinal tract. Clinical studies have reported that DNA hypermethylation in the mSEPT9 V2 promoter region in CRC. SEPT9 hypermethylation is an important biomarker in cancer diagnosis and treatment and can be detected in tumor tissue as well as in blood, plasma, stool, urine and other biological samples. However, the methods used to determine SEPT9 and other potential markers are generally time-consuming and lead to late diagnoses. Therefore, it is of great importance to develop simple, rapid and cost-effective Point-of-Care (PoC) systems for use in cancer diagnosis as well as prognosis. This presentation is planned to include two parts in which different PoC tools are developed for the analysis of methylated SEPT9 (mSEPT9) via smartphone, in CRC patients. In the first section, application of a newly designed electrochemical biosensing system with bio-functional magnetic nanoparticles (MNPs) will be defined. Then, colorimetric spot tests with the novel materials for both naked-eyes and fluorescence-based detection will be explored in the following part. Both systems are applied the samples from CRC samples obtained by Yeditepe University and then, the results are compared with the reference method and all the findings will be finally evaluated in terms of their practical uses and detection efficiency.

**Mapping the molecular diversity of the colorectal adenoma-early carcinoma transition**

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A colorectal adenoma, an aberrantly growing tissue that arises from the intestinal epithelium is considered a precursor of colorectal cancer (CRC), the third most common cancer worldwide. In our studies we have addressed A) chromosomal instability (CIN) early in adenomas by structural and numerical chromosomal aberrations; B) mutation profile among low- and high-grade adenomas and in situ carcinoma with detailed follow up and C) a whole transcriptome analysis of 18 fresh frozen colorectal adenomas and their adjacent mucosa from the Czech and Italian populations, matched for histology and sex.

Results: A) by using aCGH histologically similar colorectal adenomas showed wide variability in chromosomal instability. According to the results the patients could be clustered into four groups: 1st with the gain of MALAT1 and TALAM1 long non-coding RNAs (lncRNAs), 2nd those with numerous microdeletions, 3rd consisted of patients with a disrupted karyotype, and 4th with no CIN in adenomas. We have identified losses in genes, such as TSC2, COL1A1, NOTCH1, MIR4673, and GNAS, and gene gain containing MALAT1 and TALAM1. B) By using a high-throughput genotyping technique we observed a high frequency of pathogenic variants in the studied genes. The APC, KRAS and TP53 mutation frequencies were slightly lower in adenoma samples than in in situ carcinoma samples. By stratifying mutation frequency based on the grade, it followed the gradient low-grade adenoma-high-grade adenomas-in situ carcinoma: APC gene 42.9-56.0-54.5%; KRAS gene 32.7-32.0-45.5%; TP53 gene 8.2-20.0-18.2%. KRAS mutations were associated with the presence of villous histology and methylation of the APC promoter was significantly associated with the presence of POLE genetic variations. Our data showed the multistep model of gradual accumulation of mutations, especially in the driver genes, such as APC, TP53 and KRAS. C) The enrichment analysis showed that the most downregulated pathways belonged

to immune responses and complement activation while the most upregulated pathways were enriched predominantly in proliferation and developmentrelated Gene Ontology biological processes. In total 2,753 differentially expressed genes (DEGs) coherent between the two populations were identified. From the subset of discriminant DEGs for colorectal adenomas, 8 candidate genes AXIN2, ETV4, RNF43, CXCL12, FBLN1, GNG7, ZEB2, and SFRP1 were selected for validation on a larger population as well as for functional in vitro assays. These results suggest new candidate biomarkers for precancerous colorectal cancer stages.

Acknowledgement: This study was supported by NV18-03- 00199 and COST Action CA17118.

**Markers of chromosomal instability associated with the clinical outcome of colorectal cancer patients**

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Although extensive biomarker-driven research has identified risk predictors for disease recurrence, selection of early-stage colon cancer patients for potential disease progression is still based on clinicopathological criteria. Although more than 80% of colorectal cancers exhibit chromosomal instability (CIN), its role in clinical outcome is still uncertain. CIN-driven intratumor heterogeneity has emerged as a biomarker associated with shorter survival in some cancer types. Accordingly, we have investigated the prognostic and predictive value of copy-number intratumor heterogeneity and tumor aneuploidy, measured as the number of subclonal CNA segments and the copy-number alteration (CNA) load, respectively. In a first cohort of early-stage colon cancer (N = 84), increased levels of copy-number heterogeneity and aneuploidy are fundamentally associated with poor outcome in stage II colon cancer. In contrast, the amount of subclonal mutations does not correlate with disease relapse. In addition, the gain of the chromosome arm 13q and loss of heterozigosity at 17q are more frequently identified in tumors from patients that developed recurrence. Clustering samples from three independent patient cohorts with colorectal advanced adenomas (N = 80), stage I (N = 37) and stage II (N = 98) adenocarcinomas, we found that the CNA load accumulated a significant increase over tumor progression (Annova's $P < 0.0001$). Moreover, levels of copy-number intratumor heterogeneity also exhibited an incremental escalation from adenomas to early-stage carcinomas (Mann-Whitney's $P = 0.04$). In summary, although further studies (prospective, if possible) are needed, our data demonstrates that genomic aberrations are promising biomarkers for patient stratification in early colorectal cancer.

**SMAD4-201 transcript as a putative biomarker in colorectal cancer**

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Transcripts with alternative 5'-untranslated regions (UTRs) result from the activity of alternative promoters and they can determine gene expression by influencing its stability and translational efficiency, thus executing complex regulation of developmental, physiological and pathological processes. Transcriptional regulation of human SMAD4, a key tumor suppressor deregulated in most gastrointestinal cancers, entails four alternative promoters. These promoters and alternative transcripts they generate remain unexplored as contributors to the SMAD4 deregulation in cancer. The aim of this study was to investigate the relative abundance of the transcript SMAD4-201 in colorectal cell lines and tissues in order to establish if its fluctuations may be associated with colorectal cancer (CRC).
Relative abundance of SMAD4-201 in total SMAD4 mRNA was analyzed using quantitative PCR in a set of permanent human colon cell lines and tumor and corresponding healthy tissue samples from patients with CRC.
The relative abundance of SMAD4-201 in analyzed cell lines varied between 16% and 47%. A similar relative abundance of SMAD4-201 transcript was found in the majority of analyzed human tumor tissue samples, and it was averagely 20% lower in non-malignant in comparison to malignant tissue samples (p=0.001). Transcript SMAD4-202 was not detectable in any of the analyzed samples, so the observed fluctuations in the composition of SMAD4 transcripts can be attributed to transcripts other than SMAD4-201 and SMAD4-202.
The expression profile of SMAD4-201 in human tumor and non-tumor tissue samples may indicate the translational potential of this molecule in CRC, but further research is needed to clarify its usability as a potential biomarker for early diagnosis.

**Exploring the value of hematological parameters as predictive factors in patients with anal squamous cell cancer treated with radical chemoradiotherapy**

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Background: Historically, the treatment of choice for anal carcinoma (AC) had been abdominoperineal resection (APR). Radical radiotherapy with concurrent 5-fluorouracil plus mitomycin C chemotherapy (CRT) was later established as standard therapy, although with a failure rate of 20–30%. The aim of this study was to evaluate the outcomes after radical CRT, prognostic and predictive factors and patterns of failure.
Patients and methods: This study included 47 patients treated with radical CRT for patohistologicaly confirmed anal squamous cell carcinoma. Analyzed haematological parameters included: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and haemoglobin level. Tumor response was assessed at 24 weeks from CRT completion. Follow-up was performed every 3 months during the first two years, and every 6 months thereafter.
Results: A complete clinical response (CR) was detected in 30 patients (63.8%). Patients who did not achieve a 6-months CR and those who had a CR after 6 months but then relapsed were referred to surgical treatment. With combined CRT and surgical salvage treatment the CR rate was 80.9%. Patients with CR after 6 months had significantly longer DFS, PFS, and OS. The final logistic regression model included pretreatment haemoglobin level and treatment break period. A significant effect on the 6-month response was confirmed for PLR ($p = 0.03$).
Conclusions: Important prognostic factors associated with CR were baseline haemoglobin level and period of treatment interruptions. Potential haematological prognostic factors could be PLR and NLR, which can be routinely determined by low-cost and minimally invasive methods.
Keywords: anal cancer, chemoradiotherapy, hematological parameters.
Acknowledgements: This study is based upon work from COST Action CA17118, supported by COST (European Cooperation in Science and Technology); www.cost.eu. This study was supported by the Ministry of Education and Science of the Republic of Serbia (Agreement No. 451-03-68/2022-14/200043). MC is supported by the Science Fund of the Republic of Serbia (PROMIS TRACEPIGEN project No. 6060876).

**Altered serum levels of ceramide and sphingomyelin species in patients with rectal cancer**

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Background: Although dysregulation of sphingolipid metabolism might be associated with rectal cancer, there is a lack of studies examining sphingolipid metabolites and enzymes as biomarkers in this type of cancer. These molecules regulate different biological processes including the cellular response to apoptosis. In the maintenance of the balance between pro-apoptotic and pro-proliferative sphingolipids, acid ceramidase (ASAH1) is considered to have an essential role. This study aimed to investigate the diagnostic and predictive potential of sphingolipids in serum of patients with rectal cancer and to analyze the association of their circulating levels with the expression of ASAH1 and apoptosis score in tumor tissue. Materials and methods: The content of ceramides and sphingomyelins was analyzed by ultrafast liquid chromatography coupled with tandem mass spectrometry in serum samples of 22 patients with locally advanced rectal cancer (LARC) and 25 healthy individuals. The expression of ASAH1, pro-apoptotic BAX and anti-apoptotic BCL2 was analyzed by quantitative real-time PCR in tumor and corresponding healthy tissue samples of patients. Results: Among analyzed sphingolipids, significantly lower serum levels of C18 CER, C22 CER, C24 CER, C18 SM and C24 SM were observed in patients than in controls ($p<0.05$). For C20 CER, C22 CER and C24 CER a positive correlation with BAX/BCL2 ratio in tumor tissue was found ($r=0.619$, $p=0.018$; $r=0.694$, $p=0.006$ and $r=0.601$, $p=0.023$, respectively). Conclusion: This study showed altered serum levels of certain sphingolipid species in LARC patients, and a positive correlation between very long-chain ceramides (C20-C24) and pro-apoptotic status of tumor tissue. Although the association of sphingolipids with rectal cancer might be proposed, their diagnostic potential should be evaluated in a larger cohort of subjects.

**Whole exome sequencing of paired colorectal carcinomas and synchronous liver metastases from the same patients**

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The present study aimed to analyze the whole exome somatic mutational spectra in retrospectively collected samples of archival FFPE specimens representing paired primary tumors and synchronous liver metastases from surgically treated patients with colorectal carcinomas. We identified small genetic alterations in individual genes and pathways in patients stratified by the response to cytotoxic therapy and overall survival.
In total, samples from 20 patients with synchronous colorectal liver metastases, diagnosed and treated at a single center, were utilized for the study. The libraries were prepared from DNA samples of primary tumors and metastatic loci, pooled in equimolar fashion and sequenced on the NovaSeq 6000 platform (Illumina). The intended average on target coverage was 200x with anticipated average duplicate rates 50%. Raw FASTQ data preprocessing was performed using the Agilent Genomics NextGen Toolkit (AGeNT) and the Genome Analysis Toolkit (GATK) according to GATK Best Practices. Identification of somatic variants and short indels was done using Mutect2 v4.2.4.1, with filtering by FilterMutectCalls tool. In order to minimize the large numbers of FFPE-related artefacts, the prioritized variants had to fulfill the following conditions: 1/ VAF>20%, 2/ supported by at least five reads, and 3/ allelic frequencies in gnomAD less than 0.001. Resulting variants were annotated using the Variant Effect Predictor (VEP) v. 98.3 and their importance was based on the VEP impact prediction. For comparisons of mutation rates between sample types and patient subgroups, for creation of somatic variant plots and the pathway analysis, we employed the Maftools 2.10 R/Bioconductor package, providing the FDR adjusted p-values.
The most frequently mutated genes in the patients were APC (55% in primaries and 60% in metastases; Tier 1 in the Cancer Gene Census - CGC), TTN (50/40; frequently mutated genes in public exomes - FLAG), TP53 (50/45; Tier 1 CGC), MUC5AC (30/25; FLAG), SYNE1 (20/25; FLAG), and FAT4 (20/25; Tier 1 CGC) with TRIP11 (Tier 1 CGC) mutated in 30% of primaries, but only in 5% of metastases ($p=0.09$). Primary tumors of patients experiencing good response to chemotherapy before liver metastasis removal (CR/PR according to RECIST) had notably higher share of mutations in TRIP11 and

FAM186A than poorly responding (PD/SD) patients. Poor responders had more frequently mutated TP53, TTN, MUC3A, and ADGRV1. Patients poorly responding to chemotherapy after metastasis resection had more frequently mutated HERC1 in their metastases than those with good response (p=0.067). Regarding pathways, the WNT pathway components were mutated more frequently in primaries and Notch and Hippo pathways in metastatic loci. Higher share of mutations in WNT, Hippo, and RTK-RAS pathways was observed in primary tumors of patients with a good response to chemotherapy compared to those with poor response.
Taken together, we report subtle changes in exome mutational profile between paired primary tumors and synchronous metastases with the exception of TRIP11 (Thyroid Hormone Receptor Interactor 11), whose higher mutation rate also associates with good response to conventional chemotherapy. After functional characterization, our data may provide a lead for studies focused on utilizing this finding in precision oncology of colorectal carcinoma.
The study was supported by the Czech Ministry of Education, Youth and Sports project no. LTC19015, EU Inter-Cost Action CA 17118 (TRANSCOLONCAN), and the Czech Health Research Council, project no. NV19-08-00113.

**Molecular adenoma features to predict metachronous colorectal cancer risk: a nested-case control study**

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Introduction: In colorectal cancer (CRC) surveillance, adenoma characteristics such as size, number of adenomas, dysplasia, villous components, are used as indicators for risk of cancer and guide the surveillance interval. The current risk groups can not optimally distinguish high risk from low risk, which may result in suboptimal surveillance strategies.
Specific DNA copy number aberrations are associated with risk of adenoma-to-carcinoma progression, but these aberrations are present in only a subset of advanced adenomas. Therefore, we hypothesize that specific DNA copy number aberrations may better predict the risk of CRC than advanced adenoma.
Aim: To evaluate whether a molecularly-defined high-risk adenoma is a better risk factor for CRC than the present high risk group.
Material and methods: DNA copy number profiles were determined, by means of low-coverage whole genome sequencing, on a series of 529 adenomas, selected from a Norwegian adenoma cohort. We retrieved detailed information on adenoma characteristics and whether adenoma patients later were diagnosed with CRC or not. By univariate and multivariate regression analysis we estimated the odds ratio of CRC by comparing adenoma patients who later were diagnosed with CRC to adenoma patients not diagnosed with CRC for the present risk groups (advanced adenoma) and for the molecularly-defined high-risk adenomas.
Results: Molecular high-risk features were observed in 85/267 (32%) of advanced adenomas and in 27/262 (10%) of non-advanced adenomas. The odds ratio of CRC was 3.58 (p=2.84E-8) and 1.90 (p=0.012), when an advanced adenoma or molecular high-risk adenoma was detected at the baseline colonoscopy, respectively. In the

multivariate regression analysis only advanced adenoma was as a significant risk factor of CRC.
Conclusion: Molecularly-defined high-risk adenomas is associated with an increased risk of CRC. However, the advanced adenoma is a stronger predictor for risk of CRC.

**Studying the genetic bases of hereditary colorectal cancer and polyposis**

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Great efforts have been made in the last years to identify additional genes that explain the predisposition to colorectal cancer or polyposis observed in some individuals or families. With this objective, we have tried to identify and characterize the genetic basis of serrated polyposis; a very elusive cancer predisposing syndrome from the standpoint of genetics. On the other hand, based on the unsuccessful results obtained when trying to identify new high-penetrance genes involved in the predisposition to nonpolyposis colorectal cancer, we studied the role of low-risk alleles (polygenic risk score) in familial and early-onset nonpolyposis colorectal cancer, and the involvement of other hereditary cancer genes, in particular TP53, in the predisposition to this tumor type.

**ATF2 – an underestimated player in aggressiveness of colon cancer**
Collaboration study: Czech Republic, Turkey, Brazil, India, Germany

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The role of the stress sensor activating transcription factor 2 (ATF2) in colorectal cancer (CRC) is controversially discussed. We started our ATF2 study with an interesting finding from immunohistochemistry: there was a high intratumoral heterogeneity with loss of ATF2 at the invasion front in a cohort of 332 colon cancer patients. Low ATF2 expression was correlated with a worse prognosis. To identify the functional role of ATF2 loss, we used a panel of different in vitro and in vivo methods: CRISPR/Cas9 gene editing for ATF2, gene expression analysis, ChIP analysis, inhibitor experiments, migration and invasion assays, competition studies using mixtures of different genotypes, xenograft experiments in the chorioallantoic membrane model (CAM) and in mouse. We identified the metastasis promoter TACSTD2 (TROP2) as a novel ATF2 target gene. TROP2 expression seems to be dependent on promoter methylation. High TROP2 levels were associated with de-adhesion, the first step in metastasis, and with higher invasion and migration capabilities. ATF2low/TROP2high tumors were intrinsically stiffer. As expected for cells at the tumor invasion front, ATF2 negative tumor cells were highly resistant upon 5-FU treatment.

**Insights from a GI-cancer genetic service – from translational research to policy changes**

Yael Goldberg1,2

Identification of carriers of monogenic colorectal predisposition syndromes and clinical recommendations for surveillance are based on evidence-based data and practice guidelines and are shaped by local policy. We would present some of the new data, genetic and clinical, that has been obtained in our group during the last years. This includes identification and substantiation of MCM9 as a polyposis and early-onset cancer predisposition gene; delineation of common MUTYH genetic variants in the local population, providing insights regarding the co-inheritance of BRCA and Lynch, and about the genetic landscape of Lynch syndrome in Israel. The data obtained leads to local guidelines regarding testing algorithm in Israel, that will be discussed.

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**Molecular mechanisms for the role of Aldo-keto Reductases as biomarkers in gastrointestinal cancers**

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Deregulation of metabolic pathways has increasingly been appreciated as a major driver of cancer in recent years. Cancer-associated alterations in metabolism include enhanced glycolysis and the preferential use of metabolic pathways for the production of biomass and nicotinamide adenine dinucleotide phosphate (NADPH). Aldo-keto reductases (AKRs) are NADPH dependent cytosolic enzymes that can catalyze the reduction of carbonyl groups to primary and secondary alcohols. AKR1B1 catalyzes the conversion of excess glucose to sorbitol while AKR1B10 can reduce a number of different reactive aldehydes including retinal. High expression of AKR1B1 in the colon and AKR1B10 in the liver (but not the colon) are associated with worse prognosis. Mechanistically, AKR1B1 was shown to enhance metastatic spread; moreover, it may play an important role in modulating the tumor microenvironment for cancer progression. AKR1B10 plays a role in modulating fatty acid metabolism and activation of nutrient sensing pathways. Overall, although AKR1B1 and AKR1B10 have similar structures, their expression in different tumor types can lead to highly diverse outcomes.

Acknowledgement: The study was carried out as a part of the COST action CA17118. TÜBİTAK project no. 118Z116 provided the funding.

**SMART DRUG DELIVERY PLATFORMS FOR CANCER TREATMENT**

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Keywords: drug delivery platforms; smart and triggered delivery; cancer treatment

The presentation is intending to organized in two major parts. The first part will include some of our results related to the development of diffrent materials for cancer treatments: antitumoral composite materials based on COLL/HA and loaded with specific nanoparticles (Fe3O4, AgNPs) and substances (citostatics); porous materials loaded with citostatics; nanostructures for targeted delivery. COLL/HA composite materials were developed for bone grafting and regeneration. When the bone mass loss is associated with tumor resection, the as created defects can be filled with such composite materials. Their major role is to assure antitumoral efect and for this reason the cummulative of chemotherapy (assured by the proper delivery of cytostatics), hyperthermia (generated by the proper iradiation of the magnetite loaded in teh composite material), fotothermia (generated by AgNPs, for instance). It is important to mention that these composites can act as smart DDS and can assure personalized therapy. Magnetic nanoparticles decorated with AgNPs can be also used as targeted drug delivery systems, if proper receptors are adsorbed onto the surface. These can be loaded with specific cytostatics which are expected to be delivered intracellularly. Porous materials were also exploited as potential support and carrier for specific drugs, including cytostatics and some of them already highlighted a targeted delivery capacity. Based on these results, some directions of potential future colaborations will be highlighted.

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**The many faces of colorectal cancer immunity: implications for disease progression and therapy**

Noel FCC de Miranda, in representation of the Cancer Immunogenomics lab of the Leiden University Medical Center, The Netherlands.

The advent of checkpoint blockade immunotherapy has underscored the essential role that the immune system plays in cancer progression but, chiefly, it has consolidated the expectation that immune cells and immune-related processes can be converted into powerful therapeutic tools.
In this talk I will discuss work performed by my lab where we applied multidimensional technologies to characterize the immune contexture of colorectal cancers at early and advanced stages of tumor progression, with an eye on the development of immunotherapeutic solutions.
The activation of innate immune pathways and, in particular, the establishment of macrophage-cancer cell communities were shown to be notorious features of colorectal cancer onset and progression. Such interactions may be disrupted by the targeting of immunological axis like the ones mediated by CD47-SIRP. In advanced cancers, we identified attractive targets for immunotherapeutic intervention including neoantigen-reactive T cells as well as innate lymphocytes with hallmarks of anti-cancer immunity. Both could be exploited in the context of antigen-targeted or cellular-based therapies.
In sum, the future outlook for the application of immunotherapies for the treatment of colorectal cancer is a promising one. The expectation is that, depending on patients' characteristics and immunological and molecular features of their tumors, tailored immunotherapeutic approaches will be applicable to a significant proportion of colorectal cancer patients.

**Germline MBD4-deficiency causes a multi-tumor predisposition syndrome**

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We have reported an autosomal recessive, multi-organ tumor predisposition syndrome, caused by bi-allelic loss-of-function germline variants in the base excision repair (BER) gene MBD4. We identified five individuals with bi-allelic MBD4 variants within four families and these individuals had a personal and/or family history of adenomatous colorectal polyposis, acute myeloid leukemia, and uveal melanoma. MBD4 encodes a glycosylase involved in repair of G:T mismatches resulting from deamination of 5′-methylcytosine. The colorectal adenomas from MBD4-deficient individuals showed a mutator phenotype attributable to mutational signature SBS1, consistent with the function of MBD4. MBD4-deficient polyps harbored somatic mutations in similar driver genes to sporadic colorectal tumors, although AMER1 mutations were more common and KRAS mutations less frequent. Our findings expand the role of BER deficiencies in tumor predisposition. Inclusion of MBD4 in genetic testing for polyposis and multi-tumor phenotypes is warranted to improve disease management.

**Understanding colorectal cancer genetics through multi-omic and trans-ancestral analysis and its impact on cancer prevention**

Riki (Ulrike) Peters, Associate Director and Professor of Public Health Sciences at the Fred Hutchinson Cancer Research Center and Research Professor at School of Public Health Science at the University of Washington

Colorectal cancer is a complex disease with numerous genetic and non-genetic risk factors contributing to its development. Large-scale collaborative efforts have enabled us to identify numerous genetic risk factors as well as gene-environment interactions that point to genes in well-established pathways as well as many novel pathways. While these findings provide enormous opportunities to develop novel targeted agents for chemoprevention of cancer, they are vastly underutilized. I will discuss barriers and the need for a dedicated program that focuses on chemoprevention of cancer utilizing human genetics.

Despite considerable improvements in screening, colorectal cancer remains one of the leading causes of cancer death. To improve screening guidelines, we are developing comprehensive risk prediction models based on our large genome-wide genetic data and harmonized lifestyle and environmental risk factor data using machine learning algorithms. These models can be used to individually tailor screening guidelines to increase adherence, maximize the appropriate use of invasive technologies and reduce mortality.

We demonstrate that our prediction model is substantially more predictive of early onset (<50 years of age) versus late onset colorectal cancer, which is relevant given the rapid increase in early onset colorectal cancer incidence rates. Our harmonized lifestyle data and genetically predicted risk factors (Mendelian Randomization) provide insights into shared and non-shared risk factors for early vs. late onset colorectal cancer.

While most studies have been done in individuals of European ancestry, our current efforts focus on expanding to racially and ethnically diverse populations, which is more representative of the U.S. population. This is critical, as current genetic risk prediction models are substantially more predictive in non-Hispanic White participants.

Colorectal cancer is a biologically heterogeneous disease with somatic mutations in many driver genes. To better understand the relationship of germline genetic, lifestyle, and environmental risk factors with molecularly defined tumor subtypes, we have harmonized existing tumor characteristics from over 12,000 patients and conducted targeted DNA sequencing on over 6,500 colorectal cancer patients. These analyses provide novel insights into differences of tumor characteristics by lifestyle factors, age at onset and show that only a small number of driver genes are associated with survival. Tumor analyses have been primarily done in non-Hispanic White patients and, to a smaller extent, in Asian patients. However, very little is known about the molecular changes in other racial and ethnic groups, including African American and Alaska Native people, who have substantially higher incidence and mortality rates. Our ongoing work is addressing these gaps.

In summary, our collaborative efforts in genetic epidemiology demonstrate enormous opportunities for cancer prevention and precision medicine and point to the need to expand efforts to racially and ethnically diverse populations.