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ORAL PRESENTATIONS' ABSTRACTS

A novel combination therapy improves potency of the oncolytic adenovirus XVir-N-31 by manipulating E2F/RB dependent replication

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BACKGROUND: Genetic manipulations that are introduced into viral genomes to make them "oncolytic" often result in an attenuated replication compared to wild type virus which might correlate with inefficient therapy response. Viruses facilitate their host cells proteome for replication. In particular the retinoblastoma protein-E2F axis, responsible for the control of cell cycle progression from G1 into S-phase, is essential for adenovirus replication.

STUDY AIMS: Development of a novel combination therapy using "small molecule" inhibitors that should manipulate the host cell and thus increase replication of the oncolytic adenovirus XVir-N-31.

MATERIALS & METHODS: Different small molecule kinase inhibitors directed against checkpoint proteins, CDK4/6 or PI3K were tested in combination with the oncolytic adenovirus XVir-N-31, wild type adenovirus type 5 and derivatives from XVir-N-31. Viral genome replication, cell killing and particle formation was tested in bladder cancer and sarcoma cell lines. For promoter studies, the E2-early and E1 enhancer were cloned into reporter plasmids. Protein level were manipulated using siTool siRNA technology. Expression level of proteins was determined by western blotting and gene expression by qPCR. Animal experiments were performed in NMRI-Foxn1 nu/nu and Rag2-/- c-/- mice.

RESULTS: The combination of CDK4/6 inhibitors with XVir-N-31 correlates with an increase in viral genome replication, particle formation and cancer cell killing compared to monotherapy. A novel, E2F trapping, adenovirus vector shows improved early replication. Mechanistically, degradation of the retinoblastoma protein and the transcription factor E2F by CDK4/6 inhibitors induces an earlier and increased expression of essential viral genes. In mouse models, the combination therapy not only reduces tumor size and increases survival of animals but importantly induces an abscopal effect.

CONCLUSION: The combination of CDK4/6 inhibitors and oncolytic adenoviruses improve the oncolytic potency of XVir-N-31 and induces a therapeutically relevant systemic antitumor effect.

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Affinity-proteomics for the discovery of novel biomarkers and therapeutic targets

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Protein profiling plays an essential part in today's biomedical research, striving to improve patients' quality of life by using molecular signatures for more precise diagnostic means and treatment guidance.

At Sciomics, we have established an affinity-proteomics platform for robust protein biomarker discovery and verification. Using our advanced, fully immuno-based scioDiscover assay covering

1,438 proteins with high sensitivity novel biomarker candidates can be identified from minimal sample amounts, only a few microliters of plasma or serum.

Having the ability to analyze tissue, cells as well as body fluid derived samples with the same platform and coverage in a robust manner enables the discovery of innovative biomarker candidates as well as to gain deep insights into the disease biology.

Here, we present the platform, its application in a variety of oncological projects (e.g. pancreatic cancer, bladder cancer and endometrial cancer) as well as our protein biomarker development pipeline.

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AKR1B and AKR1C Enzymes as Biomarker Candidates of Endometrial Cancer

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BACKGROUND: Endometrial cancer (EC) is the most frequent gynecological malignancy in the developed world. There is a great need for prognostic biomarkers for pre-operative stratification of EC patients with high risk of progression and recurrence, who need radical surgery and adjuvant chemo/radio therapy from those with low risk, who have low chances to develop metastases and do not need radical surgery with lymphadenectomy. Prognostic biomarkers would also reduce overtreatment of EC patients with low risk for progression and would lower concurrent unnecessary burden to these patients. Enzymes of the Aldo-Keto Reductase (AKR) subfamilies 1B (AKR1B1 and AKR1B10) and 1C (AKR1C1-AKR1C3) are known to have important roles in progression and chemoresistance of different cancers including EC. Study Aims. To evaluate the potential of AKR1B1, AKR1B10 and AKR1C3 as prognostic biomarkers of endometrial cancer.

MATERIALS AND METHODS: We evaluated the immunohistochemical (IHC) staining of AKR1B1, AKR1B10 and AKR1C3 in tissue paraffin sections from 123 well-characterized patients with EC, including 101 patients with endometrioid EC and 12 patients with serous EC and examined possible correlations between expression of these proteins and other clinicopathological data, including using machine learning methods.

RESULTS: Significantly higher immunohistochemical levels of AKR1B1, AKR1B10 and AKR1C3 were found in adjacent non-neoplastic endometrial tissue compared to endometrioid EC. Patients with IHC staining of both, AKR1B1 and AKR1B10, above the median values showed significantly better overall and disease-free survival compared to all other patients. Multivariant Cox analysis recognized a strong AKR1B1 and AKR1B10 staining as a statistically important survival prediction factor in patients with endometrioid EC and not in patients with serous EC. Also patients with AKR1C3 above median level had better overall survival (hazard ratio, 0.19; 95% confidence interval, 0.06–0.65, $p = 0.008$) and disease-free survival (hazard ratio, 0.328; 95% confidence interval, 0.12–0.88, $p = 0.027$). Machine learning based multi-variate models identified additional factor combinations for survival prediction.

CONCLUSIONS: Our results suggest that AKR1C3, AKR1B1 and AKR1B10 have protective roles in endometrioid EC and represent prognostic biomarker candidates. After validation in larger cohorts

in multicentre studies AKR1C3, AKR1B1 and AKR1B10 may contribute to stratification of patients for more personalized treatments.

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Are urine biomarkers useful to reduce the number of follow up cystoscopies in bladder cancer patients?

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INTRODUCTION: Cystoscopy, the gold standard for bladder cancer surveillance, is an invasive technique with patient discomfort and possible complications. We have previously reported and validated several gene expression classifiers in exfoliated urinary cells with a diagnostic accuracy equal or superior to the current gold standard (cystoscopy combined with cytology). However, these classifiers lack sensitivity in low-grade recurrences and were only evaluated in case-control cohorts. Thus, in this study we aimed to improve our previous urine gene expression classifiers focusing on the detection of non high-risk non-muscle invasive bladder cancer (NMIBC), and develop a new classifier able to decrease the frequency of cystoscopies during bladder cancer (BC) patients' surveillance.

METHODS: A total of 597 urines from BC patients, controls and patients in follow up for BC (PFBC) were included. The study has three phases. In the urinary biomarker discovery phase, 84 urines from BC and control patients were retrospectively included and analyzed by RNA sequencing. In the classifier development phase, a total of 132 selected genes from previous phase were evaluated by nCounter® in 214 prospectively collected urines from PFBC (98 with tumor). A diagnostic classifier was generated by logistic regression. Finally, in the classifier validation phase, a multicentric and international cohort of 248 urines (134 BC and 114 non-recurrent PFBC) was used to validate classifier performance. A total of 521 genes were found differentially expressed between non high-risk NMIBC samples and all other groups ($p<0.05$).

RESULTS: An 8-gene diagnostic classifier with an AUC of 0.893 was developed. Validation of this classifier in a cohort of PFBC achieved an overall sensitivity (SN) and a negative predictive value (NPV) of 96% and 97%, respectively (AUC=0.823). Notably, this accuracy was maintained in non high-risk NMIBC group (SN=94%; NPV=98%).

CONCLUSIONS: The 8-gene expression classifier has high SN and NPV in a real clinical scenario. The use of this classifier can reduce the number of follow up cystoscopies in PFBC, although assessing its final place in clinical setting is necessary.

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BioEndoCar: Biomarkers for Diagnosis and Prognosis of Endometrial Carcinoma

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BACKGROUND: Project BioEndoCa (2018-2022) was funded by the ERA-NET Transcan 2 initiative and included partners from The Netherlands, Germany, Poland, Estonia and Slovenia and clinical collaborators from Czech Republic, Italy and Slovenia. BioEndoCar addressed the lack of non-invasive diagnostic and prognostic biomarkers for endometrial cancer (EC), the most frequent gynecological malignancy in the developed world.

STUDY AIMS: Development of diagnostic and prognostic algorithms based on targeted proteomics, targeted and nontargeted metabolomics and integration of omics data and clinical data for further clinical validation and translation into clinical application.

MATERIALS AND METHODS: Patient recruitment and sample collection tool place at six medical centres (University Medical Centre Ljubljana, University Medical Centre Maribor, Slovenia; Maastricht University Medical Centre, The Netherlands; Lublin Medical University, Poland; University Hospital Brno, Czech Republic and University Genoa, Italy). The consortium employed semi-targeted proteomics (Sciomics GmbH, Germany) and metabolomics (Helmholtz Zentrum München, Germany) approaches to analyze the levels of 1008 different proteins in a total of 302 samples using high content antibody microarrays and the levels of > 900 metabolites and 630 metabolites in 400 and 440 samples using nontargeted and targeted approaches, respectively. Bioinformatics/ biostatistical analyses derived diagnostic/ prognostic algorithms based on metabolites, proteins and clinical data.

RESULTS: BioEndoCar (clinical study NCT03553589) enrolled more than 600 patients with EC and control patients and collected their blood samples and clinical data, and created BioEndoCar biobank, an infrastructure available for additional studies and collaborations. Diagnostic and prognostic algorithms based on blood metabolites, proteins and clinical data were constructed using three different machine-learning approaches. The best diagnostic performance with ROC AUC 0.76-0.82 were found for non-targeted followed by targeted metabolomics and combined dataset with sensitivities from 69% to 79% and specificities from 69% to 76%. Prognostic algorithms revealed unexpectedly low performance with the best AUC of 0.629.

CONCLUSIONS: After additional validation in multicenter studies, diagnostic algorithms may contribute to development of non-invasive tests for early diagnosis of cancer. Biomarkers are also needed for preoperative stratification that may help improving treatment of patients therefore BioEndoCar consortium aims to continue with discovery of prognostic models and is currently searching for funding that would allow continuation/ expansion of the project.

BioEndoCar (https://bioendocar.eu/)

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Biomarkers in urology

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Biomarker can be described as a value of a test (laboratory, imaging, physical) which depends on one or more patophysiological processes in a patient. In urology biomarkers have been used for more than 60 years. Most extensively studied and clinically used biomarkers are prostatic specific antigen (PSA) determined in serum for prostate diseases and urinary cytology obtained from urine for bladder and upper urinary tract cancerous diseases. Usage of biomarkers has also been important in testicular tumors particularly in follow up of the disease.

While main serum components are tightly regulated by the homeostatic mechanisms, concentration of urinary ingredients vary and depend on the state of hydration of an individual. Development, research, and clinical use of urinary biomarkers should therefore consider this fact. Furthermore three additional problems complicate development and use of biomarkers for cancerous diseases in urology: presence of a substance, which is not specific for a cancer, but for an organ (e.g. PSA), minimal concentration, which can be detected by laboratory means and subjective scoring in some biomarkers (cytology). Genetic biomarkers have a potential of being specific for a disease, however tumors have a variable genetic footprint, which can change in metastatic sites. A panel of a number of genetic biomarkers is probably necessary to detect tumors reliably.

Our group has been researching clinical use on biomarkers in prostate cancer and bladder cancer for more than 15 years. In the field of prostate cancer, we have evaluated proPSA, PHI and thymidine kinase, while research activity is still ongoing for the Mitomic test. In the field of bladder cancer our research has focused on comparison of urinary cytology as established biomarker to nuclear matrix protein 22 and BladderChek, as well as Bladder Xpert cancer test. Results of studies on biomarkers performed by our group will be briefly presented in the lecture.

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Cancer genetic screening and personalized cancer risk assessment – Slovenian experience

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The principal national institution for the comprehensive management of cancer in Slovenia is the Institute of Oncology Ljubljana. The fundamental mainstay of the Institute is multidisciplinary approach to cancer treatment, cooperation and effective integration in the network of Slovenian health care. Since 1950, the Institute holds as well the National Cancer Registry, with obligatory cancer registration and represents valuable data storage for the verification of anamnestic data of cancer diagnoses of the deceased family members. As such, the Institute served as a perfect founder of cancer genetic service. In 1994, the idea of providing cancer genetic testing for medullary thyroid

cancer patients, and later on in 1999 for hereditary breast and ovarian cancer, grew into the todays' service. After the referral from primary or secondary level, a patient with suspected hereditary cancer syndrome may receive genetic counseling, multidisciplinary assessment, genetic testing/screening, personalized cancer screening or prophylactic surgery, as well as targeted treatment and psychological support, all at one place.

Besides genetic screening of high-risk individuals, in 2010 personalized cancer screening was organized at the Institute and nowadays more than 1000 individuals are actively invited and screened for breast and other cancers according to the hereditary cancer syndrome and current guidelines.

The Institute holds the National registry of tested individuals from cancer families, where the data from all Slovenian genetic centers is stored in order to closely monitor and research the burden of hereditary cancer syndromes. In 23 years of cancer genetic service, more than 12000 individuals received genetic counseling and the registry reported more than 2200 carriers of pathogenic variants in selected cancer genes that represent approximately 0.1% of the population.

We still have many challenges to face. The first one is the optimization of the current strategies to identify carriers of pathogenic germline variants that predispose for cancer and the second is the implementation of evidence based individualized cancer screening that would be population based, not just opportunistic, so we would overcome current inequalities. In parallel, we must aim to maintain the high quality services despite the high workload volumes that we are facing and will be facing in the future.

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Cell-Free DNA Fragmentation Patterns in a Cancer Cell Line

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BACKGROUND: Cell-free DNA (cfDNA) fragments are constantly being released by cells into bodily fluids. These fragments carry important genetic, biological and pathological information which can be used to inform on their tissues of origin. One of the key sources of information encoded within these fragments is their epigenetic features which include DNA methylation, histone modifications, and fragmentation profiles of cfDNA.

STUDY AIMS AND MATERIALS AND METHODS: We compared the cfDNA fragmentation patterns of cultured human bone cancer (143B) cells by employing electrophoresis assays of increasing sensitivity. This included four automated capillary electrophoresis assays from Agilent, i.e. the DNA 1000 and High Sensitivity DNA kits for the Bioanalyzer system and the dsDNA 915 and dsDNA 930 kits for the Fragment Analyzer system. We also included an optimized manual agarose gel electrophoresis protocol.

RESULTS: Our findings showed that 1) with increasing resolving power, the sizing methods were able to reveal additional multiples of nucleosomes (up to 7 nucleosomes with manual agarose gel electrophoresis) ii) the cfDNA laddering pattern observed with commonly used methods extends beyond 1-3 nucleosome multiples, and iii) the modal size and relative concentration of the high molecular weight (HMW) cfDNA populations are exaggerated due to the limiting resolving power of electrophoretic methods in these size ranges, and that the large peaks/bumps corresponding to these populations instead consist of several smaller, low concentration poly-nucleosomal subpopulations that continue the DNA laddering pattern as shown in the smaller populations. Furthermore, we also found that the relative contribution of the increasingly longer cfDNA populations decay exponentially, revealing a power-law distribution. This suggested that cfDNA is subject to a stochastic inter-nucleosomal DNA cleavage process which results in shorter fragments accumulating more rapidly due to the degradation of larger populations which then feeds the shorter populat.

CONCLUSIONS: These findings may explain why the size profiles of DNA populations originating from apoptosis, accidental cell lysis, necrosis, and purported active release have historically been reported as looking similar. Our results also made it clear that by using different sizing methods diverse size profiles were revealed and that one should be cautious of drawing conclusions based on the results of only a single sizing method.

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Circulating Tumor DNA (ctDNA) as a Cancer Biomarker: Will it Replace Standard Protein Biomarkers?

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Protein-based biomarkers are widely used in monitoring patients with diagnosed cancer. These biomarkers however, lack specificity for cancer and have poor sensitivity in detecting early recurrences and monitoring therapy effectiveness. Emerging data suggests that the use of circulating tumor DNA (ctDNA) has several advantages over standard biomarkers. Thus, following curative-intent surgery for cancer, the presence of ctDNA is highly predictive of early disease recurrence while in metastatic cancer an early decline in ctDNA following the initiation of treatment is predictive of good outcome. Compared with protein biomarkers, ctDNA provides greater cancer specificity and sensitivity for detecting early recurrent/metastatic disease. Thus, in patients with surgically resected colorectal cancer, multiple studies have shown that ctDNA is superior to CEA in detecting residual disease and early recurrence. Similarly, in breast cancer, ctDNA was shown to be more accurate than CA 15-3 in detecting early recurrences. Other advantages of ctDNA over protein biomarkers in monitoring cancer patients include a shorter half-life in plasma, an ability to predict likely response to specific therapies and identify mechanisms of therapy resistance. In contrast to proteins however, ctDNA biomarkers are more expensive to measure, less widely available and have longer turn-around times for reporting. Furthermore, ctDNA assays are less well standardized. Because of their advantages, it is likely that ctDNA measurements, will in the future, enter clinical use where they will complement existing biomarkers and imaging in managing patients with cancer. Hopefully, these combined approaches will lead to a better outcome for patients.

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Clinical Implications of CAR-T Cell Therapy

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BACKGROUND: CAR-T cell therapy is a novel therapeutic approach changing the landscape of hematologic malignancies. The first commercial CAR-T cell products were licensed for acute lymphoblastic leukemia and non-Hodgkin's lymphoma. The scope of new study results, indications and licensed products is increasing leading to new treatment options not only for relapsed/refractory disease but also in second even first line treatments.

STUDY AIMS: Presenting current state of CAR-T cell therapy in hematology and our institution experience with commercial product as well as efforts of implementing production of academic CAR-T cell therapy.

MATERIALS AND METHODS: We performed a relevant literature review and retrospectively analyzed our patient data. The project of implementing our own CAR-T cell production is presented.

RESULTS: Tisagenlecleucel is the first and most widespread CAR-T cell product for acute lymphoblastic leukemia and diffuse large B cell lymphoma. At the time of writing there are six approved CAR-T cell therapies by FDA/EMA with several waiting for the approval. The other indications include mantel cell lymphoma, follicular lymphoma and multiple myeloma. Several hundred studies with different, improved CAR-T cell products are being conducted mainly in China, United States and European Union for hematologic and solid tumors. Treatment results are encouraging in patients with advanced disease and every effort is being made to study this approach in earlier stages of disease. Our transplant center is a medium sized with experience in CAR-T cell therapy since 2020 on 13 patients. With the aim of extending this therapy to patients without commercial indication we decided to develop our own academic program.

CONCLUSIONS: CAR-T cell therapy is the new and efficient treatment for some advanced hematologic conditions. Commercial products are effective but not available to all potential patients. Academic CAR-T cell therapies can be implemented in such circumstances.

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Clinical importance of BRCA 1/2 gene mutations in ovarian cancer

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Ovarian cancer is a disease with poorest prognosis in gynaecologic oncology. Despite agressive surgical approach and adjuvant systemic treatment (chemotherapy and bevacizumab), majority of patients have relapse of the disease. The- median progression free survival (PFS) is up to 18 months, while median overall survival (OS) is 40 months and 5 – year OS is only 40%. Until recently, there was no clinicaly relevant predictive biomarker to tailor treatment in these patients.

The introduction of poly-ADP-ribose polymerase (PARP) inhibitors into treatment of ovarian cancer showed that BRCA 1/2 gene mutations represent important biological target for treatment with PARP inhibitors. Olaparib is the first PARP inhibitor that showed activity in relapsed ovarian cancer in maintenance treatment after response to platinum based chemotherapy. In patients with relapsed BRCA 1/2 gene mutated ovarian cancer maintenance treatment with olaparib meaningfully improved PFS compared to placebo. Two other PARP inhibitors, niraparib and rucaparib, showed similar improvement of PFS. PARP inhibitors have shown activity in BRCA 1/2 non-mutated relapsed ovarian cancer also, but benefit in patients with BRCA 1/2 mutated tumors is substantionaly better.

Since relapsed disease is incurable, all efforts should be made to improve first-line treatment with the aim of cure of the disease. Recently, PARP inhibitors have been implemented into first-line therapy of ovarian cancer. Study SOLO-1 showed impressive activity of maintenance treatment with olaparib in first-line BRCA 1/2 mutated ovarian cancer. Patients with mutations in BRCA 1/2 genes (germline or somatic) who recieved olaparib (after initial surgery and chemotherapy) had meaningful improvement of PFS (56 months vs. 14 months; HR 0.33) compared to placebo. In addition, niraparib and rucaparib also showed clinical benefit in first-line setting regardless of BRCA 1/2 gene mutations, but benefit of treatment with PARP inhibitors is much better in BRCA 1/2 mutated disease.

The detection of BRCA 1/2 gene mutation (germline or somatic) has become standard procedure at diagnosis of ovarian cancer. The aim for detection of BRCA gene 1/2 mutations is maintenance therapy with PARP inhibitors in first line offering best survival and potential cure of disease.
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Design of a national screening programme

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Preventive medicine and public health share many common goals. First, both strive to promote quality of life by preventing disease. This goal is accomplished through health promotion programs and prevention of common diseases. Prevention occurs at three levels. The first level is primary prevention. These are preventive measures that are undertaken to prevent the onset of illness and injury. Secondary prevention entails measures that lead to early diagnosis and prompt treatment of illness or injury. Tertiary prevention involves measures aimed at minimizing disability after disease symptoms have appeared.

Screening is defined as the presumptive identification of an unrecognized disease or defect through tests, exams, or other procedures that can be applied rapidly and easily. Screening tests differentiate apparently healthy persons who may have a disease from those who probably don't have the disease.

Periodic health screening can lead to early detection and diagnosis of a disease. This early detection then leads to earlier treatment with a goal of decreasing mortality and morbidity related to that disease. Screenings can be cost-effective if the disease is common enough and the test is accurate enough. It's also cost-effective if affordable treatments that work are accessible to those patients whom test positive.

The best screening protocols generally incorporate a patient history, physical exam, and laboratory test. Positive results of the screening test will trigger a diagnostic work-up and preventive or treatment interventions.

Screening tests are used for a presumptive identification of an unrecognized disease or illness. Diagnostic tests, on the other hand, are used to determine the presence or absence of a disease when the patient is showing symptoms of the disease. In some circumstances, the same test can function as either a screening or diagnostic test.

A good screening test must meet several important criteria. It needs to be simple and quick to administer. It should be inexpensive and safe to use. It also needs to be readily available, along with an accessible plan of treatment in place in case of positive results. A good screen must be acceptable to the population in which it will be used. It must also be well researched and proven to be valid, reliable, and to have good predictive values.

Two central concepts in evaluation of tests are validity and reliability. Validity is defined as how well the test result corresponds to the "true" condition of the patient, i.e. whether or not the person has the disease. Reliability, on the other hand, evaluates how consistent or reproducible the test results are over time or under different testing conditions. Sometimes, we use a series of tests to screen for a condition (the presence of Down syndrome in a fetus).

Screening and diagnosis are not the same thing. Screening is conducted on asymptomatic patients, while diagnosis is conducted on patients showing some signs or symptoms of the target disease.

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Detection of BRAF mutations by droplet digital PCR in circulating tumor DNA of non-metastatic melanoma

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BACKGROUND: Implementation of adjuvant therapies in surgicaly treated non-metastatic melanoma improved treatment outcomes in some patients. However, adjuvant therapy can be associated with risk of toxicity. Therefore, there is an unmet need to better identify patients at high risk of recurrence.

PATIENTS AND METHODS: We carried out an ultrasensitive droplet digital PCR (ddPCR)-based detection of BRAFV600Emutated circulating tumor DNA (ctDNA) from blood samples prospectively collected before surgery, 1 hour after surgery, and then serially during follow-up.

RESULTS: In 80 patients (stages III), BRAFV600E mutations were detected in 47.2% of tissue, in 37.7% of ctDNA samples collected before surgery, and in 25.9% of ctDNA samples collected 1 hour after surgery. Patients with detected ctDNA in blood collected 1 hour after surgery compared to patients without detected ctDNA had higher likelihood of melanoma recurrence ($P < 0.001$) and shorter median disease-free survival (P ¼ 0.001) and overall survival (P ¼ 0.003).

CONCLUSIONS: Ultrasensitive ddPCR can detect ctDNA in pre- and post-surgical blood samples from patients with resectable melanoma. Detection of ctDNA in post-surgical samples is associated with inferior treatment outcomes.

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Detection of somatic mutations with ddPCR from liquid biopsy of CRC patients

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With the ever-increasing use of biological drugs in cancer treatment, tumor mutation status has become an essential part of molecular diagnostics that enables optimal treatment response. Genetic screens for somatic mutations, that arise spontaneously within tumor cells, are performed on genes including KRAS, BRAF, and NRAS that are known to be frequently mutated in CRC tumors and confer resistance to therapies directed against the EGF signaling pathway. Considering that these mutations provide a selective advantage for tumor cell survival, detecting somatic mutations present in wild-type backgrounds with as low as $< 0.1\%$ is of potential diagnostic utility. Currently, the main source of tumor sample DNA for mutation detection is paraffin-embedded tissue. However, in recent years liquid biopsy has become a promising alternative. Liquid biopsy is obtained from venous blood, which represents a source of cell-free DNA (cfDNA) that can be isolated from either plasma or serum fractions. While the liquid biopsy is not included in current protocols for mCRC patient

management, it has been extensively studied as a non-invasive method for treatment response and patient monitoring. Its use in routine practice however needs to be technically and clinically validated.

The aim of the study was to determine the cfDNA levels and the KRAS and BRAF mutation status of a group of patients who underwent surgical removal of primary CRC. Since the source of cfDNA in the bloodstream can be of various origins (different necrotic/apoptotic processes), we compared cfDNA concentrations of CRC patients to two different groups (hemorrhoid patients and healthy individuals). cfDNA was isolated from serum and its concentration was measured with our custom-designed human gDNA ddPCR assay which enables efficient and specific measurement of low concentrations of human DNA. The highest concentration of cfDNA was measured in CRC patients group (average 0.44 ng/ul), while both hemorrhoid patients and healthy individuals had significantly lower average concentrations, 0.25 ng/ul (* $p = 0.01$) and 0.08 ng/ul ($p = 0.0001$), respectively. Elevated cfDNA concentration can be an indicator of pathological or necrotic processes occurring in the body. However, these kinds of results must be interpreted with caution since other factors in addition to cancer can be the cause of elevated cfDNA serum concentrations.

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Diagnostic algorithms ensure recognition of Ph-like acute lymphoblastic leukemia molecular markers

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Philadelphia chromosome (Ph)-like acute lymphoblastic leukemia (ALL) is characterized by an expression profile similar to Ph+ ALL, but lacking the BCR::ABL1 fusion gene. It is a biologically and clinically challenging subtype of B-ALL, independently associated with adverse outcome. Exact genomic profiling of B-ALL subgroups and Ph-like recognition is crucial not only for prognostication, but even more due to the new therapeutic options given by targeted therapies. Since there is still no single standardized diagnostic test enabling prompt recognition of Ph-like ALL, integration of multiple existing techniques is another approach. Diagnostic algorithms combining different widely available techniques can ensure its reliable detection.

We present our own algorithm, which integrates the following methods: chromosome banding analysis, flow cytometry, RT-PCR, FISH, and next generation sequencing (NGS). We retrospectively analyzed cytogenetic and molecular genetic data of patients in which B-ALL was confirmed by immunophenotyping first. Next, we upgraded the analysis by CRLF2 rearrangement (CRLF2-r) detection by FISH and proceeded by NGS testing by a panel of genes altered in B-ALL. Based on age, we separated patients into three groups: pediatric cases (<20 years), young adults (20-39 years), and adults (>39 years).

CRLF2-r was tested in the vast majority (91%) of B-ALL samples without recurrent genetic abnormalities which are mutually exclusive with Ph-like ALL. CRLF2-r was confirmed in 10% of tested cases, and 3.4% of all B-ALL patients. Due to a lack of material, NGS was done only in a half of potentially Ph-like cases. In 10% of tested patients other Ph-like fusions were found by NGS. The whole group with the confirmed Ph-like ALL shared the main characteristics of this genetic subgroup. Patients were predominantly males, young adults with normal karyotype, and poor disease course. The overall frequency of Ph-like ALL in our population is not considerably higher than 10%, which is in accordance with previously reported data for the adult European population.

We verified particular steps on ALL samples routinely analyzed in our laboratory by comparing obtained data with existing literature. The obtained frequencies, genetic and patients' characteristics

are in concordance with the literature data, ensuring that the proposed algorithm allows reliable detection of Ph-like ALL.

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Eliminating remnant half-life-dependent antigen: An innovative approach for a more accurate tumor marker follow-up

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OBJECTIVES: Tumor markers provide insights into the course of disease and response to therapy in carcinoma patients, allowing physicians to determine changes in marker levels during disease. We aimed to evaluate the value of newly produced CA 19-9 levels in determining the response to treatment and prognosis in patients with pancreatic adenocarcinoma.

METHODS: In a patient with pancreatic carcinoma, remnant CA 19-9 levels were determined by calculating the amount of biological decay of half-life-dependent CA 19-9. The amount of newly produced CA 19-9 was determined by subtracting the remnant CA 19-9 concentration from the actual CA 19-9 concentration.

RESULTS: The calculated levels of newly produced and remnant CA 19-9 varied according to the sampling frequency and biological half-life of the tumor marker. Throughout the disease, the shape and gradient of the curve for the level of newly produced CA 19 9 were shifted compared with those for the actual measured serum levels.

CONCLUSIONS: The level of newly produced CA 19-9 significantly differs from the actual measured serum levels. The amount of newly produced CA 19-9 provides a more realistic and accurate indication of the effects of treatment and prognosis throughout the course of disease.

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Elucidating the role of ETS2 in colorectal cancer

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BACKGROUND: ETS2 belongs to the largest family of transcription factors and has several implications in health and disease. Oncogenic roles of ETS2 have been shown in esophageal squamous cell carcinoma, hypopharangeal cancer, prostate and breast cancer. Conversely, a tumour suppressive role has been seen in hepatocellular carcinoma, non-small cell lung cancer and gastric cancer. Regulation of distinct pathways in these two conditions enables it to carry out its unique context based functions. RNA-Seq data across different cancers has revealed that colorectal cancer (CRC) shows highest levels of ETS2. However its mechanism of action and the pathways associated have not been explored yet.

STUDY AIMS: In this study, we investigated the expression of ETS2 in CRC biopsy samples and its role in CRC progression.

MATERIALS AND METHODS: We carried out our study on clinical samples and CRC cell lines using Immunohistochemistry, Immunofluorescence, Lentiviral based gene silencing, plasmid based constitutive overexpression, Proteomic analysis, Flow cytometry, Western Blotting and Real Time PCR assays

RESULTS: In our investigation, we found a significantly elevated expression of ETS2 in CRC biopsies at mRNA and protein levels. Further, to assess its role in CRC, gene silencing studies were carried out that revealed G2/M arrest. Cells progressed through initial G1 phase but were stalled later, due to reduction in pCDC25B and CDC16, despite an elevated expression of cyclin B1. Proteomic evaluation of ETS2 overexpression revealed a novel Ral family protein interaction, implicating its role in mitosis. Sphere formation assay showed increased expression of ETS2 in 3D cultures attributing it to a stemness role. Migration and invasion were also found to be altered upon silencing ETS2. These results cumulatively demonstrate the role of ETS2 in colorectal cancer progression.

CONCLUSIONS: ETS2 has been implicated to have novel functions in CRC through regulation of the mitotic machinery. Additionally, we demonstrate that ETS2 is a crucial factor in colorectal cancer progression. Further studies to delineate the mechanism of ETS2 in regulating stem cell markers in cancer are being explored.

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Establishing accuracy- and precision-ROC-curves for bioassay validation including their interrelations with sensitivity/specificity ROC-curves

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INTRODUCTION: "traditional" ROC-curves provide information about recall (= sensitivity) and specificity.

STUDY AIM: A measurement system is considered valid if it is both accurate and precise. Construction of ROC-curves for accuracy and for precision allow direct comparison with "traditional" ROC-curves.

METHODS AND PATIENTS: ROC-curves have been made using data from a study which applied the UBC® Rapid Test as a point of care test (POC-assay) for detection of urinary bladder cancer, including diagnostics with a quantitative reader system, compared to the visual test only. Sensitivity/specificity (SS-), accuracy/specificity (AS-), precision/specificity (PS-), and F1-Score-ROC-curves were established from setting various cut-off values over the complete concentration range of the UBC® Rapid Test, based on data from patients (n = 1243) with or without clinical proven urinary bladder cancer. For direct comparison, all curves were plotted in a single diagram and compared to the visual test results.

RESULTS AND DISCUSSION: The SS-AS-PS-ROC-plot for the UBC® Rapid Test reader system demonstrated an inverse relationship between precision and recall, and a correlation where the increase of one is at the cost of reducing the other. While the recall curve includes an area under the curve (AUC) with respect to the diagonal at its base, an area over the curve (AOC) can be calculated for precision at the opposite diagonal. With decreasing accuracy, precision was only 0.50 in the beginning, including a fast decrease to 0.0. Accuracy was 0.92 and constantly decreased to 0.0, correlating to specificity in a nearly straight line. An "AOC" area could be calculated for accuracy as well. Sensitivity/precision optimum was 30 μg/l. At recall of 0.659, specificity of 0.63, accuracy of 0.634, and a precision of 0.124, the estimated visual test cut-off was 6.2 μg/l. Those results fitted well into the corresponding ROC-curves, showing a good conformity.

CONCLUSIONS: Accuracy- and precision-ROC curves allow direct comparison to "traditional" recall ROC-curves, comparison of different assays, and investigation of the interrelations between these parameters in a single SS-AS-PS-F1-Score-ROC-plot.

KEYWORDS: Accuracy ROC-curve; precision ROC-curve; F1-Score-ROC-curves; SS-AS-PS-F1-Score-ROC-plot.

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EV-associated DNA as biomarker: potential and challenges

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Clonal evolution and heterogeneity of tumors that survived the initial therapy or gained additional mutations independent of treatment stress is the primary cause of relapse in cancer. Therefore, sensitive and reliable methods to accurately measure the mutational shifts between diagnoses during therapy and relapse could provide helpful information on the disease progression. Extracellular vesicles (EVs) such as exosomes (diameter: 70-150 nm) released from tumor cells have emerged as potential valuable biomarkers as they have been illustrated to feature disease-specific nucleic acid signatures representing the pathological state of the individual cells. While cancer cells release relatively high amounts of EVs compared to healthy cells, mutational profiling of EV-DNA could provide a sensitive method to track the evolution of rare cancer sub-clones. A significant limitation in the field of studies dealing with cancer EVs is the heterogeneity in preparing corresponding samples. Currently, our project aims to evaluate and identify novel tumor-derived EV-DNA diagnostic and prognostic potential. Additionally, to develop a sensitive diagnostic assay, we plan to go a step further and create a novel method, finally allowing the preparation and analysis of purified EV subpopulations. Subsequently, this method will be validated and qualified for the diagnostic studies of cancer patient samples.

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Final results of a 2:1 control-case observational study using immunotherapy in addition to first-line hormone therapy, in ER+ metastatic breast cancer

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BACKGROUND: ER+/HER2- breast cancer is the most common type of metastatic breast cancer. It is considered immunologically "cold"; therefore, immunological therapy is not suitable for it. In this setting, therapies interfering with E2 signalling have been seminal in reducing breast cancer mortality. Nevertheless, acquired resistance occurs in about 30-50% of these patients.

STUDY AIM: We conducted a pilot study that combines immunotherapy (cyclic interleukin-2 interferon-beta sequence) and hormone therapy (HT) to overcome endocrine resistance in metastatic breast cancer.

MATERIALS AND METHODS: The final results of a 2:1 control-case retrospective observational study are here shown following 22 additional months of postoperative follow-up and 6 further controls. There were 95 controls and 42 cases in total. The 95 controls were ER+/HER2- metastatic breast cancer patients who underwent first-line HT with aromatase inhibitors (AIs) or fulvestrant. Twenty-eight of them (28.9%) also received biological drugs including cyclin kinase inhibitors (CKIs). The 42 cases were ER+ metastatic breast cancer patients who received interferon betainterleukin- 2 immunotherapy in addition to first-line HT. Selective estrogen receptor modulators/down-regulators (SERMs/SERDs) were used for HT in 39 (92.9%) of them and AIs in the remaining 3.

RESULTS: Median progression-free survival (PFS) and overall survival (OS) were significantly longer in the 42 studied patients who received hormone immunotherapy (HIT) than in the 95 controls (median time 33 vs. 18 months, $P = 0.002$, and 81 vs. 62 months, $P = 0.019$). In the analysis adjusted for disease-free interval (DFI), hormone receptor, HER2 status, visceral involvement, AIs, and biological therapy, the PFS and OS hazard ratio (HR) further increased in favor of the 42 cases ($P = 0.004$ and $P = 0.044$ respectively). In the same ER+/HER2- metastatic breast cancer patients treated with both AIs

and CKIs, a median PFS ranging from 25.3 to 28.18 months and a median OS of 37.5 months were observed.

CONCLUSIONS: This study strongly suggests multi-center randomized clinical trials should be performed to enter our proposed immunotherapy into clinical practice.

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Final results of the german multicenter-study: urinary based rapid tests in comparison to cytology

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BTA stat®, Alere NMP22® BladderChek®, UBC® rapid test and UBC® rapid test visual are urine-based rapid tests for the presence of urinary bladder cancer (BC). uromonitor® is a urine-based test measuring FGFR3, KRAS and TERT mutation. This multicentre study is the first to compare the performance of all available rapid tests with urine cytology.

488 patients with cystoscopy-verified bladder cancer (BC), 79 patients with no evidence of disease and 221 healthy controls were enrolled in this prospective study. Bladder cancer group included 175 low-grade non-muscle invasive, 185 high-grade non-muscle-invasive, and 71 high-grade muscle invasive tumors. Urine samples were analyzed by voided urine cytology, uromonitor®, BTA stat®, Alere NMP22® BladderChek®, and UBC® Rapid test. UBC Rapid® test was assessed qualitatively and quantitatively using the point-of-care (POC) system concile® Ω100 POC reader using a cutoff of 10 ng/ml.

BTA stat®, Alere NMP22® BladderChek®, UBC® rapid test, UBC® rapid test visual, uromonitor®, and cytology showed a sensitivity of 75.4%, 30.6%, 70.3%, 44.7%, 49.1%, 56.1%, and a specificity of 71.1%, 96.7%, 79.0%, 94.0%, 88.7%, and 85.7% respectively. BTA stat® and quantitative UBC® Rapid test proved to be the best dual combination with the highest overall sensitivity (58.7%) and a specificity of 88.6%.

Sensitivity increased in cytology, uromonitor®, NMP22®, BTA stat® and qualitative and quantitative UBC® Rapid test to 70.3%, 55.1%, 44.6%, 84.8%, 57.2%, and 79.7%, respectively, for estimating the risk for high-grade BC.

BTA stat® and the quantitative UBC® rapid test showed higher sensitivity in detecting BC compared to urine cytology, but at the expense of lower specificity. A dual combination of these two tests outperforms urine cytology in terms of higher sensitivity and specificity, making them a potential alternative for the detection of BC.

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Genetic analysis of archival tissue when testing for hereditary cancer predispositions

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BACKGROUND: In families, where genetic testing for hereditary cancer predispositions is undertaken, it is preferable to test cancer patients first, followed by targeted testing of their healthy relatives, if necessary. Such an approach yields most informative results whilst minimizing the number of unnecessary genetic tests. In cases where cancer patients are already deceased, testing formalin-fixed-paraffin-embedded (FFPE) tumour or non-tumour tissue samples is increasingly being used as an alternative to testing unaffected family members.

STUDY AIMS: To identify and present cases of successful DNA analysis of FFPE tissue samples from deceased individuals using next generation sequencing (NGS) based approaches.

MATERIALS AND METHODS: In order to identify cases where FFPE tissue DNA of deceased patients was used for genetic testing in the clinical setting, we obtained relevant clinical information from the archives of the Department of Clinical Cancer Genetics, Institute of Oncology Ljubljana. Identification of germline and somatic genetic variants was performed using targeted next-generation sequencing or Sanger sequencing of DNA samples, isolated from FFPE tumour or healthy/non-neoplastic tissue samples.

RESULTS: We present two cases of FFPE genetic testing in families undergoing genetic assessment for hereditary cancer. In Family 1, the proband was a healthy 28-year-old female, whose mother had developed a clear-cell renal carcinoma aged 41 and died within a year. NGS-based panel testing of the mother's tumour tissue revealed a pathogenic variant c.263G>T p.(Trp88Leu) in the VHL gene, absent from her non-neoplastic tissue, rendering genetic testing in the proband unnecessary. A healthy 53-year-old proband in Family 2 reported her mother had developed a breast carcinoma aged 35 and died aged 42. The mother's FFPE non-neoplastic tissue sample collected nearly 40 years previously was successfully analysed and revealed a pathogenic variant in BRCA2 (c.7806-2A>G p.?), which our proband had not inherited.

CONCLUSIONS: We demonstrate the usefulness of genetic analysis of FFPE samples from affected deceased individuals in families with suspected hereditary cancer syndromes. Causative variants were identified using tissue samples in Family 1 and Family 2, a somatic VHL variant and a germline BRCA2 variant, respectively. More accurate estimates of cancer risk for probands were achieved using FFPE genetic testing than would have been possible had only healthy.

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Genetic testing of ovarian tumour samples at the Institute of oncology Ljubljana

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In Slovene population, ovarian cancer (OC) is the is the sixth leading cause of cancer death among women. Hereditary breast and ovarian cancer (HBOC), is often in association with inherited variants in BRCA1 and BRCA2 as well as in other breast and/or ovarian cancer susceptibility genes. BRCA1/2 mutated or homologous recombination deficient epithelial OC (EOC) can be treated with PARP inhibitors in different treatment settings. Consequently, the detection of germline and somatic

pathogenic/likely pathogenic variants (PV/LPV) in BRCA1/2 genes is important for treatment decisions as well as cancer risk assessment. The aim of our study was to compare testing workflows in EOC patients using germline and tumor genotyping of BRCA1/2 and other HBOC susceptibility genes.

Patients with advanced non-mucinous EOC, who responded to platinum-based chemotherapy, were included in the study. DNA extracted from blood and FFPE tumor tissue were genotyped using Illuminas NGS panels.

Germline or somatic PV/LPV in BRCA1/2 genes were detected in 21,8% of 170 non-mucinous EOC patients. Additionally 6.4% had PV/LPV in other HBOC genes. Sensitivity of tumor genotyping for detection of germline PV/LPV was 96.2% for BRCA1/2 genes and 93.3% for HBOC genes. With germline genotyping-only strategy, 58.8% of HBOC PV/LPV and 68.4% of BRCA1/2 PV/LPV were detected. By tumor genotyping-only strategy, 96.1% of HBOC PV/LPV and 97.4% of BRCA1/2 PV/LPV were detected.

Genotyping of tumor first, followed by germline genotyping seems to be a reasonable approach for detection of somatic and germline PV/LPV in HBOC susceptibility genes in non-mucinous EOC patients, giving highest yield in shortest time frame.

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Gut Microbiome and Fecal Transplantation in Cancer Tretment - State of the art literature review

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The wide genetic diversity of the human microbiome is associated with numerous physiological and pathological pathways and enables its unique ability to adapt to varying environments. The microbiome structure is typically analyzed by next-generation sequencing. The functional analysis includes the influence on various metabolites such as short-chain fatty acids (SCFAs) and bile acids within the intestine. The intestinal microbiota plays an important role in the production of SCFAs (butyrate, acetate, and propionate). The interplay of these metabolites can have a significant effect on the immune system.

Dysbiosis has been related to several pathological conditions like inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), colorectal cancer (CRC), etc. and several other extra-intestinal conditions like type 1 & 2 diabetes, obesity, and more.

Restoration of dysbiosis has emerged as a promising therapeutic approach. Several approaches have been investigated to achieve this goal, including prebiotics and probiotics, fecal microbiota transplantation (FMT), extracellular vesicles, immune modulation, microbial metabolites, dietary interventions, and phages.

Immunotherapy to inhibit the programmed cell death–1 (PD-1) checkpoint protein in metastatic melanoma patients has demonstrated durable complete response (CR) rates of 10 to 20%. Extensive research efforts have been done to overcome the resistance to anti–PD-1 therapy. One of the most promising approaches involves the modulation of the gut microbiota. Several studies indicated that the immune status of cancer patients, as well as their response to therapies, are associated with different patterns of the gut microbiome.

It has been reported recently that FMT from PD-1 responder patients into non-responders, resulted in clinical benefit, by turning some of these patients into responders. Thus, modulating gut microbiota could turn into a promising approach for improving cancer immune therapy.

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How to find measurable residual disease markers in hematological malignancies?

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BACKGROUND: Measurable residual disease (MRD) is an important biomarker in malignancies used for prognosis, prediction, monitoring, and assessment of response to treatment. Detection of MRD during morphologic remission after standard chemotherapy is a strong prognostic factor for subsequent relapse. MRD monitoring requires a reliable marker determined at the time of diagnosis in addition to a very sensitive technique. Two well-established techniques are sensitive enough: multiparametric flow cytometry and qPCR. In acute myeloid leukemia (AML), almost half of patients have a specific and reliable molecular MRD marker. In mutant NPM1 three different transcripts can be tracked. In AML with core binding factor and in acute promyelocytic leukemia, fusion genes are used as MRD markers. qPCR is the most widely used technique for sensitive monitoring of these markers. The sensitivity of the qPCR assay is as high as 10-5. In the remaining half of AML patients without above mentioned MRD markers, NGS panels of genes commonly mutated in AML is used to detect variants that can be followed.

STUDY AIM: To determine the proportion of AML patients with potential MRD markers detected by NGS.

RESULTS: We analyzed our cohort of AML patients in whom standard molecular MRD markers had been previously excluded. In only 1/48 patients, no variant was found using a panel of 75 genes (Archer VariantPlex Myeloid). In 21/49 patients, variants were detected in the ASXL1, TET2, and DNMT3A genes, which are not suitable MRD markers because they may be part of clonal hematopoiesis. However, they were not observed as the only variant in any of the patients and can be used as an additional marker. Only one variant was detected in 2/48 patients, whereas up to 9 variants were detected in others. One of the challenges of NGS analysis in AML is also the detection of germline variants. Consistent with literature data, DDX41 was also the most common germline variant in our group.

CONCLUSIONS: In recent years, NGS has become an important technique in the routine diagnosis of AML, not only to classify the disease but also to detect potential MRD markers. In the majority of AML patients, an appropriate MRD marker or a combination of them can be found.

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Identifying bladder cancer biomarkers using –omics

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Uncovering epigenetic, genetic, transcript, protein, peptide or metabolic alterations are critical for further understanding of the critical pathways leading to cancer at each specific progression step. The advent of high-throughput techniques has accelerated the discovery of the molecular alterations underlying carcinogenesis and tumor progression. Nowadays it is possible to characterize molecular events at the DNA, RNA, protein or metabolite level. These approaches are relevant to identify biomarkers that may aid for the diagnosis as well as the subcassification of tumors and select indolent lesions from aggressive tumors. We have designed and optimized several –omics strategies at different levels to uncover molecular alterations underlying carcinogenesis and cancer progression and identify profiles and individual biomarkers that meet the clinical needs of bladder cancer patients. At the DNA level, we utilized epigenetic high-throughput technologies such as methylation arrays in tissue samples to identify molecular profiles differentially expressed between tumors and matching normal urotheilium. These analyses revealed a profile of 84 hypermethylated genes differentially expressed

between tumors and matching urothelium plus identified SOX9 and PMF1 as novel genes methylated in bladder cancer. At the genomic level, we optimized the use of CGH arrays in urinary samples to identify DNA gains and losses that may aid for bladder cancer diagnostics. These genomic analyses revealed the ampification of prefoldin in bladder cancer. At the RNA level, we profiled miRNAs in tissue samples comparing matching tumors and normal urothelium. miRNA profiles identified the association of miR.143, miR-222 and miR-452 with tumor progression and clinical outcome and as urinary markers. At the protein level several proteomic approaches were designed to identify biomarkers. We optimized iTRAQ and SILAC approaches in vitro to identify molecular alterations related to bladder cancer metastases. These analyses identified filamin and CUL3 associated with bladder cancer progression. In serum samples antibody arrays and protein arrays were applied to uncover novel protein and antibodies related to bladder cancer. In the urine we optimized DIGE to uncover novel protein biomarkers. Selected candidates from each approach were validated in independent series of patients by independent techiniques to prove the utility of novel candidates as biomarkers. In summary, our studies uncovered molecular alterations underlying carcinogenesis and cancer progression and identified profiles and individual biomarkers that may meet clinical needs of bladder cancer patients.

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Imaging and clinical biomarkers for the prediction of survival in patients with hepatocellular carcinoma treated with transarterial chemoembolization

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BACKGROUND: Transarterial chemoembolization (TACE) is the standard treatment of intermediate-stage hepatocellular carcinoma (HCC). Current prognostic factors of survival in patients treated with TACE are mainly based on clinical assessment. Apart from the well-known clinical factors related to tumor stage and liver function, remarkably few data are available upon other prognostic factors of survival. Moreover, treatment induced hypoxia after TACE has been shown to stimulate tumor angiogenesis through upregulation of hypoxia-inducible factor proteins which induce expression of proangiogenic factors, making them a possible important biomarker of survival. Thus, careful selection of patients likely to respond and benefit from TACE using a noninvasive imaging and clinical biomarker seems important.

METHODS: The mechanism of TACE is a selective delivery of a high local concentration of a chemotherapeutic drug mixed with embolic material, which results in strong local cytotoxic and ischemic effects. Computed tomographic perfusion imaging (CTPI) is a dynamic, contrast-enhanced, minimally invasive functional radiologic imaging technique. The basis for the use of CTPI in oncology are the microvascular changes in angiogenesis which reflect as increased tumor vascularization. Expression of individual proangiogenic factors correlates with tumor angiogenesis, increased capillarization of sinusoids, degree of differentiation, local aggressiveness, and extrahepatic spread of HCC.

RESULTS: In our previous studies we demonstrated that CTP parameters, Child Pugh class, ascites and tumour burden have been linked to survival rates and can be used to select appropriate candidates for TACE. Survival was statistically significantly longer in patients with hepatic blood flow (BF) lower than 50.44 ml/100 ml/min ($p = 0.033$), hepatic blood volume (BV) lower than 13.32 ml/100 ml ($p = 0.028$) and time to peak (TTP) longer than 19.035 s ($p = 0.015$). Child Pugh class, ascites and tumour number showed statistically significant differences with respect to survival ($p = 0.008$; $p = 0.016$ and $p = 0.001$). In our ongoing study we have been prospectively investigating the association between a change of several serum proangiogenic factors after TACE as biomarkers of survival.

CONCLUSION: Ideal biomarkers for HCC are those that help identify suitable candidates for different therapeutic modalities. CTP and clinical biomarkers can be used to predict survival to TACE. So far, there is still a need for specific biomarkers to assess prognosis of treatment.

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Implementing the TCGA classification endometrial cancer: consequences on medical and surgical approaches

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Endometrial cancer (EC) is the fourth most widespread malignancy in developed countries and the first gynecologic cancer in the United States and its incidence is expected to double in the next 10 years. In the last years, the Cancer Genome Atlas (TCGA) results have renovated the EC classification: four genomic classes with different oncological outcomes [POLE (good prognosis); copy number-high tumors with TP53 mutations (poor prognosis); hypermutated tumors with microsatellite instability, MSI (good to intermediate prognosis) and copy number-low (good to intermediate prognosis)]. This innovative way to define the EC has led to a new tendency to reframe EC clinical and pathological history. After this, the TGCA molecular classification received an external and real life-based validation by on several evidences that confirmed the validity of this new EC stratification (as PORTEC 3, etc). Then, a fundamental step was the transition from the tumor DNA sequencing to immunohistochemistry (IHC) analysis of the molecular classification with the ProMisE study that outlined four different prognostic EC sub-groups almost superimposable to the TCGA genomic classes. These subgroups were defined: POLE-mutated (good prognosis), p53-abnormal (poor prognosis), mismatch repair deficient, MMRd (good to intermediate prognosis) and p53-wild type (good to intermediate prognosis). Noteworthy, the p53-wild type, also known as "no specific molecular profile" (NSMP), represents the most prevalent group of EC (39-64% within the overall EC population). So, other molecular markers were investigated to further understand the shadow areas that still persist. Recently, on these bases, the ESGO/ESTRO/ESP guidelines classification system was introduced, combining the pathological and clinical features to the new molecular information in the EC risk stratification and management. Waiting for the result of PORTEC 4a study that will define the correct therapeutic management with adjuvant therapy and the EUGENIE trial that will indicate the proper surgical staging for each molecular class, the aim of my presentation will focus to describe the updates about the classification and management of EC in the context of this new molecular era.

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Inhibition of proteasome and immunoproteasome synergizes with targeted therapy against chronic lymphocytic leukemia cells

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BACKGROUND: Chronic lymphocytic leukemia (CLL) is characterized by a progressive accumulation of CD5+/CD19+ B cells. Over the last decade, the treatment has led to the advent of targeted therapies. As resistance to these therapies occurs even after robust and deep clinical responses, the demand for novel targets in CLL remains high.

STUDY AIMS: To address the problem of resistance in CLL, we evaluated constitutive proteasome (cP) and immunoproteasome (iP) as new targets in CLL and proteasome inhibitors (PIs) as novel compounds to be used alone or in combination with targeted therapy for the treatment of CLL.

MATERIALS AND METHODS: The cytotoxicity and selectivity of PIs was determined using metabolic activity assay PrestoBlue. The effect of PIs on the activity of the NFκB pathway was determined using Ramos-Blue NFκB reporter cells and translocation of p65-NFκB in primary CLL cells was determined using imaging flow cytometry. Flow cytometry and caspase activity assay were used to detect hallmarks of apoptotic cell death.

RESULTS: A series of 11 PIs was evaluated in patient-derived CLL cells (n=87). All PIs demonstrated concentration and time-dependent cytotoxicity against CLL cells. The EC50,48h values of cP inhibitor carfilzomib and iP inhibitor ONX-0914 were 2 nM and 10 nM, respectively, demonstrating that PIs are cytotoxic in low nanomolar concentrations. We showed that PIs induce apoptotic cell death in CLL cells as evidenced by the detection of several hallmarks of apoptosis, such as disruption of mitochondrial membrane potential, caspase-dependent mechanism of cytotoxicity, activation of caspase 3/7, and display of apoptotic morphology. Mechanistically, PIs suppressed the TNFα-induced activation of NFκB in Ramos-Blue cells and also suppressed the PMA/ionomycin-induced translocation of NFκB in patient-derived CLL cells. Importantly, carfilzomib and ONX-0914 demonstrated cytotoxic activity independently of genetic background even against cells carrying del(17p) and unmutated IGHV. We next screened the CLL biobank to identify targeted therapy-insensitive CLL samples and observed that PIs in combination with targeted therapies also induce synergistic cytotoxicity against hard-to-treat CLL cells (n=15).

CONCLUSIONS: We provided the rationale to consider cP and iP as new targets in CLL and cP/iP inhibitors as novel compounds for the treatment of resistant CLL.

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Integrating biomarkers into the ENDORISK prediction model

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BACKGROUND: Bayesian networks (BNs) are machine-learning–based computational models that visualize causal relationships and provide insight into the processes underlying disease progression, closely resembling clinical decision-making. Preoperative identification of patients at risk for lymph node metastasis (LNM) is challenging in endometrial cancer, and although several biomarkers are related to LNM, incorporation in clinical practice could be improved.

STUDY AIMS: Development and external validation of a preoperative BN to predict LNM and outcome in endometrial cancer patients and implementation of the BN in clinical practice.

MATERIALS AND METHODS: A BN was developed using score-based machine learning in addition to expert knowledge using a retrospective multicenter cohort from the European Network for Individualized Treatment of Endometrial Cancer (ENITEC). This network, called ENDORISK, was externally validated in four study cohorts: the Molecular Markers in Treatment in Endometrial Cancer (MoMaTEC), the PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study cohort, and study cohorts from Tübingen (DE) and Brno (CZ). For inclusion into the network, different histological, molecular and clinical biomarkers were tested and validated with LNM and 5-year disease-specific survival (DSS) as main outcome measures. The BN is currently being updated with The Cancer

Genome Atlas (TCGA) molecular subgroups, myometrium invasion on imaging and the sentinel node procedure. As preparatory steps for implementation of ENDORISK in clinical practice, qualitative research is performed to get doctors and patients perspectives of the BN.

RESULTS: A BN was constructed including preoperative tumor grade; immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), p53, and L1 cell adhesion molecule (L1CAM); cancer antigen 125 serum level; thrombocyte count; imaging results on lymphadenopathy; and cervical cytology. The network was externally validated in the four independent cohorts including 1502 patients. The area under the curve (AUC) for the prediction of LNM varied between 0.82 and 0.85 and for 5-year DSS between 0.70 and 0.86. The network was well-calibrated. Four focus groups with gynaecologists and nine interviews with patients have been executed. These are currently being analysed.

CONCLUSION: This ENDORISK network illustrates how BNs can be used for individualizing clinical decision-making in oncology by incorporating easily accessible and multimodal biomarkers. Currently additional research is being executed in advance of a prospective feasibility study for implementation in clinical practice.

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Methylation profiles for bladder cancer subclassification

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Bladder cancer is a heterogeneous disease. Patients with low grande non-invasive bladder tumors usually present good prognosis while tumors with high grade and T1 stage represent the non-invasive lesions with the highest risk to recur and progress into invasive disease. Those with muscle-invasive disease usually have a worse prognosis with higher risk for methastasis and shorter overall survival. To date, it remains difficult to subclassify bladder tumors based on tumor and grade staging and to prognose which tumors will be cured and which will recur, progress or die from the disease so that more aggressive therapy can be administered. We aimed at identifying and validating potential methylation profiles and candidates that may associate with cancer progression and serve as diagnostic and prognostic biomarkers to select which patients will be cured and those who may recur, progress or die from the disease. First, methylation array profiling comparing tumors and matching normal urothelium revealed methylation profiles associated with bladder carcinogenesis. SOX9 and PMF1 hypermethylation was identified and validated to be associated with disease progression and clinical outcome. Second, methylation profiles using methylation specific multiplexed ligation polymerase assays (MS-MLPA) were differentially expressed in tumors representing each step in bladder cancer progression in association with tumour stage, and clinical outcome and showed clinical utility as urinary biomarkers. Furthermore, they served to subclassify non-mascle invasive disease as well as pT1 high-grade lesions and muscle-invasive tumors. Third, we designed methylation specific polymerase chain reactions (MS-PCR) for individual candidates such as KiSS-1, myopodin. Their methylation was associated with tumor progression and survival in independent series of tumors. Immunohistochemistry expression patterns of selected proteins epigenetically silenced by methylation such as PMF-1 or myopodin were revealed associated to tumor progression and clinical outcome at the protein level as well. Furthermore, they were revealed useful as urinary diagnostic biomarkers. In summary, our study not only revealed epigenetic alterations by hypermethylation in carcinogenesis and along bladder cancer progression, but also served to subclassify bladder tumors along each step in disease progression revealing the diagnostic and prognostic biomarker utility of methylation profiles and individual candidates.

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Methylation status of mismatch repair genes in endometrial and colorectal cancer

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BACKGROUND: Lynch syndrome (LS) is the most common hereditary form of colorectal and endometrial cancer and is associated with a germline pathogenic variant in mismatch repair (MMR) genes. Besides family history data and clinicopathological features there are several laboratory-based strategies that help establish diagnosis of LS, including tumor testing for presence of microsatellite instability (MSI), BRAF V600E mutation and MLH1-promoter methylation status. Patients with tumor that is MSI-high or has abnormal/deficient MMR protein expression on immunohistochemistry without concurrent MLH1-promoter hypermethylation or BRAF V600E mutation are suspected for LS.

STUDY AIMS: Our aim was to select LS related colorectal and endometrial cancer patients based on MLH1-promoter-methylation status. The final confirmation of LS was performed using germline testing of MMR genes.

MATERIALS AND METHODS: Patients diagnosed with colorectal or endometrial cancer with immunohistochemically determined loss of MLH1/PMS2 (n=249) between 2019 and July 2022 were included in the study. Methylation-specific MLPA for MMR genes was performed on DNA extracted from FFPE tumor samples. For germline testing, DNA was extracted from blood and NGS sequencing was performed using Nextera DNA Library Preparation Kit in combination with Illumina's TruSight Hereditary Panel.

RESULTS: Among 249 tumors a MLH1-promoter hypermethylation was detected in 154 (61.8%) tumors, indicating sporadic cancer. Unmethylated MLH1-promoter was detected in 95 patients (38.2%). Among patients with unmethylated MLH1-promoter 62 were patients with colorectal cancer and 33 with endometrial cancer. Until today 32/95 patients with unmethylated MLH1-promoter (33.7%) were referred for germline genetic testing of MMR genes. Three patients (9.4%) had a germline pathogenic variant in one of the MMR genes and therefore confirmed LS.

CONCLUSIONS: Tumor testing for MLH1-promoter methylation status is relevant approach for detecting molecular abnormalities related to LS and therefore useful to distinguish suspected LS related colorectal and endometrial cancers from sporadic MMR deficient cancers.

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microRNA-532 quantitatively regulates the translational activity of FOXM1 in colorectal cancer

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BACKGROUND: Cancer is alteration of cell cycle regulatory networks in mammalian cells. It has resulted in high mortality across the world. Study of cell cycle regulators is essential for understanding dysregulation in this process. Forkhead Box M1 (FOXM1), a master cell cycle regulator and a transcription factor, is a promoter of cell proliferation, exhibiting high expression levels in various cancers. MicroRNAs are 18-22 base-long small RNA molecules that play a dynamic role in regulation of cancer and directly regulate the translation by binding the 3'UTR of their target gene/genes. In a previous study conducted in our lab, FOXM1 was shown to bind with promoter regions of several microRNAs.

STUDY AIMS: To find novel microRNA/microRNAs regulating the expression of FOXM1 in CRC cells and elucidate respective regulatory pathways in colorectal cancer.

METHODOLOGY: Database mining was done to list the microRNAs that possibly regulate FOXM1 protein expression. From them, seven were selected by frequency of occurrence. Their interaction with FOXM1 3'UTR was evaluated by dual luciferase reporter assay. We studied regulatory role of selected microRNA by transiently overexpressing it in CRC cell lines. Total protein was collected from these cells and analyzed for LC-MS/MS to find differentially expressing proteins. Finally, hsa-miR-532 overexpressing lines were also evaluated for their role on cell proliferation and cellular migration.

RESULTS: Data mining revealed potential candidates such as hsa-miR-149, hsa-miR-370 and hsa-miR-532 that might have direct role in FOXM1 regulation. Dual luciferase reporter assay revealed that among selected microRNAs, hsa-miR-532 was able to bind to 3'UTR region of FOXM1, indicated by consistent decrease of luciferase activity in three different cell lines. Overexpression of hsa-miR-532 in HCT116 and HT29 cells led to decrease in translational expression of FOXM1. It also impacted expression of Cyclin B1 that is under direct regulation from FOXM1. LC-MS/MS analysis revealed that overexpression of microRNA can directly impact the regulation of several important proliferation/ migration related proteins.

CONCLUSION: We discover that hsa-miR-532 is an important microRNA that directly regulates the expression of FOXM1 and is involved in cellular proliferation and migration of CRC cells. Our study suggests that microRNA532 quantitatively regulates the translational activity of FOXM1.

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Molecular mechanisms, biomarkers and emerging therapies for chemotherapy resistant triple negative breast cancer

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BACKGROUND: Triple-negative breast cancer (TNBC) is associated with high recurrence rates, high incidence of distant metastases, and poor overall survival (OS). Taxane and anthracycline-containing chemo-therapy (CT) is currently the main systemic treatment option for TNBC, while platinum-based chemotherapy showed promising results in the neoadjuvant and metastatic settings. An early arising of intrinsic or acquired CT resistance is common and represents the main hurdle for successful TNBC treatment.

STUDY AIM AND METHODS: A scientific literature search has been made regarding the many mechanisms recently uncovered that can lead to the development of chemoresistance. Breast cancer, triple-negative, chemoresistance, bio-markers, and emerging therapies were the used key words.

RESULTS: Mechanisms involved in chemoresistance include cancer stem cells (CSCs) induction after neoadjuvant chemotherapy (NACT), ATP-binding cassette (ABC) transporters, hypoxia and avoidance of apoptosis, single factors such as tyrosine kinase receptors (EGFR, IGFR1), a disintegrin and metalloproteinase 10 (ADAM10), and a few pathological molecular pathways. Some biomarkers capable of predicting resistance to specific chemotherapeutic agents were identified and are expected to be validated in future studies for a more accurate selection of drugs to be employed and for a more tailored ap-proach, both in neoadjuvant and advanced settings. Recently, based on specific biomarkers, some therapies were tailored to TNBC subsets and became available in clinical practice: olaparib and talazoparib for BRCA1/2 germline mutation carriers larotrectinib and entrectinib for

neurotrophic tropomyosin receptor kinase (NTRK) gene fusion carriers, and anti-trophoblast cell surface antigen 2 (Trop2) antibody drug conjugate therapy for heavily pretreated metastatic TNBC (mTNBC). Further therapies targeting some pathologic molecular pathways, apoptosis, miRNAS, epidermal growth factor receptor (EGFR), insulin growth factor 1 receptor (IGF-1R), and androgen receptor (AR) are under investigation.

CONCLUSIONS: Some of the reported investigational therapies showed showed promising results and are under evaluation in phase II/III clinical trials. Emerging therapies allow to select specific antiblastics that alone or by integrating the conventional therapeutic approach may overcome/hinder chemoresistance.

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Molecular profiling of ovarian tumor microenvironment

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BACKGROUND: Ovarian carcinomas (OvCas) comprise low-grade (lg, 6-10%) and high-grade (hg) tumors. Bor- derline ovarian tumors (BOTs) are rare and present low malignant potential. BOTs may often be misdiagnosed as invasive. At diagnosis, ~60% of lgOvCas are accompanied by BOTs. BOTs and lgOvCas are primarily chemoresistant, while hgOvCas recur usually as chemoresistant. Molecular characteristics of BOTs and lgOvCas are poorly recognized. In hgOvCas, it is necessary to improve diagnosis and patient outcomes.

STUDY AIMS: We aim to comprehensively, molecularly characterize borderline, lg, and hg ovarian tumors to better understand their biology and lay grounds for new diagnostic and therapeutic methods.

MATERIAL AND METHODS: Snap-frozen and formalin-fixed paraffin-embedded ovarian tumor samples from previously untreated patients with BOTs, lg, and hgOvCas and samples of normal ovarian epithelium were subjected to 1) Whole Transcriptome Sequencing, by RNAseq (~150 frozen OvCa samples, from patients subsequently treated with platinum and cyclophosphamide or platinum and taxanes). 2) Next Generation Sequencing of a panel of 44 genes (117 hgOvCas, 35 lgOvCas and 75 BOTs; 3) DNA methylation profiling with Infinium® Methylation EPIC (Illumina) arrays (80 hg serous (s)OvCas, 29 lgsOvCas, 52 sBOTs and 5 normal ovaries). Clinical data were collected. Up-to-date bioinformatic tools were applied.

RESULTS AND CONCLUSIONS: 1) Expression analysis: We identified protein-coding genes and long non-coding RNAs with an expression level significantly related to overall survival, disease-free survival, treatment sensitivity and complete remission. Gene ontology analyses revealed pathways related to the above clinical end-points. Several identified genes are annotated in the Ensembl database by transcript numbers only, which suggests their functions are still unknown. 2) Mutation analysis: Mutation profile differs between BOTs and ovarian cancers, especially in genes involved in the Fanconi anemia pathway. 90 new SNP and non-SNP variants with moderate/high impact on protein function were identified. 3) Methylation analysis: The most dramatic differences in methylation patterns occur between normal ovarian epithelia and BOTs, and concern genes involved in the regulation of adhesion, intercellular junctions, and cytoskeleton organization. Only a small

percentage of methylation changes differentiated BOTs from lgOvCas. Later changes (lgOvCa vs hgOvCa) mostly consist in the methylation degree.

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mRNA-Seq of an Early-Onset Colorectal Cancer revealed enriched expression of SERPINA3 involved in the regulation of stemness and EMT markers

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BACKGROUND: Colorectal cancer (CRC) incidence stands third among all cancers globally, with the mean age of diagnosis being 65 years. Interestingly, during the past 20 years, there has been a rise in the incidence of Early-Onset Colorectal Cancer (EOCRC) in those under the age of 50. EOCRC stand out as a unique molecular subtype of colorectal cancer due to their aggressive behaviour and poor prognosis. However, the molecular alterations or markers of EOCRC, which could differ from the conventional CRC, are less explored.

STUDY AIMS: To explore the molecular drivers in EOCRC and their functional relevance.

MATERIALS AND METHODS: Using paired-end mRNA-sequencing, we examined the differentially expressed genes in a young colorectal cancer patient. To identify the altered genes enriched in the gastrointestinal epithelial maintenance bioprocess, differentially expressed genes were analysed using the DAVID and AmiGO databases. Target gene expression in tumour samples was examined in comparison to matched normal tissues. Additional knockdown studies clarified their function in stemness and EMT. Treatment using pro-inflammatory cytokine IL6 indicated immune response alteration in gene expression.

RESULTS: Whole mRNA sequencing and bioinformatics database analysis revealed up-regulation of SERPINA3 gene enriching Gastrointestinal epithelial maintenance bioprocess. Further validation showed SERPINA3 up-regulation was exclusive to early-onset CRC. SERPINA3 silencing led to significant deregulation of stem cell-regulatory genes and EMT markers in HT29. SERPINA3 was found to have a strong nuclear expression in patient samples and showed elevated expression upon IL6 treatment in HT29. Additionally, the PROGgeneV2 database showed a poor prognosis for SERPINA3 high-expressed groups.

CONCLUSIONS: Our results demonstrate the mechanistic role of SERPINA3 in stemness and EMT. The nuclear localisation of SERPINA3 suggests its role as a transcription factor or regulator. It also elucidates the unexplored function of SERPINA3 in the inflammation-related aspects of colorectal cancer.

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Mutational spectrum in patients with metastatic gastrointestinal stromal tumours

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About 85% of malignant GISTs harbour activating mutations in one of the genes, KIT or PDGFRA, while 10% to 15% of all GISTs have no detectable KIT or PDGFRA mutations (KIT/PDGFRA wild-type GIST), hence could have alterations in genes of the succinate dehydrogenase (SDH) complex or in BRAF, PIK3CA, or rarely RAS family genes. Clinical benefit of tyrosine kinase inhibitors (TKIs) depends on GIST genotype, therefore molecular characterization of GIST has a crucial role in overall management of GIST.

The aim of this retrospective study was to molecularly characterize primary tumour samples of 116 patients with metastatic GISTs from the Slovenian Cancer Registry treated with TKIs between 2002 and 2020 at the Institute of Oncology Ljubljana and to assess their treatment outcome.

Direct Sanger sequencing was performed in tumour samples of all patients included in this study to determine mutation spectrum of KIT gene (exons 9, 11, 13 and 17) and PDGFRA gene (exons 12, 14 and 18). Moreover, all KIT/PDGFRA wild-type GISTs as determined by Sanger sequencing were profiled by a targeted next-generation sequencing (NGS) approach (TruSight Oncology 500 DNA Kit by Illumina) and their expression of SDH complex was assessed by immunohistochemistry.

Direct Sanger sequencing results in 95 (82%) patients with mutations in KIT and 5 (4%) in PDGFRA while 16 (14%) GIST patients had no mutation in either of the analysed genes. A total of 7 out of 16 KIT/PDGFRA wild-type GISTs that have been additionally genotyped by NGS (44%) were found to carry a mutation, either in KIT or PDGFRA and they all responded to imatinib. Only 5 out of 16 of KIT/PDGFRA wild-type GISTs (31%) were confirmed to be KIT/PDGFRA/BRAF/SDH/NF1 wild-type also by NGS, and none of them responded to TKIs.

Mutation frequencies of KIT and PDGFRA genes observed in Slovenian patients are comparable with published series. Location of the mutation in KIT exon 11 is associated with a favourable clinical outcome while the type of detected mutation is not significantly important. For patients with KIT/PDGFRA wild-type GIST the reliability of direct Sanger sequencing results is insufficient and therefore it is recommended that NGS becomes a requirement for their treatment decision.

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Personalized Treatment for Cancer: How Biomarkers are Showing the Way

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Personalized treatment can be defined as using the biological characteristics of a patient's disease in order to administer the most effective therapy at the optimum dose. To provide personalised treatment for cancer, 4 main types of biomarkers are necessary. These include prognostic biomarkers to identify who should or should not receive adjuvant treatment, predictive biomarkers to identify the most appropriate therapy, toxicity biomarkers to identify potential severe toxicity and monitoring biomarkers to assess real-time response to treatment. Validated prognostic biomarkers include uPA, Oncotype DX and MammaPrint for lymph node-negative breast cancer, MSI for stage II colon cancer and AFP, HCG and LDH for advanced germ cell tumors. Clinically used predictive biomarkers include ER and HER2 for endocrine and anti-HER2 therapy, respectively in breast cancer,

mutation status of BRAF for anti-BRAF therapy in melanoma, KRAS/NRAS mutation status for anti-EGFR antibodies in colorectal cancer and EGFR mutational status for anti-EGFR TKIs in non-small cell lung cancer. Recommended biomarkers for predicting response to immunotherapy using checkpoint inhibitors include PD-L1, MSI and tumor mutational burden. For upfront identification of patients at high risk of suffering from severe therapy-related toxicity in colorectal cancer, specific variants of dihydropyrimidine dehydrogenase (DPD) may be measured for predicting toxicity from fluoropyrimidines and uridine diphosphate glucuronosyltransferase*28 (UGT1A1*28) for predicting toxicity from irinotecan. Therapy monitoring biomarkers include CEA for colorectal cancer, PSA for prostate cancer, CA 125 for ovarian cancer and CA 15-3, CEA, TPA and TPS for breast cancer. The use of prognostic, predictive, toxicity and monitoring biomarkers can thus help match each patient to the most effective and least toxic therapy as well as futile unnecessary costs.

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Plasma levels of matrix metalloproteinases and their tissue inhibitors as potential diagnostic and prognostic biomarkers in colorectal cancer

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BACKGROUND: Several studies have shown that the expression of both MMPs and TIMPs is increased in oncological diseases and is positively correlated with tumor stage. Some MMPs and TIMPs have been repeatedly studied as candidate circulating biomarkers in solid tumors including colorectal cancer, with promising results. In most studies, MMPs were determined in blood serum; however, due to the high concentration of MMP in leukocytes and herefore high risk of false positive results, serum is not a suitable material for determining MMP levels and blood plasma should be used instead.

STUDY AIMS: To evaluate the potential of selected MMPs and TIMPs, determined in blood plasma, as diagnostic and prognostic biomarkers of colorectal cancer.

MATERIALS AND METHODS: 148 patients with colorectal cancer and 68 age-matched controls were recruited into the study. Preoperative blood samples from patients and blood samples from controls were collected, serum and plasma were separated and immediately frozen at -80°C. Classical tumor biomarkers (CEA, CA 19-9) as well as other biomarkers (cytokeratins, growth factors) and vitamin D were measured by automated immunoassays in blood serum samples; matrix metalloproteinases MMP-2, -7, -8, -9, -10 and tissue inhibitors of metalloproteinases TIMP-1, -2, -3 and -4 were measured in blood plasma.

RESULTS: Plasma MMP-8 and MMP-9 levels were significantly higher in patients compared to controls. When comparing patients with early-stage cancer to patients with advanced-stage cancer, MMP-7 and MMP-8 levels were significantly higher in advanced stages. The best AUCs were found for MMP-9 (0.6760) and MMP-8 (0.6542). The hazard ratio was calculated and MMP-8 and TIMP-1 were evaluated as the best prognostic markers. A risk score including both traditional tumor markers and candidate biomarkers was calculated using a multivariate model.

CONCLUSION: Plasma MMP-8 and TIMP-1 seem to be promising biomarkers for diagnostic and prognostic purposes in colorectal cancer, however their clinical value was not confirmed by this study. A risk score including MMP-8 and classic tumor biomarkers could be useful in clinical decision-making.

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Prognostic value of blood based protein biomarkers in lung cancer - a critical review and update

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BACKGROUND: Lung cancer is the leading cause of cancer mortality, with estimated 1.8 million deaths, worldwide. Significant therapeutic and technical progress lead to rapid changes in the management of NSCLC to a more personalized treatment approach, requiring a biomarker-driven patient selection. Here we discuss relevant serum protein biomarkers in late staged non-small cell lung cancer with regards to their prognostic significance, with special attention to the preconditions, conduct and the existing problems with prognostic biomarker studies, improvements already implemented and remarks for future evaluations.

METHODS: We searched the PubMed database to summarize the results of prognostic studies on serum tumor markers carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), neuron specific enolase (NSE), squamous cell carcinoma antigen (SCCA), CA125, CA19-9, CA15-3 and pro-gastrin-releasing peptide (proGRP) since 2009.

RESULTS: Especially CYFRA21-1 showed high prognostic value in NSCLC patients treated with chemotherapy, immunotherapy, targeted therapy or combinations thereof in early and advanced stages. In contrast, contradicting results were observed for CEA. CA 125 may be a promising marker for prognosis in NSCLC patients, however studies including the marker in patients treated with immunotherapy are scarce. Combinations of tumor markers are proposed to provide a more significant prognostic value than single markers did.

CONCLUSION: Protein serum tumor markers, especially CYFRA21-1, showed prognostic potential in advanced staged NSCLC. However, the study designs, preanalytics, analytics, and the evaluation and interpretation of results remain heterogeneous. A comparison of the results and consecutive integration in clinical guidelines is difficult. Therefore, we propose the generation of a catalogue for criteria to perform consistent, comparable studies on basis of the REMARK guidelines.

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Proteomic Tissue-based Classifier for Early Prediction of Prostate Cancer Progression

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Even though ~40% of screen-detected prostate cancers (PCa) are indolent, advanced-stage PCa is a lethal disease with 5-year survival rates around 29%. Identification and or development of biomarkers for earlier detection of aggressive cancer is a key challenge. Starting with 52 candidate biomarkers, selected from existing PCa genomics datasets and known PCa driver genes, we applied targeted mass spectrometry to quantify proteins that significantly differed in primary tumors from PCa patients treated with radical prostatectomy (RP) across three study outcomes: (i) metastasis 1-year post-RP, (ii) biochemical recurrence 1-year post-RP, and (iii) no progression after 10 years post- RP. Sixteen proteins that differed significantly in an initial set of 105 samples were evaluated in the entire cohort ($n = 338$). A five-protein classifier which combined FOLH1, KLK3, TGFB1, SPARC, and CAMKK2 with existing clinical and pathological standard of care variables demonstrated significant improvement in predicting distant metastasis, achieving an area under the receiver-operating characteristic curve of 0.92 (0.86, 0.99, $p = 0.001$) and a negative predictive value of 92% in the training/testing analysis. This classifier has the potential to stratify patients based on risk of aggressive, metastatic PCa that

will require early intervention compared to low-risk patients who could be managed through active surveillance.

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sIL-2R – an Immuno-biomarker for Prediction of Metastases in Uveal Melanoma

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BACKGROUND: Uveal Melanoma (UM) is the most common primary intraocular malignancy in adults. High serum levels of sIL-2R have been reported in acute inflammations and metastatic cancers.

STUDY AIM: This study evaluated the potential of high / increasing sIL-2R levels in predicting UM metastases.

PATIENTS AND METHODS: The study included a total of 1,546 sera samples of subjects from three groups: 119 healthy Controls (73 subjects), 566 UM 10 year (10y) Disease-Free (DF) (220 patients), 861 Metastatic UM (268 patients). Patients were followed-up biannually with liver ultrasound and liver function tests for the presence of metastases (Mets). Blood samples to measure the levels of sIL-2R were obtained at the time of primary diagnosis, soon after initial treatment (enucleation, brachytherapy), every 6 months, 10 years from diagnosis, at Mets confirmation by CT and after additional treatments.

RESULTS: Significantly higher sIL-2R levels were detected for the Mets patients compared to healthy controls and 10y DF patients. Compared to the upper limit of the normal level of sIL-2R)1,000 U/ml), its levels in metastatic UM were in 61%, in 10y DF UM - 25% and in the Controls – in 6.25% of subjects. High levels of sIL-2R in Metastatic patients, decreased significantly post treatments. Individual kinetics of markers, indicated similar trends of sIL-2R compared to other markers we have shown to be sensitive in UM, as OPN (Osteopontin) and S-100 (S-Protein 100), predicting metastases, which were confirmed on liver imaging.

CONCLUSION: Significantly higher sIL-2R levels, were evident in all UM patients with Mets. Significant increases in sIL-2R levels on serial evaluations indicated and predicted UM Mets, enabling earlier treatment of Mets, to improve survival.

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Single EV analysis by imaging flow cytometry and identification of new biomarkers

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In recent years, extracellular vesicles (EVs) have been recognized as a new class of potential biomarkers for the progression or severity of various diseases. While for the detailed characterization, conventional analytical methods require upfront preparation of EVs from biological fluids, imaging flow cytometry (IFCM) performed on the AMNIS ImageStream MKII allows analyses of EVs in various types of non-processed body fluids at the single EV level.

Following optimization of protocols and the qualification of more than 40 different commercial antibodies for the single EV analysis, we are able to dissect the heterogeneity of EVs in different sample types, now. To this end, we already detected different EV compositions in serum samples of severe and mild SARS-CoV2 patients as well as in the synovial fluids of orthopedic patients with aseptic joint effusion (AJE) and prosthetic joint infection (PJI).

Aiming to explore the power of our platform in qualifying EVs as biomarkers in oncology, we have started to compare plasma samples from breast cancer, lung cancer and head and neck cancer patients. Combing different EV subpopulations with clinical data facilitate an additional level in diagnostics.

Thus, EV population analyses combined with clinical data reflected differences in disease stages, subtypes, forms and prognosis. Consequently, IFCM provides an excellent platform for the discovery of EV-based biomarkers in inflammatory and oncologic diseases.

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Targeting cancer related genes and miRNAs using gene editing technology

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Cancer related deaths are increasing annually worldwide. During last decades, extensive researches have revealed many genes and miRNAs that are orchestrating tumor initiation, progression and metastasis. Recently, Gene editing has emerged as one of the recent promising tools for gene therapy. From the most important gene editing enzymes are zinc finger nucleases (ZFNs), homing meganucleases and transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9). Recently, many studies were conducted to introduce new treatment modalities for cancer treatment using the gene editing technology, either by targeting genes or miRNAs. These targeted therapies will introduce new therapeutics options that are much potent and specific.

The crisper technology was widely applied to introduce therapeutic options in cancer treatment, additionally, it was used in cancer immunotherapy. For example, CRISPR technology has introduced an alternative to the conventional clinical drug, Herceptin, by targeting HER2 in breast carcinoma. Moreover, it was used in CAR-T cell generation and immune cell checkpoint inhibition. Researchers are seeking to fight many hard diseases by the use of CRISPR technology, however, many challenges still exist. Some of these challenges include the requirement of PAM sequence, the possibility of on target deletion or addition, off target effects, Cas9-DSB complex.

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The challenge to predicting immunotherapy response to the Bacille of Calmette-Guerin (BCG) in high risk T1G3 bladder cancer

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Patients with bladder tumors with high grade and T1 stage represent the lesions with the highest risk to recur, progress into invasive disease and shorter survival. These are usually treated by surgery plus intravesical instillations of the Bacille of Calmette Guerin (BCG). The mechanism by which BCG mediates immune response has not been fully characterized. To date, it also remains difficult

to predict which tumors will be cured and which will fail therapy and recur, progress or die from the disease so that more aggressive interventions can be administered. We aimed to unclear the BCG mechanisms involved in BCG therapy and identify and validate potential BCG predictive biomarkers that may help to select which patients will be cured and those who may recur, progress or die from the disease. First we utilized high-throughput technologies such as transcript arrays in tissue samples and identified molecular profiles that predicted successful BCG response and distinguished patients more likely to recur, progress or die of the disease. We identified transcript profiles differentially expressed in two independent series or tumors in association with age, tumor size, recurrence, progression and disease specific survival. We selected several candidates for further validation in independent series of patients by independent techiniques from transcript profiles related to BCG response taking disease specific survival as clinical endpoint. In selected genes showing expression loss, their epigenetic silencing was tested after azacytidine exposure in vitro. For methylation analyses, the clinical validation of selected candidates was verified designing new methylation specific polymerase chain reactions (MS-PCRs using independent series of bladder tumours. Immunohistochemistry expression patterns of individual selected proteins such as ezrin were revealed associated with an early recurrence after 3 months initiating BCG immunotherapy (p= 0.041), progression (p=0.009), and disease-specific survival (p=0.006) in an independent series of bladder tumours spotted onto tissue microarrays of patients treated with BCG. Our study not only served to uncover molecular mechanisms associated with BCG immunotherapy in bladder cancer, but also revealed the predictive biomarker utility of transcript profiles and individual candidates such as ezrin in BCG response.

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The Diagnostic and Prognostic Value of sIL-2R as an Immune Biomarker in Head and Neck Cancer

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BACKGROUND: Head and neck cancer (HNC) patients are usually diagnosed with advanced disease and multimodality therapies are required, as well as prognostic biomarkers to predict their response and assess survival.

AIM: In this study, we aimed to evaluate the ability and clinical significance of the immune Biomarker sIL-2R in HNC patients, to assess therapy response and prognosis.

MATERIALS AND METHODS: We evaluated 328 blood samples from 145 head and neck cancer patients (HNC) from several subgroups: 84 larynx carcinomas pre- and 39 post-therapy, 46 oral cavity carcinomas pre- and 29 post-therapy, 12 nasopharynx carcinomas, 16 parotid and other salivary gland carcinoma patients. The control group included 45 healthy subjects. Serum sIL-2R levels were evaluated by ELISA assays and correlated to disease stage, lymph nodes, response to therapy, survival and cancer differentiation.

RESULTS: Significantly higher sIL-2R levels were recorded in all HNC patients, as opposed to controls, in advanced versus early-stage disease that decreased following therapy. sIL-2R distinguished best, in comparison to other tumor markers, between HNC patients and controls. Survival was strongly associated to lower sIL-2R levels in patients entering the study.

CONCLUSION: sIL-2R is a sensitive immune marker for HNC patients. Its levels correlate to disease stage, assess response to therapy and are predictive of recurrence or better survival. We suggest, therefore, using sIL-2R as a reliable prognostic marker in HNC patients as a single marker, or in a combined panel of biomarkers.

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The use of RNAseq in discovery of splicing abnormalities

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BACKGROUND: Pathogenic variants in susceptibility genes that interrupt RNA splicing are a well-documented mechanism of hereditary cancer syndromes development. However, if a variant beyond the canonical GT-AG splice site lacks RNA based evaluation, it will likely be characterized as variant of uncertain significance (VUS)

STUDY AIMS: The aim of the study was to reclassify VUS based on bioinformatical and experimental evaluation in patients who have undergone genetic testing for hereditary cancer syndromes at our clinic.

MATERIALS AND METHODS: 732 patients underwent NGS panel testing using hybrid capture library preparation with TruSight Hereditary Cancer Panel (Illumina). Bioinformatic tools for splicing prediction NNSplice, MaxEntS-can, GeneSplicer, SpliceSiteFinder-like (SSF), SpliceAI and SPICE were used. RNAseq was performed using an in-house developed approach using long-range PCR and deep NGS sequencing (Dragoš et al., 2021).

RESULTS: Among 732 patients, 12 VUS were bioinformatically predicted to alter splicing. Using RNAseq, we have evaluated their spliceogenicity. One variant showed complete splicing defect, nine variants caused partial or uncertain splicing aberration, and two variants had no impact on mRNA splicing.

CONCLUSIONS: Based on the functional characterization of VUS, using RNA sequencing, we have successfully reclassified 50% of investigated variants. 25% of our variants were upgraded to likely pathogenic leading to improved clinical management of the patient and the family members.

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The utility of epigenetic analysis of cfDNA in treating colorectal cancer patients

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Liquid biopsies present an exciting opportunity for frequent, minimally invasive monitoring of tumor progression, evolution and response to therapy. Our team has developed novel approaches for studying cfDNA, which generate an unprecedented breadth of data on tumor dynamics. Using cell-free chromatin immunoprecipitation followed by sequencing, we are able to infer gene expression patterns in the cells that released cfDNA; and using nanopore sequencing we are able to rapidly detect both DNA methylation patterns and copy number variations in cfDNA. Using Multiplexed, single-molecule, epigenetic analysis of plasma-isolated nucleosomes we gain insight on complex interactions of different epigenetic modifications at fine resolution.

These novel biomarker will be useful in interrogating complex clinical scenarios of tumor heterogeneity; response to treatment; resistance mechanism and more, and will facilitate clinical decisions. In addition, it will shed light on tumor biology and advance treatment development. Overall, this project will develop innovative, sensitive and specific liquid biopsy biomarkers for tumors. Our approach

can evolve into a novel comprehensive liquid biopsy modality for cancer both in late and early stage disease.

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Tumor biomarkers in COVID-19 patients: are they of use in prognosis of disease severity?

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BACKGROUND: Coronavirus disease 2019 (COVID-19) is a rapidly evolving infectious/ inflammatory respiratory disorder whose progression and outcome is hard to predict. It is important to estimate the severity and prognosis of the disease in order to decide on optimal clinical management. Since tumor biomarkers have shown an elevation in various inflammatory conditions in the lungs they might be suitable biomarkers for prognosis evaluation.

STUDY AIMS: to evaluate selected tumor biomarkers as potential biomarkers of COVID-19 severity and prognosis.

MATERIALS AND METHODS: We conducted a retrospective non-randomised study that included three groups of patients diagnosed with COVID-19, without a medical history of cancer: Group 1 - 30 patients with mild to moderate disease, Group 2 - 30 patients on mechanical ventilation who have recovered, Group 3 - 30 patients who died on/with covid-19. Five tumor markers (CEA, CA 15-3, CYFRA 21.1, HE4) were measured using automated immunoassays (CLIA). We compared tumor biomarker levels among groups and calculated ROC curves.

RESULTS: Levels of mucin-type tumor biomarkers CA 125 were significantly higher in Group 2 compared to Group 1 (CA 125 - mean 40.5 vs. 23.9 kIU/L, p=0.0015, CA 15-3 kIU/L mean 42.7 vs 24.5 , p=0.0015). Levels of CEA, CYFRA 21.1 and HE4 were significantly higher in Group 3 than in Group 1 (11.4 vs 3.6 µg/L, p=<.0001; 13.0 vs 3.5 µg/L, p=<.0001; and 422.9 vs.182.4 pmol/L, p=<.0001, respectively).

CONCLUSION: Our results confirm outcomes of other studies that tumor biomarker levels are elevated in pulmonary fibrosis and might predict the life-threatening outcome of covid-19 disease and help in treatment decisions. Studies with larger patient groups are needed in order to support these findings.

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Tumor marker - as a parameter of aggressiveness of tumor disease

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AIM OF THE STUDY: The aim is to ensure whether any markers can be used to live as markers of the severity and aggressiveness of the cancer and thus to estimate the prognosis of cancer diseases.

GROUPS OF PATIENTS AND METHODS: Groups of 300 patients with lung cancer, 220 females with breast cancer, 300 patients with colorectal cancer, 150 females with ovarian cancer and 500

males with prostate cancer were included. A total of 12 different biomarkers were examined. Marker combinations depended on the primary tumor diagnosis.

RESULTS AND CONCLUSIONS: Optimal combinations of markers were found for the monitored markers. The applicability of optimal combinations has been verified in routine practice.

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Use of biomarkers in diagnosis and presurgical patient stratification

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BACKGROUND: Endometrial cancer (EC) incidence has been rising over the past 10 years. Early and accurate detection of the disease is an important contributor to survival. Diagnosis of EC patients is favored by the presence of symptoms like abnormal vaginal bleeding in women, which affects 11M women in the EU and US yearly, but only 5-10% of these women will be diagnosed with EC. Current EC diagnostics is based on the observation of cells in an endometrial biopsy, but this fails in 31% patients requiring additional more invasive procedures to be diagnosed, and inaccurately identifies histological type and grade of EC in 55% of patients compromising the treatment-decision making.

STUDY AIMS: Elucidate protein biomarkers in gynaecological fluids to accurately diagnose EC.

MATERIALS AND METHODS: Biomarkers have been discovered through literature review and a discovery phase and validated by using targeted Mass Spectrometry. Biomarkers have been evaluated in five clinical retrospective studies in uterine fluids from 358 patient specimens. A subset of biomarkers are also validated in an independent retrospective clinical study in a cohort of 250 samples using ELISA technology.

RESULTS: Our research has permitted to identify 5 protein biomarkers, which expression in uterine fluid permits a quantitative and accurate diagnosis with 97% negative predictive value, 99% sensitivity, 79% specificity and 87% positive predictive value. Moreover, it provides an objective assessment of the endometriod vs. non-endometrioid histological subtype of cancer to guide treatment planning and optimize surgical intervention.

CONCLUSION: These results are the basis to develop an in vitro diagnosis for accurate, faster, and minimally invasive diagnosis that will improve patient outcomes, reduce patient morbidity, and lower the cost of care.

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Validation of cancer markers with emphasis on screening

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BACKGROUND: Tumor markers are generally considered unsuitable for screening purposes due to the numerous false positives that need to be sorted out by other costly methods.

STUDY AIMS: To show that at least in part, the low specificity of cancer markers is due to poor evaluation practices regarding: (a) diagnostic errors in the selection of the gold standard samples, (b) the assay error around the cutoff, (c) the unrealistic importance assigned to false positives in retrospective case studies, (d) the prevalence error related to overestimation of malignancy in patients with a positive cancer marker test, (e) the infrequent use of cancer marker combinations despite numerous publications indicating their advantage, (f) using absolute cutoff values rather than a personalized cutoff baseline for each patient, (g) the disregard in the statistical estimation of cancer risk of other predictors such as weight loss, asthenia, etc, and (h) the interpretation of the results in terms of sensitivity and specificity rather than using statistics that provide an estimation of the probability of having cancer.

MATERIALS AND METHODS: Different statistical tools including Kappa, error estimation, Logistic Regression and plain arithmetic were used on datasets (blinded or not blinded) of different cancer markers or combinations of markers.

RESULTS AND CONCLUSIONS: Taking into account the abovementioned issues results in a significant reduction of errors and therefore in the corresponding improvement in the performance of cancer markers. Also, some medical practice issues emerge from the analysis: For example, CA125 is considered inadequate for screening because it detects only ~50% of ovarian cancer in early stages. Yet, since >80% of ovarian cancers stages I & II can be cured, if CA125 detects half of them, the lifesaving benefit would still be very significant (>30%). The possibility of using saliva as the first fluid to screen for cancer and then using a blood test for confirmation purposes is also discussed.

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Warburg's mitochondrial HK2-VDAC1 complex disruption is a promising new weapon in cancer drug discovery

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Disruption of Hexokinase-2 and Voltage-dependent anionic channel-1 (HK2-VDAC1) complex is an effective therapeutic strategy to contain tumor growth, and small molecules such as methyl jasmonate (MJ) have been shown to destabilize the HK2-VDAC1 complex resulting in cancer cell death. However, molecular interactions underlying the disruption of the HK2-VDAC1 complex are poorly understood. In the current work, we present the molecular dynamic simulation data describing the details of key molecular interactions involved in the MJ-mediated disruption of the HK2-VDAC1 complex. The HK2-VDAC1 complex is prepared from the existing X-ray crystal coordinates and the missing 16 residues of HK2 at the N-terminal were modeled. This 16-residue region of HK2 anchors VDAC1to form a complex. To perform the100 ns of MD simulations of HK2-VDAC1 bound molecules, VDAC1 is surrounded by DPPC membrane to mimic possible physiological membrane conditions. This built model was tested in silico to predict specific binding sites and interactions for ATP and a known inhibitor methyl Jasmonate (MJ) which can cleave the HK2-VDAC1complex, to deplete the ATP production and glycolysis in the cancer cell. From the observation of the study, the molecule binding pocket (S1) was identified in the intersection region of HK2 and VDAC1, and an alternate pocket was also identified at the HK2 N-terminal helix and VDAC1 interaction region (S2). Molecular docking of the ATP results to bind at the S1 pocket selectively over the S2 pocket whereas the MJ molecule binds to both S1 and S2 pockets differentially. Furthermore, to understand the binding, and stability and to confirm the possible binding site of MJ in the HK2-VDAC1 complex, molecular dynamics simulations of the ATP and MJ bound to the HK2-VDAC1 complex with DPPC membrane were performed for 100 nanoseconds. From the binding free energies from MM-PBSA calculations, the ATP binds strongly at the S1 pocket (-35.095 kJ/mol) selectively over the MJ

molecule (-36.265 kJ/mol). In the case of the MJ molecule, results suggest that S2 pocket (-70.669 kJ/mol) is more preferential over the S1 pocket (-36.265 kJ/mol). The strong binding interactions at S2 pocket residues corroborate the selectivity and stability of the MJ molecule over poor interaction in S1 pocket.

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Whole genome cell-free tumor DNA liquid biopsy for ultrasensitive detection and monitoring of central nervous system tumors

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Liquid biopsy offers a noninvasive approach to monitor cancer burden during therapy and surveillance. However, in brain tumors, blood-based liquid biopsy methods have been unsuccessful due to a low circulating cell free tumor DNA (ctDNA) burden, blood brain barrier, and low number of mutations in coding regions. In contrast with targeted panels, whole genome sequencing (WGS)-derived patient specific mutational signature from a matched tumor-normal WGS can provide a personalized, highly sensitive and specific approach to detect mutations in ctDNA and provide blood-based monitoring in brain tumor patients. Furthermore, it can be performed on lower amount of peripheral blood since WGS requires less sequencing depth compared to targeted ctDNA panels. We have profiled a cohort of 28 extra- and intra-axial adult and pediatric brain tumors including meningiomas (11), medulloblastomas (5), ependymomas (2), neurocytoma (1), and low- and high-grade gliomas (9). Tumor DNA was extracted from archival pathology tissue, normal DNA from unsorted white blood cells, and ctDNA from 1-2 mL of post-surgery plasma. WGS was performed with 40x coverage for Tumor-Normal DNA and 20x for ctDNA. Using WGS of matched Tumor-Normal and plasma samples, we derived a personalized mutational pattern using SNVs, indels and copy numbers for quantification and ultra-sensitive detection of ctDNA in plasma samples. An AI-based error suppression model was implemented to filter out the noise in the cell free DNA (cfDNA) while the personalized mutational signature was used to detect the ctDNA in the cfDNA and to amplify the somatic signal to determine the Tumor Fraction. A patient-specific personalized genome-wide compendium of somatic mutations could be established across all tumor types of brain tumors and ctDNA tested at the time of diagnosis, during the therapy or surveillance period. The ctDNA Tumor Fraction (TF) was compared to the clinical status and MR-based imaging. All subtypes of brain tumors contained sufficient number of mutations to derive personalized mutational signatures and TF corelated with clinical status. TF levels corelated with the disease course on imaging at given time points reaching a 10-4 minimal residual disease detection sensitivity. We conclude that patient-specific WGS tumor signature in ctDNA from blood can be used for sensitive monitoring of adults and children with brain tumors.

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