1

Drug repurposing screen reveals glioblastoma cell line susceptibility to statins

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Background: Glioblastoma has been extensively molecularly profiled, although this knowledge has not yet been translated into improved survival outcomes. We used a bioinformatics approach to identify potential novel therapeutic strategies for glioblastoma.
Objectives: Comprehensive online datasets which have assessed up to 1376 cancer cell lines in multiple ways were interrogated to identify potential drug candidates for glioblastoma.
Methods: Datasets included were from the cancer cell line encyclopedia (mRNA expression), the Achillies project (cell viability following Crispr-Cas9 knockout) and PRISM (drug treatment). A t-test comparing cell viability of glioblastoma cell lines versus other cancers was used to identify potential drug candidates, followed by the use of multiple statistical tools to investigate potential mechanism of action and status of biomarkers.
Results: Fluvastatin and pitavastatin were amongst the drugs with the most significant effects against glioblastoma cell lines while also being FDA approved. These effects were found in both glioblastoma cells and other cancer types with a mesenchymal-like expression phenotype. The anti-cancer properties of statins have previously been attributed to the inhibition of HMG-Coa reductase. Here, we found their effects correlated with the gene knockout of UBIAD1, which participates in non-mitochondrial ubiquinone and menaquinone-4 synthesis. We tested the effects of statins on patient-derived glioblastoma cell lines with a mesenchymal (n = 2) and non-mesenchymal phenotype (n=2). Mesenchymal-like glioblastoma cells lines were found to be particularly susceptible to multiple statins.
Conclusion: Statins appeared to be especially effective against glioblastoma lines and the effect could be linked to the direct or indirect inhibition of UBIAD1.

2

DIFFERENTIAL EXPRESSION OF CHECKPOINT MARKERS IN THE NORMOXIC AND HYPOXIC MICROENVIRONMENT OF GLIOBLASTOMAS USING DIGITAL SPATIAL PROFILING

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Aim: Glioblastoma is the most common primary malignant brain tumor in adults with an overall survival of only 14.6 months. Hypoxia is known to play a role in tumor aggressiveness but the influence of hypoxia on the immune microenvironment is not fully understood. The aim of this study was to investigate the expression of immune-related proteins in normoxic and hypoxic tumor areas by digital spatial profiling.
Methods: Tissue samples from 10 glioblastomas were stained with a panel of 40 antibodies conjugated to photo-cleavable oligonucleotides. The free oligo-tags from normoxic and hypoxic areas were hybridized to barcodes for digital counting. Differential expression patterns were validated by Ivy Glioblastoma Atlas Project (GAP) data and an independent patient cohort.
Results: We found that CD44, Beta-catenin and B7-H3 were upregulated in hypoxia, whereas VISTA, CD56, KI-67, CD68 and CD11c were downregulated. PD-L1 and PD-1 were not affected by hypoxia. Focusing on the checkpoint-related markers CD44, B7-H3 and VISTA, our findings for CD44 and VISTA could be confirmed with Ivy GAP RNA sequencing data. Immunohistochemical staining and digital quantification of CD44, B7-H3 and VISTA in an independent cohort confirmed our findings for all three markers. Additional stainings revealed fewer T cells and high but equal amounts of tumor-associated microglia and macrophages in both hypoxic and normoxic regions.
Conclusion: We found that CD44 and B7-H3 were upregulated in areas with hypoxia, whereas VISTA was downregulated together with the presence of fewer T cells. This heterogeneous expression should be taken into consideration when developing novel therapeutic strategies.

3

Digital image analysis is a robust method for evaluation of tumor-associated macrophages in breast cancer

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Introduction:
Tumor-associated macrophages (TAMs) may be of prognostic value in breast cancer. The analytical validity of CD68 as a potential biomarker, however, needs to be tested before clinical use.
The objective was to investigate agreement between 1) estimation of CD68+ TAMs by “eyeballing” and digital image analysis (DIA) and 2) assessment of TAMs on tissue-micro-arrays (TMA) and whole-slides (WS).
Material and Methods:
Tumor-containing tissue blocks and TMA (1 core/patient) were available from 1167 breast cancer patients. Immunohistochemistry for CD68 was performed on TMA (991 pts.) and on corresponding WS from 234 patients. Area fractions of CD68+ TAMs were estimated by two observers using light microscopy (“eyeballing”) and by DIA.
Results:
Interobserver agreement for “eyeballing” on WS was moderate with intraclass-coefficient of 0.72 (95%Cl: 0.65-0.78), and Kappa coefficient of 0.53, when grouping the results into three categories (0-0.10, 0.11-0.30, >0.31). Few tumors (21/991) showed high levels of TAMs (> 0.31).
A strong significant correlation was found between DIA and “eyeballing” for both WS and TMA ($p<0.0001$, Kruskal-Wallis test). Correlation between TMA vs. WS was also highly significant by ”eyeballing” and by DIA with Spearman correlation coefficients of 0.55 and 0.62 ($p<0.0001$), respectively.
Discussion and Conclusions:
The correlation between “eyeballing” and DIA as well as the association between TMA and WS by both methods was highly significant. The interobserver agreement by “eyeballing” was moderate. This indicates that CD68 assessment by DIA may be useful for delivering a robust and quantifiable estimate and securing the analytical validity of CD68 as a potential biomarker.

4

Fund af lymfeknuder efter yderligere fiksering af tumorskiver fra tarmresektater med kolorektal cancer – et pilotprojekt

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Introduktion: Antallet af lymfeknuder og lymfeknudemetastaser har betydning for stadieinddeling og prognose. Formålet med dette kvalitetsudviklingsprojekt var at undersøge, om primært uidentificerede lymfeknuder i selve tumorskiverne fremtræder tydeligere efter yderligere fiksering i formalin.
Materialer og metoder: I alt 10 resektater injiceret med methylenblåt blev inkluderet. Tidligere neoadjuverende behandling var et eksklusionskriterium. Ved primær udskæring blev antallet af lymfeknuder i tumorskiverne registreret. Efter yderligere minimum 2 dages fiksering blev tumorskiverne atter undersøgt, og antallet af lymfeknuder, der kunne findes henholdsvis visuelt, palpatorisk og slutteligt ved finkæmning af fedtvævet, blev registreret særskilt. Tumorskiverne blev fotograferet før og efter ekstra fiksering. Samme person foretog begge udskæringer på alle præparater.
Resultater: Fem højresidige, 3 sigmoideum og 2 rektumresektater blev inkluderet. Ved sekundær udskæring blev identificeret 2–10 ekstra lymfeknuder pr. resektat fordelt på 1–4 ekstra lymfeknuder identificeret visuelt (median 2.5), ingen ved efterfølgende palpation, men ved finkæmning af fedtvævet 1–7 ekstra lymfeknuder (median 2). Lymfeknuderne var alle <5 mm, blåfarvede og uden metastaser.
Diskussion og konklusion: Alle supplerende lymfeknuder var uden metastaser, og ændrede således ikke stadieinddelingen. Lymfeknuderne fremtrådte tydeligere efter yderligere fiksering. Dette skyldtes en kombination af intensiveret blåfarvning og bedre fiksering af det omgivende væv, som var skåret i skiver. For at kunne sammenligne skiverne før og efter ekstra fiksering, blev supplerende finkæmning af fedtvævet først foretaget ved sekundær udskæring. Det kan ikke afgøres om de ekstra lymfeknuder identificeret visuelt kunne være fundet ved finkæmning ved primær udskæring. Projektet har ikke givet anledning til ændring af praksis på afdelingen.

5

Collision metastases of colorectal and prostatic adenocarcinoma in 2 lymph nodes – a case report

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Introduction: Colorectal cancer is a common cancer and a leading cause of cancer mortality. Localized lymph node metastases at the time of diagnosis are frequent. Prostatic cancer is the second most common cancer in males. Metastases to mesenteric lymph nodes are rarely encountered. Collision metastasis of 2 different primary cancers to 1 lymph node is very unusual.
Material and methods: We report a case of a 78-year old male diagnosed with 2 synchronous adenocarcinomas in sigmoid colon and rectum. During the diagnostic process, a PET-CT scan revealed a small FDG positive focus in prostate. Biopsies confirmed a diagnosis of prostatic adenocarcinoma.
Results: The patient underwent surgery and by histopathological examination 2 primary adenocarcinomas were diagnosed, stage T4a and T2, respectively. Metastases with 2 distinct morphological patterns were present in 23 out of 44 mesenteric lymph nodes. In 3 lymph nodes, the morphology was consistent with colorectal adenocarcinoma and in 22 with prostatic adenocarcinoma. Collision metastasis containing both colorectal and prostatic adenocarcinomas was identified in 2 of the lymph nodes. Immunohistochemical (IHC) staining confirmed the primary origin of both components of the metastases.
Discussion and conclusion: Collision metastases including colorectal and prostatic adenocarcinoma has been reported in 5 previous published cases. Since the identification can potentially affect the therapy of patients, it is important to recognize. IHC staining is recommended in case of uncertain primary origin. In this case, an already existing diagnosis of prostatic cancer and plenty of lymph nodes with extensive involvement of metastases of prostatic adenocarcinoma was helpful.

6

Development of an automated method to obtain reproducible counts of lymphocytes in patients with colorectal cancer

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Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide. Although clinical outcome varies among patients diagnosed within the same TNM stage, it is central in treatment decisions and follow-up programmes. Assessment of Tumor-infiltrating lymphocytes provides valuable information evaluating survival outcomes. The aim of this study was to develop a fully automated method for quantification of subsets of T-lymphocytes in the invasive margin (IM) and central tumor (CT) based on artificial intelligence.
Method and materials: The cohort consisted of 163 consecutive patients diagnosed with colorectal adenocarcinoma. One representative slide from the surgical specimen was selected from each patient for double-labelling immunohistochemical staining with cytokeratin in combination with CD3 or CD8, respectively. Visiopharm Quantitative Digital Pathology software was used to develop Application Protocol Packages (APPs) for segmentation into the tissue classes; background, normal epithelium, cancer epithelium, and surrounding tissue followed by identification of CT and IM as well as the subsequent quantitative analysis of immune cells.
Results: Fully automated counts for CD3+ and CD8+ T-cells were obtained in 93% and 92% of the cases, respectively. The remaining cases required manual editing of the CT and IM. No correlation was observed between T-stage, histological subtype, differentiation of tumor or tumor buds and requirement for manual editing.
Discussion and conclusion: The development of a fully automated method for counting CD3+ and CD8+ lymphocytes in a cohort of patients with CRC provided excellent results eliminating not only observer variability in lymphocyte counts but also identified the regions of interest for the automated analysis.

7

Implantation af intestinal slimhinde på tyndtarmsserosa - en kasuistik

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Sygehistorie: 84-årig kvinde indlægges dag 0 obs. akut abdomen.
Dag 1: Laparaskopi konvertet til åben kirurgi med resektion af perforeret sigdoideum med divertikulitis og udtalte adhærencer til tyndtarm og uterus. I den forbindelse opstår tyndtarmslæsion, der oversyes.
Dag 2: Åben kontrol, hvor der bemærkes grøntligt sekret fra tyndtarmssegment i afstand fra læsionen. Perforation kan trods grundig søgning ikke identificeres.
Dag 4: Gentaget åben kontrol, hvor der fortsat set grøntlig sekret fra samme tyndtarmssegment. Segmentet reseceres på mistanke om perforation.
Makroskopisk findes et tyndtarmsresektat målende 17 cm. Normal slimhinde. Serosa fibrinbelagt og hæmoragisk. Ingen tegn på perforation. Der bemærkes et område med mere fast væv og indtrækning på serosa, hvorfra der tages ekstra snit.
Mikroskopisk ses upåfaldende mucosa. Serosa er i flere snit pusbelagt og akut og kronisk inflammeret, hvor inflammationen strækker sig fra serosa og ind i den yderste del af muscularis propria. I snittene fra det makroskopisk beskrevne område ses tydeligt fastsiddende, ikke-neoplastisk intestinalt epitel på serosaoverfladen med rigelige Panethceller, men uden villi. Immunhistokemi for SATB2 er negativ.
Der lader til at være sket en mekanisk og inflammatorisk betinget implantation af intestinal slimhinde på tyndtarmens serosa med følgende sekretion fra epitelet, hvilket klinisk er tolket som perforation.

8

Brain Tissue Based Diagnostics of Rare and RT-QuIC-negative Prion Disease Subtypes

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Introduction. Prion diseases are rapidly progressing, neurodegenerative protein misfolding diseases. The commonest prion disease caused by misfolding cellular prion protein is sporadic Creutzfeldt-Jakob disease (sCJD). Current sCJD classification consists of 14 molecular subtypes defined by misfolded prion protein isoform, patient's genotype at polymorphic codon 129 in the prion protein gene and distinct neuropathological features such as spongiosis severity and misfolded prion protein accumulation pattern in different brain regions.
Material and methods. Real time quaking-induced conversion (RT-QuIC) method is widely applied for ante-mortem diagnostics of prion disease, and it is an excellent tool providing quick and highly accurate (>90%) positive/negative-type of answers based on cerebrospinal fluid sample analysis of sCJD suspected patients. However, the RT-QuIC method cannot distinguish between different disease molecular subtypes, and, most importantly, does not detect some of the rarer subtypes.
We present 3 recent, unique sCJD cases that would have been impossible to diagnose without neuropathological and molecular examination of brain samples.
Results. The 3 cases include the first in the world sCJD molecular subtype VV1 with 1-Octapeptide Repeat Deletion polymorphism, the first in Denmark Variably Protease Sensitive Prionopathy, and a case of sporadic Fatal Insomnia presenting with Parkinsonism. RT-QuIC test was negative in 2 of the cases and was not performed in the last case.
Discussion and conclusion. It is strongly encouraged to still consider autopsy-based diagnostics in patients with suspected sCJD or an atypical fast progressing dementia.

9

Right heart failure caused by carcinoid heart disease associated with a non-metastasizing pancreatic neuroendocrine tumour – a case report

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Right heart failure may be a rare manifestation of neuroendocrine neoplasia.
We report the case of an elderly man with symptoms of heart failure. Transthoracic echocardiography showed enlarged right-sided cavities, and a right-sided heart catheterization revealed a hyperdynamic circulation with high cardiac output. After initial supportive treatment, the patient's condition deteriorated, and he died. A medical autopsy found a previously undiagnosed, 15 mm diameter, grade 1 neuroendocrine tumour in the head of the pancreas without evidence of metastasis. The right-sided cardiac valves and the aortic valve were sclerotic.
To our knowledge, this is the first case of right heart failure caused by right-sided cardiac valve sclerosis associated with an undiagnosed, apparently non-metastasizing pancreatic neuroendocrine tumour without symptoms of carcinoid syndrome. Carcinoid heart disease is associated with an increased mortality and should be suspected in patients presenting with idiopathic right heart failure.

10

CD163 er en potentiel markør for sarkoidosegranulomer.

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Introduktion: Sarkoidose er en systemisk sygdom, som er kendetegnet ved dannelse af ikke-nekrotiserende granulomer, hvori makrofager er en essentiel bestanddel. Makrofager inddeles i to subtyper, M1 og M2, der blandt andet adskiller sig i ekspressionen af overfladeproteiner, hvor CD68 er markør for M1 subtype, og CD163 er markør for M2 subtype. I dette kvalitetssikringsprojekt undersøges, om sarkoidosegranulomer adskiller sig fra non-sarkoidosegranulomer, baseret på niveauet af CD68 og CD163 positive makrofager ved at benytte digital billedanalyse og semikvantitativ gradering.
Materialer og metoder: Materialet blev fremskaffet fra Regionshospitalet Randers ved søgning på granulomatøse sygdomsdiagnoser i tidsintervallet d. 01.01.2017 til d. 25.05.2020. Der blev foretaget immunhistokemisk farvning med CD68 (PG-m1) og CD163 (MRQ26) på væv fra 7 patienter med sarkoidosegranulomer og 29 patienter med non-sarkoidosegranulomer. To observatører vurderede graden af CD68 og CD163 positive reaktioner som følgende: 0 (ingen immunreaktion), 1 (svag immunreaktion), 2 (moderat immunreaktion), 3 (stærk immunreaktion). Herudover blev materialet analyseret ved hjælp af digital billedanalyse software (Visiopharm) og makrofagernes ekspressionsniveau blev kvantificeret som arealfraktioner.
Resultater: Niveauerne af CD68 var ens i sarkoidosegranulomerne og non-sarkoidosegranulomerne. Derimod var niveauet af CD163 signifikant lavere i sarkoidosegranulomerne end i non-sarkoidosegranulomerne både ved digital billedanalyse ($p=0,0445$) og i den semikvantitative graderingen ($p=0.0133$).
Diskussion og konklusion: Vores resultater viser, at CD163 kan være en potentiel markør til at adskille sarkoidosegranulomer fra non-sarkoidosegranulomer. Da sarkoidose kan være en vanskelig diagnose at stille morfologisk, kan CD163 understøtte en korrekt diagnosticering. Disse resultater skal dog valideres yderligere i en større kohorte, da vi har et lavt antal sarkoidosegranulomer.

11

Evaluating the use of PHH3 as a tool in mitotic activity counting on surgical resection specimens in breast cancer.

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Introduction:
Assessing mitotic activity in invasive breast cancer is an essential prognostic factor.
However, there is a certain degree of inter observer variation when evaluating mitotic count.
Phosphohistone H3 (PHH3) is a marker of mitotic activity, The aim of this study is to evaluate the use of PHH3 as a supplement to counting mitoses on HE-slides.
Material and methods:
We collected slides from 30 resection specimens of invasive breast carcinomas selected from a period between June and September of 2021. We stained two consecutive slides from each specimen with HE end PHH3.
Mitoses were counted on the HE slide and subsequently the corresponding PHH3 slide and, lastly, the number of mitotic figures on the HE slide was counted in the area of the tumor with the highest density of PHH3 signals (field diameter 0,55 mm).
For each step, we monitored how long it took to evaluate the number of mitoses/PHH3 signals.
Results:
Mean number of mitoses was respectively 5,57 (n1), 9,03 (n2) and 7,23 (n3) for each consecutive step with a mean difference between n1 and n3 of 1,8 (p 0,001). In four cases, the discrepancy between n1 and n3 was large enough to assign a higher number of points for mitotic count and possibly warrant a change in grading of the tumor.
Discussion:
The study shows, that PHH3 may be useful in identifying areas with a higher mitotic count in tumors with heterogenous proliferation. However, the data are not conclusively reproducible.

12

Mutational analysis in the diagnosis of an uncharacteristic sex cord-stromal tumor of the ovary: a case report

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This case report details the difficult diagnosis of an ovarian sex cord-stromal tumor in a pregnant 28-year-old woman. She had a previous history of follicular thyroid cancer at age 11. At 25 weeks of gestation, she was admitted with complaints of abdominal pain. Sonographic and magnetic resonance imaging revealed a 13 cm cystic-solid tumor in the pelvic cavity. At 31 weeks of gestation, the tumor had grown considerably, now 18 cm, and the patient experienced increasing pain. A cesarian section combined with a unilateral salpingo-oophorectomy was performed. Histology and immunohistochemistry showed a sex cord-stromal tumor of the ovary with an uncharacteristic morphology. Differential diagnoses were luteoma of pregnancy, a juvenile granulosa cell tumor, a Sertoli-Leydig cell tumor (SLCT) of poor differentiation, or a sex cord-stromal tumor, NOS (not otherwise specified). SLCTs are very rare neoplasms that account for less than 0.2% of all ovarian cancer, and up to approximately 60% of SLCTs are associated with germ-line and/or somatic mutations in the DICER1 gene. The other differential diagnoses are only rarely associated with DICER1-mutations. Given the patient's previous history, we speculated that she might have the hereditary cancer predisposition DICER1 syndrome. Mutational analysis of the tumor tissue demonstrated two mutations in the DICER1 gene (c.988C>T, p.Q330 and c.5127T>A, p.D1709E). Thus, we were able to reach the diagnosis of a poorly differentiated SLCT of the ovary. A subsequent genetic evaluation of peripheral blood from the patient showed a germline mutation in the DICER1-gene confirming DICER1 syndrome in the patient.

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Basal cell carcinoma of Prostate with MSMB-NCOA4 fusion and a probable Basal cell carcinoma in-situ: Case report

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Introduction:
Basal cell carcinomas of prostate (BCCP) are very rare. Most arise in the transition zone, thus associated with lower urinary tract symptoms and rarely associated with elevated Prostate Specific Antigen (PSA). These features make diagnosis difficult. Basal cell carcinoma in situ (BCCIS) is a non-existent histological lesion, but here is an attempt to describe it through this case. On the molecular front, BCCP has shown association with the microseminoprotein beta (MSMB) gene, but microseminoprotein beta–nuclear receptor co-activator 4 (MSMB–NCOA4) gene fusion, has never been reported in BCCP as per the latest literature.

Material and methods: Histological material submitted after prostatectomy and needle biopsies of abdominal wall metastasis were subjected to detailed examination. Prostatic needle biopsy performed in 2005 was also retrieved. Molecular analysis (Archer FusionPlex Solid Tumor Panel), Whole Exome Sequencing (WES) and Single Nucleotide Polymorphism Arrays were performed.

Results: A 74-year-old man presented with hematuria and previous diagnosis of prostatic hyperplasia. He underwent a prostatectomy. Pathological examination revealed a diffusely infiltrative tumor with non-acinar adenocarcinoma morphology and many glandular structures - basal cell carcinoma in-situ (BCCIS)? Tumor was diagnosed as BCCP. Molecular examination detected MSMB–NCOA4 fusion. Patient presented with metastasis to abdominal wall 8 months post prostatectomy.

Discussion and conclusion: BCCP is an aggressive type of prostate cancer which might be challenging to diagnose based on routine protocols. This results in delayed diagnosis and treatment and thus poor prognosis. Diagnosis of BCCIS, if agreed upon its existence needs to be studied in larger cohort as a precursor lesion.

14

Malassezia folliculitis: A case study of an uncommon but important differential diagnosis

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Introduction

Malassezia folliculitis, also known as Pityrosporum folliculitis, is an inflammation of hair follicles caused by the endogenous yeast Malassezia sp. In young patients, who are most often affected, it is typically seen as itchy, monomorphic papules and pustules with perifollicular erythema on the upper back and chest. However, in older patients it can affect the forehead and cheeks and clinically be mistaken as acne or neoplasia.

Material and Methods

A 87-year-old patient was referred to a dermatologist with a 3x3 cm lesion on the right cheek. Clinically tentative diagnose of insect bite reaction was suggested and a punch biopsy was performed. Based on conventional microscopy and immunohistochemical stainings urticarial reaction and solar elastose were sugested. Over the following 2,5 years the disease progressed slowly and punch biopsies were twice performed without the correct diagnosis.

Results

A fourth punch biopsy revealed an acanthotic epidermis with mild hyperkeratosis, dilation of hair follicles with follicular plugging, superficial edema of the dermis and marked actinic elastosis. Furthermore, in the dilated hair follicle a number of characteristic budding conidia was seen by conventional PAS-stain. This confirmed the diagnosis of Malassezia folliculitis. The patient was successfully treated with topical econazole cream.

Discussion and conclusion

Although the condition presents with small papules and pustules, this alone should not be confused with acne, especially in older, immune incompetent patients. This is an uncommon but important differential diagnosis in unexplained facial lesions in older patients, which must not be overlooked.

15

A nationwide population-based study on the prognostic significance of margin clearance in Whipple resection specimens with pancreatic ductal adenocarcinoma

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Background: Although resection margin status is considered a prognostic factor after a Whipple procedure for pancreatic ductal adenocarcinoma (PDAC), the definition of microscopic margin involvement (R1 resection) is unclear. In this nationwide population-based study, we investigated the overall minimum margin clearance associated with improved survival and evaluated whether one or more of the resection margins are of independent prognostic significance.

Material and methods: Data from 351 patients who underwent a Whipple procedure for PDAC in the period 2015-2018 were retrieved from the Danish Pancreatic Cancer Database. Surgical specimens were evaluated using a standardised pathological protocol involving multicolour inking, axial slicing, and assessment of the circumferential margins. The margin clearances were stratified by 0.5 mm increments (range 0.5-≥3.0 mm), thereby dichotomising the patients into groups with R0 (no microscopic tumour remaining) and R1 resections.

Results: When categorised according to margin widths of <0.5 mm, <1.0 mm, <1.5 mm, <2.0 mm, <2.5 mm and <3.0 mm, R1 resections were detected in 32%, 54%, 70%, 74%, 84% and 84% of cases, respectively. In multivariable analyses, an overall margin clearance of ≥1.5 mm was associated with improved survival compared with a clearance of <1.5 mm (HR 0.65 95% CI 0.48-0.89). Moreover, when evaluating the margins separately, posterior margin clearance of ≥1.5 mm was associated with improved survival (HR 0.73 95% CI 0.56-0.96).

Discussion and conclusion: Margin clearance of at least 1.5 mm is associated with improved survival following the Whipple procedure for PDAC. Furthermore, assessment of the posterior margin provides isolated prognostic information.

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BRAFV600E expression is homogenous and associated with non-recurrent disease and better survival in primary melanoma

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Background: More than 50 % of the melanoma patients harbor a mutation in the BRAFV600E gene that activates MAPK cancer signaling pathway. The mutation status guides in treatment selection. However, the relationship between the BRAFV600E gene expression and BRAFV600E protein intratumoral distribution, on one side, and clinicopathological factors and patient outcomes, on the other, is not fully described.
Methods: We performed immunhistochemical staining to investigate the expression of BRAFV600E in 166 cutaneous superficial spreading melanomas and compared to gene expression levels using NanoString analysis.
Results: 97 (49%) melanomas stained positive for BRAFV600E, with nearly 100% intratumoral homogeneity observed. Positive BRAFV600E expression was significantly associated with non-recurrent disease and was found to be an independent predictor of better prognosis in multivariable analyses. Furthermore, presence of TILs, SLNB negativity and low Breslows thickness were all independent predictors of better prognosis. We observed no difference in the BRAF mRNA levels in BRAFV600E negative and BRAFV600E positive melanomas, respectively. Validation in a larger publicly available cohort confirmed only a weak correlation (spearman 0.4) between BRAFV600E mRNA and protein levels, and no differences in mRNA between BRAFV600E mutated and non-mutated patients.
Conclusion: Our findings indicate that BRAFV600E is homogeneously present throughout the whole tumor and is associated with non-recurrent disease and better survival in primary melanoma. We also show that BRAFV600E mutation does not result in higher transcriptional levels, suggesting that activation of MAPK signaling pathway in BRAFV600E mutated patients can be attributed to the increased biochemical activity caused by the mutation.

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LAG3 and TIGIT expression on Tumor-infiltrating lymphocytes in cutaneous melanoma

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Background: Tumor-infiltrating lymphocytes (TILs) in melanoma represents the host's immune response against cancer cells and is associated with improved overall survival.. However, many TILs express targetable inhibitory immune checkpoint (IC) proteins such as PD1 and CTLA4, that can inhibit the T cell response.

Methods: The study cohort consisted of FFPE tissue from 166 patients diagnosed with cutaneous superficial spreading melanoma in Region Zealand. NanoString analysis of 790 genes related to tumor and immune response was performed.

Results: A comparison of melanomas with presence (n=120 and absence n=46) of TILs revealed 36 differentially expressed genes of which all where highly expressed in melanomas with TILs. In order to identify biological pathways we performed STRING Enrichment analysis. Mainly one large cluster was identified and all genes were involved in immune response/regulation. The 10 top-ranked Hub genes (with highest degree of connectivity) were identified, including LAG3 and TIGIT. Blockade of both proteins are currently being tested in clinical trials.

Conclusion: Melanomas with TILs show upregulation of many immune related genes, some favoring and others inhibiting an immune response. IC genes LAG3 and TIGIT are upregulated in patients with TILs. Immunohistochemial subtyping of LAG3 and TIGIT positive TILs reveal their spatial context.

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Histologic findings in a case of Erdheim-Chester disease –a rare systemic disorder.

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Introduction
Erdheim-Chester disease (ECD) is a rare non-Langerhans histiocytosis characterized by severe fibrosis accompanied by infiltration of CD68-positive and CD1a-negative macrophages, multinucleated giant cells and proliferation of lymphocytes. ECD is associated with mutations of kinase-signalling-pathways e.g. BRAF, NRAS and KRAS. ECD can involve all organ systems and is considered a potential severe multi-systemic disease with life-threatening manifestations.

Material and methods
We report the autopsy findings in a 48-year-old woman. Death was thought to be heart failure due to an inoperable heart tumour diagnosed four years earlier. If it was neoplastic or reactive had not been finally concluded. Malignancy was excluded.

Results
Autopsy revealed a massive fibrous tumour-like growth involving heart, pericardium and lungs. The growth surrounded the thoracic and abdominal aorta, as well as the renal arteries and the systemic veins. Infiltration was also seen in retroperitoneum, perirenal fat capsules and mesentery of the small intestine, as well as the peripancreatic and periadrenal tissue.
Histopathology showed fibrous proliferation with a mixture of lymphocytes, plasma cells and abundant histiocytes, including multinucleated giant cells and BRAF V600E mutation. Very characteristic bronchovascular fibrous bundles were observed in the lung sections. The morphologic findings are compatible with ECD.

Discussion and conclusion
Until now ECD has been reported in <1000 cases. ECD is often misdiagnosed as a reactive process. Also, in this patient, where unfortunately first by examining autopsy material a definite diagnosis was reached.

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Skleroserende polycystisk adenose

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Introduktion
Skleroserende polycystisk adenose (SPA) er en sjælden og relativt nybeskrevet tumordannelse som udgår fra spytkirtelvæv. I det følgende beskrives det histologiske billede som er mangfoldigt, hvorfor man sommetider må overveje differentialdiagnoserne acinic cell karcinom, epitelial-myoepitelialt karcinom og adenoidcystisk karcinom, som i lighed med SPA kan indeholde både celler med myoepitelial og duktal differentiering.

Materialer og metoder
En case fra rutinediagnostikken med en ensidig parotistumorexcision fra en 42-årig mand blev gennemgået. Der blev foretaget HE-oversigtsfarvning samt immunhistokemisk undersøgelse som angivet i tabel 1 i rutine diagnostisk øjemed.

Resultater
Ved HE-farvning sås en kapselafgrænset ekspansivt voksende tumor opbygget af glandulært epitel omgivet af myoepitel. Der var relativt velformede ductusstrukturer med varierende grader af cystisk dilatation. Det glandulære epitel varierede fra lavt kubist til højt cylindrisk og indeholdt ikke fremtrædende zymogengranula. Der var en sparsom komponent af hyalin fibrose, mest udtalt i relation til kapslen, men fokalt også mellem ductusstrukurerne.

Diskussion og konklusion
En række immunfarvninger blev foretaget hvoraf fire støttede diagnosen SPA, fem ikke var konklusive da der i litteraturen ikke kan findes oplysninger om det, og en enkelt farvning som ikke støttede diagnosen. Acinic cell karcinom og epitelialt-myoepitelialt karcinom var mindre sandsynligt pga. reaktionsmønstrene for CD117 og DOG1. Adenoidcystisk karcinom kan immunhistokemisk ikke sikkert adskilles fra SPA, men tumorens ganske monomorfe opbygning taget i betragtning, er adenoidcystisk karcinom mindre sandsynligt. I litteraturen fremgår det at der ikke er fuldstændig enighed om, hvorvidt SPA er en neoplastisk eller reaktiv proces.

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Mesonephric-like adenocarcinoma - a case report

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Introduction
Mesonephric-like adenocarcinomas (MLA) are rare, clinically aggressive neoplasms of the uterine corpus and ovary, representing <1% of endometrial carcinomas. Although their molecular, morphologic and immunohistochemical profiles have been recently defined in WHO Classification of Female Genital Tumors in 2020, the pathogenesis of MLAs are poorly understood.

Case presentation
A 75-year-old woman with atypical vaginal bleeding and an endometrial mass detected by transvaginal ultrasound, underwent endometrial curettage and subsequently a total abdominal hysterectomy, that revealed a mesonephric-like adenocarcinoma of the endometrium. The tumor showed closely packed, glandular formations with >5% solid growth and absence of squamous differentiation. The glandular epithelium was pleomorphic, with hyperchromatic, mild to moderately atypical nuclei and numerous mitosis. Immunohistochemically, the tumor showed a positive reaction for CKAE, TTF-1, GATA3 and PAX8, no reaction for ER, CK20, CDX2, WT1, chromogranin, synaptophysin or napsin and wild type reaction for p53, focal reactivity for CK7 and P16 and a luminal reaction for CD10. Furthermore, the tumor had a proliferation rate of 50 % identified with ki67, supporting the diagnosis.

Discussion
MLAs are challenging to diagnose, because a variety of growth patterns can be present within the same tumor. It remains unclear whether they represent mesonephric (Wolffian) carcinomas arising in the endometrium/ovary, supported by the closely morphological and immunohistochemical resemblance to other mesonephric adenocarcinomas and a luminal CD10 reaction, illustrated in the abovementioned case or endometrioid (Müllerian) carcinomas that differentiate along a mesonephric pathway and closely imitate mesonephric carcinomas. Further investigation is therefore needed to understand these tumors.

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Do pathologists have an interest in intervening with the public?- and how can we do it

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Introduction:
Pathologist work in the laboratory interacting with the clinicians. The patients we provide with the broad spectrum of diagnosis mandatory for the correct treatment do not know us. Often the patients do not know how and which kind of complex analysis the diagnosis is based on. We would like to present our idea of presenting pathology in an informal way aiming to reach out and meet the general population.
Material and methods:
During the last years, we have been involved in different small projects focusing on this topic. We included some of our material from the competition: Kreativ Patologi, which led to a collection of interesting and funny pictures from the daily work of pathologists. The collection inspired us to present selected material to group of people outside the laboratory. We managed to collaborate with Galleri Rasmus, a Gallery well known on Funen. This resulted in collaboration with two different artists, who integrated pathology-images in their art. The pictures were presented at an exhibition combined with a workshop.
Results:
We experienced at huge interest in our work and an audience eager to know about “how the body looks” through a microscope. A platform of interacting with the public that do not exists today.
Discussion and conclusion:
We wish to present our experience and ideas. We look forward to an open discussion with the audience. The pathologists are the doctors the patients never meet, and for many years, we have not reflected on this fact.

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Ileocecal intussuception caused by a tubulovillous adenoma in the small intestine

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Introduction
In this case study, we present a case of ileocecal intussusception in a 67 year old woman. The woman presented with a 12-hour history of intermittent abdominal pain, greatest in the right side of the abdomen. She had no past medical history of concern. Computed tomography scan showed ileocecal invagination. A right hemicolectomy was performed with primary ileotransverse anastomosis. Intussusception are most often found in children, and only 5% of all cases occur in adults. 90% of cases in adults are secondary to lesions in the bowel wall including carcinomas or benign neoplasms. Tumors are quite common in the colon, whereas it is much more rare in the small-intestines.
Results
In this case study a rare tubulovillous adenoma (TVA) in the terminal ileum (TI) is diagnosed as the cause of invagination. All steps are presented: clinical assessment, radiologic evidence, surgical treatment, and lastly the pathological diagnosis of a TVA.
Discussion and conclusion
This case study is unique due to the rarity of both TVAs in the small intestines as well as intestinal invagination in adults. Uniquely, we have been able to thoroughly document evidence of the diagnosis. We present extensive radiological imaging as well as macroscopic and histological pictures of this rare TVA in the TI causing ileocecal invagination.

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Reliable assessment of CD20 in breast cancer requires whole slide sections instead of tissue microarrays

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Background:
Tumor infiltrating B-lymphocytes may be diffusely scattered or form nodular lymphoid structures especially in the tumor-periphery. We aimed to test the use of tissue microarrays (TMA) as compared to whole-slide sections (WS) in evaluation of CD20 using digital image analysis (DIA) in breast cancer (BC).

Material and Methods:
Immunohistochemistry for CD20 was performed on TMA (1-mm core/patient) and corresponding WS from 221 BC patients. DIA based on the convolutional neural network U-Net automatically quantified the CD20-index of WS and TMA within 4 regions: 1) in direct contact with tumor-epithelial cells; 2) in stroma margin (20 µm) of tumor-epithelial cells, 3) in tumor-related stroma, and 4) in the whole lesion. Bland-Altman analysis and Wilcoxon signed-rank tests compared CD20-indices of WS and TMA.

Results:
For all 4 regions, a higher CD20-index was detected on WS as compared with TMA ($p<0.001$). The variation in CD20-indices was widest in the tumor-related stroma and most narrow in the tumor-epithelium. The mean differences between CD20-indices of WS and TMA were 0.15% (95% limits of agreement: -0.74; 1.03)% for tumor-epithelium contact; 0.65% (-3.62; 4.9)% for stroma margin of tumor-epithelium; 1.33% (-5.86; 8.52)% for tumor-related stroma, and 1.00% (-4.7; 6.7)% for whole lesion.

Discussion and Conclusion:
Low CD20-indices were predominantly observed on TMA and high CD20-indices on WS. The results indicate that TMA can underestimate presence of CD20 positive cells in BC and that B-lymphocytes occurring in nodules in the periphery of the tumor can be missed on TMA.

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Dødsårsager ved intrauterin fosterdød efter føtal autopsi på Rigshospitalet. En opgørelse.

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Introduktion: Hovedformålet med føtal obduktion og placentaundersøgelse efter intrauterin fosterdød (IUFD) er, at bidrage til opklaring af dødsårsagen af hensyn til forældrenes sorgbearbejdning og med henblik på planlægning af eventuelle kommende graviditeter. I 2014 udgav DSOG en guideline for håndtering af IUFD i Danmark med et nyt nationalt klassifikationssystem (INCODE-DK) til bestemmelse af sandsynlige og mulige dødsårsager ved IUFD.
På Afdeling for patologi, Rigshospitalet (RH), har vi siden 2014 systematisk anvendt INCODE-DK ved udarbejdelse af svar på obduktion efter IUFD. Formålet med aktuelle opgørelse er at få overblik over kvaliteten af disse obduktioner ud fra andelen af forklarede dødsfald.
Materialer og Metoder: I perioden 14.2.2014-31.10.2021 inkluderedes alle fosterobduktioner med gestationsalder (GA) på mindst 22 uger, hvor der klinisk var konstateret fosterdød inden forløsningen.
Resultater: I perioden udførtes i alt 1604 fosterobduktioner, heraf var 301 efter IUFD (GA 22-42 uger). Dødsfaldet var forklaret i 203 (67,4%) tilfælde, med sandsynlig dødsårsag i 113 tilfælde (37,4%) eller mulig dødsårsag i 90 tilfælde (29,9%). Dødsårsagen var uforklaret i 98 tilfælde (32,6%). I de forklarede tilfælde var ”patologisk placenta” langt den hyppigste dødsårsag (159 tilfælde, 55,8%).
Diskussion og konklusion: Vores opgørelse over brugen af INCODE-DK på RH i en 7½ årig periode har vist, at vi påviser en sandsynlig eller mulig dødsårsag i godt 2/3 af tilfælde med IUFD. Denne opklaringsprocent er i tråd med internationale studier, hvor opklaringsprocenten dog er yderst varierende. Resultatet understreger vigtigheden af føtal obduktion inklusiv placentaundersøgelse ved IUFD samt den store anvendelighed af INCODE-DK som arbejdsredskab for føtalpatologer i Danmark.

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Impact of new molecular criteria on diagnosis and survival of adult glioma patients

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Introduction: The fifth edition of WHO classification of Tumors of the Central nervous system (WHO-CNS5) released in 2021 has integrated molecular parameters to further refine CNS tumor classification. Objectives: This study reclassified a retrospective cohort of adult glioma patients according to WHO-CNS5, and assessed if overall survival (OS) correlated with the revised diagnosis. Further, we evaluated the diagnostic impact of 850k methylation analysis performed in the cohort. Methods: Adult glioma patients (n=228) diagnosed at the Department of Pathology, Aarhus University Hospital between 2017 and 2019 was reclassified according to the new criteria. All patients had routine diagnostic NGS analysis performed. 850k methylation analysis was performed in 29 patients. Survival data is described using Kaplan-Meier plots and Cox proportional hazard model. Results: 19 patients were reclassified. Specifically, diffuse astrocytic glioma, IDH wildtype, with molecular features of glioblastoma (DAG-G) were reclassified as glioblastoma (n=15), and lower grade IDH mutant (IDHmut) astrocytomas (WHO grade 2-3) were reclassified as astrocytoma IDHmut, WHO grade 4 (n=4). Shifts to glioblastoma were because of TERT mutation (n=9), TERT mutation and 7+/10- (n=3), EGFR amplification (n=2), and EGFR amplification and TERT mutation (n=1). Shifts to astrocytoma, IDHmut, grade 4 were due to CDKN2A/B co-deletion (n=4). Survival analysis showed no significant difference in OS for reclassified DAG-G in the whole group (p=0.558) and for TERT mutations only (p=0.451), compared to glioblastoma, IDH wildtype. 850k methylation analysis resulted in revised diagnosis (n=2), confirmed diagnosis (n=15) and no match (n=12). Conclusion: The OS of reclassified DAG-G correlated with the revised diagnosis.

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Deeper sections reveal residual tumor cells in rectal cancer specimens diagnosed with complete pathological regression following neoadjuvant treatment

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Introduction

Guidelines for diagnosing pathological complete regression (pCR) in rectal cancer after neoadjuvant treatment vary, and there is currently no consensus on the appropriate number of sections to examine per tissue block. The consequence of systematic use of deeper sections on diagnostic accuracy and prognosis for patients with ypT0 rectal cancer was investigated in this retrospective study.

Materials and Methods

All patients who underwent neoadjuvant therapy and surgical resection that were diagnosed with ypT0 rectal cancer from 2015 to 2020 in our department were included (n = 23). In accordance with current British guidelines, three additional deeper sections were cut from each tissue block from the primary tumor site and reviewed for presence of residual tumor cells.

Results

Additional sections revealed residual viable tumor cells in seven patients (30.4%). Of the seven patients, three patients (42.9%) later had local recurrence or distant metastasis during the follow-up period, compared with one patient (6.25%) with no residual tumor cells in deeper sections (p = 0.07). A nonsignificant reduction in disease-free survival (p = 0.08) was observed in the patients with residual tumor.

Discussion and conclusion

Systematic use of deeper sections in evaluation of tumor regression in rectal cancer reveals the presence of residual tumor cells in a subset of patients originally diagnosed with pCR based on a single section per FFPE block. Although the results are not statistically significant, it cannot be excluded that accurately distinguishing complete from near-complete response may be clinically relevant for prognostic prediction.

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Development of an invasive ductal carcinoma within a complex fibroadenoma

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Fibroadenomas are among the most common benign tumors of the female breast with peak incidence in the 2nd and 3rd decade of life. Fibroadenomas are generally not considered premalignant and are not reported as an independent risk marker for breast cancer. Carcinoma in situ may occasionally be present within a fibroadenoma, and invasive cancer originating in the surroundings may be observed to extend into a fibroadenoma. Cancer arising within a fibroadenoma is, however, unusual with a reported incidence ranging from 0.02-0.125% and less than 250 reported cases. Complex fibroadenomas have been associated with a higher relative risk of developing breast cancer, but this may be related to the presence of associated benign proliferative changes.
We present a case of invasive ductal carcinoma arising within a complex fibroadenoma in a 56 years old female with a growing lump in the upper lateral quadrant of the right breast. The lump had been present for 20 years and was classified as a benign lesion by mammography and verified by several biopsies. The patient underwent lumpectomy, and histopathological examination incidentally showed a 27 mm invasive ductal carcinoma completely encapsuled within a complex fibroadenoma. The patient subsequently underwent sentinel node procedure without sign of metastasis.
Fibroadenomas are not associated with an increased risk of breast cancer but may rarely be involved with malignancy. The presented case illustrates that detection of premalignant and/or malignant changes within a fibroadenoma may be delayed due to benign radiological and clinical findings.

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Glomus tumors in children: Two case reports

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Glomus tumors are mesenchymal neoplasms, accounting for less than 2% of soft tissue tumors, being even more rare in children. Such tumors are composed of cells resembling perivascular modified smooth muscle cells and are typically located in the cutaneous tissue of the distal extremities, but tumors have occasionally been reported in visceral areas. Glomus tumors are usually benign with malignant cases typically more deeply localised. Glomus tumors is a diagnostic challenge when they present in unusual localizations and/or with atypical morphology. Recently a MIR143-NOTCH2 fusion has been reported in some glomus tumors.
We report two cases of glomus tumors in children; I) a fourteen-year old male with a soft tissue mass in the foot. A biopsy revealed a tumor that was histologically and immunohistochemically consistent with a glomus tumor of uncertain malignant potential. Six months later a lung metastasis was discovered. II) an eleven-year old male with respiratory stridor. A bronchoscopy identified a tracheal tumor. Initially a carcinoid tumor was suspected. The biopsy revealed a tumor with spindle cell features. Among differential diagnosis were carcinoid tumor, synovial sarcoma and a leiomyomatous tumor. Archers fusion panels detected a MIR143-NOTCH2 fusion. The tumor was finally diagnosed as a glomus tumor of uncertain malignant potential.
Even though glomus tumors are rare they do appear in children. Some cases are easily diagnosed but in certain challenging cases detection of NOTCH gene rearrangement is desirable to obtain a correct diagnosis.

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Can we reduce the workload in melanoma sentinel node (SN) examination? –a national study of pathology reports from 590 patients

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Introduction: With the implementation of the 2021 melanoma SN pathology guideline overall workload has increased, going from examining nodes at 5 levels (50µm apart) and using less expensive immunohistochemical stains before to now examining 6 levels (150µm apart) and using more expensive stains. Detecting SN metastases are important for correct tumor staging, and metastasis diameter (metastasis >1mm) help determine candidates for adjuvant therapy.
Material and Methods: We reviewed 970 melanoma SN pathology reports nationwide (590 patients) to answer the following for each SN: Was a metastasis detected? If yes, in which level(s)? Was the metastasis >1mm? If yes, in which level(s)?
Results: The updated guideline detected metastases in 117 (23%) and metastases >1mm in 38 (7%) of patients. Examining only 3 levels (300µm apart) would have detected metastases in 111 (22%) and metastases >1mm in 36 (7%) of patients. The 6 undetected metastases with 3 levels measured <0.1mm, 0.1mm, 0.4mm, and 0.1mm respectively and included 2 metastases with missing data on metastasis levels (metastases measured 0.4mm and 0.8mm). The 2 undetected metastases >1mm measured 1.1mm and included 1 metastasis with missing data on metastasis levels (metastasis measured 1.0mm). When 6 levels are the gold standard, sensitivity for detecting metastases and metastases >1mm with 3 levels is 97% (specificity 100%).
Discussion and conclusion: Reducing the amount of examined levels from 6 to 3 levels does not have great impact on SN metastasis detection rate, and we recommend that future melanoma SN guidelines consider this to reduce overall workload of SN examination.

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Uncovering the glioblastoma tumor-microenvironment by high-end multiplexing with imaging mass cytometry

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Introduction and Aim:
Glioblastoma is one of the most aggressive cancers, and hypoxia plays an essential role in its tumor-microenvironment. Tumor-associated microglia and macrophages (TAMs) have been reported to constitute up to 30 % of the cells, a fraction that is even higher in hypoxic areas. Single-cell mRNA sequencing of glioblastoma tumors has revealed vast heterogeneity, but the spatial aspects are not entirely defined yet. The aim of this study was to uncover differences between the hypoxic and normoxic tumor-microenvironment of human glioblastoma by high-end multiplexing with imaging mass cytometry.
Materials and Method:
A tissue microarray (TMA) with normoxic and hypoxic areas from 4 IDH-wildtype glioblastomas was prepared based on the hypoxia marker hypoxia-inducing factor 1 alpha (HIF1 alpha). The TMA was stained with 18 metal-tagged antibodies covering TAMs, lymphocytes, immune checkpoints, vessels, tumor cells and proliferation. The Hyperion-CYTOF technology was used to ablate the samples and the images were analyzed by MCD viewer, Visiopharm software, and customized R scripts.
Results:
Single-cell analysis of 160 fields covering around 45,000 cells in the glioblastoma microenvironment revealed multiple cellular phenotypes. It was revealed that proliferating TAMs (IBA1+, Ki67+) were more frequent in hypoxia, whereas proliferating vessels (CD34+, Ki67+) were more frequent in normoxia. Moreover, proliferating stem-like tumor cells (OLIG-2+, Ki67+) were more frequent in normoxia.
Conclusion:
Our study revealed multiple cellular phenotypes in the glioblastoma microenvironment. The TAM, endothelial and tumor cell phenotypes revealed may play a critical role in glioblastoma biology but this needs to be clarified in future studies.

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Expression of GDNF and its receptors in glioblastoma

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Introduction
Neurotrophic factors are important for brain development, but their role in glioblastoma, the most aggressive primary brain cancer, is not fully understood. We focused on glial cell line-derived neurotrophic factor (GDNF), which has been shown to play a role in growth and migration in glioblastoma. The aim of this study was to investigate the expression of GDNF and its primary receptor GFRA1 as well as GDNF family receptors GFRA2-4 and RET.

Material and Methods
Tissue sections from 10 glioblastoma patients were stained with antibodies against GDNF (frozen tissue) and each of the receptors (formalin-fixed paraffin-embedded tissue). The area fractions of positive receptor staining were quantified with a software-based classifier. Expression of GFRA1 in astrocytic tumor cells and tumor associated microglia and macrophages was revealed with double immunofluorescence stainings using GFAP and Iba1, respectively.

Results
GDNF staining in a varying number of cells was seen in 5 of the 10 samples. Mean area fractions of the receptors were 31 % (range 7-56) for GFRA1, 3 % (0,2-7) for GFRA2, 0,4 % (0,01-2) for GFRA3, 0,1 % (0,02-0,3) for GFRA4 and 1 % (0,1-2) for RET. Co-expression of GFRA1 and GFAP was widely present, while co-expression of GFRA1 and IBA1 was limited.

Discussion and conclusion
Our results suggest that GDNF and GFRA1 are widely expressed in glioblastoma tissue, but GFRA2-4 and RET are also expressed and may play a role in glioblastoma biology. Future experimental studies are needed to clarify the role of GDNF and its receptors in glioblastoma biology.

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2 års erfaringer med multidisciplinær konference mhp. exomsekventering af fostre og børn

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I slutningen af 2019 blev det muligt at lave exomsekventering på AalborgUH. Føtalmedicinere, føtalpatologer, klinisk genetikere og klinisk laboratoriegenetikere besluttede en ugentlig multidisciplinærteamkonference (MDT) med henblik på at drøfte fostre og børn mhp. indikation for exomsekventering. De aftalte indikationer var misdannelser i mindst 2 organsystemer eller mistanke om gendefekt, som ikke kan findes ved anden undersøgelse, samt normalt resultat ved array-CGH. Undervejs fandt vi behov for at drøfte andre kliniske, genetiske og patoanatomiske problemstillinger, og indikationerne blev udvidet til at omfatte drøftelse af relevans af et specifikt panel, komplicerede array-CGH-svar, abnorme ultralydsfund eller lignende. Hvis MDT fandt indikation for yderligere undersøgelse, fik parret tilbudt genetisk rådgivning.
Vi har i perioden september 2019 til september 2021 drøftet 54 patienter. Hos 19 fandtes ingen indikation for yderligere undersøgelse. 30 tog imod tilbud om genetisk rådgivning hvoraf 18 fik lavet exomsekventering. Dette gav i 8 tilfælde forklaring på fænotypen, hos 3 fund af usikker betydning, 6 var med normalt resultat og 1 blev afbrudt efter revurdering af ultralydsfundene.
Erfaringer med denne MDT er positive. Der er sparet mange konsultationer, idet spørgsmål har kunnet løses uden at henvise forældre, og der er en lavere tærskel for drøftelse af mindre abnorme fund. Samtidig har det betydet, at føtalpatologen har været velorienteret om de diagnostiske overvejelser, såfremt en føtal obduktion blev rekvireret.

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Impaired vitamin d receptor signaling in flaring ulcerative colitis due to a specific loss of crypt top epithelial cells

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Background: Low serum levels of vitamin D, 25(OH)D, are linked to an adverse clinical outcome of ulcerative colitis (UC) although the underling mechanisms remain unknown. The active form of vitamin D, $1,25(OH)_2D$, affects cells via the transcription factor, vitamin D receptor (VDR). We studied VDR signaling in intestinal epithelial cells (IECs) from UC and controls to explore novel therapeutic implications.
Methods: Sigmoid colon biopsies were obtained from patients with UC (n=65) and controls (n=20). Total gene expression was evaluated by RNA-array, and VDR expression by immunohistochemistry. These analyses were complemented by single cell (sc)RNA-sequencing analysis (n=8). Patient derived colonic organoids were stimulated by $1,25(OH)_2D$ or vehicle control. Transcriptional effects were studied by RNA-sequencing and VDR/chromatin binding by chromatin-immunoprecipitation combined with sequencing (ChIP-seq). A CRISPR/CAS9 knockout of VDR in organoids was performed to assess dependency of VDR.
Results: VDR was highly expressed in IECs situated at the top of crypts. This cell population was reduced in biopsies from inflamed UC colon, where the overall VDR expression was significantly reduced. After 24h stimulation with $1,25(OH)_2D$, 398 genes were differentially expressed. Analysis of our scRNA-seq dataset revealed these genes primarily to be expressed at VDR+ crypt top cells.
Conclusions: VDR and genes regulated by VDR are primarily expressed by crypt top IECs, which form an essential component of the gut barrier. As VDR signaling controls several processes in IECs as well as the terminal differentiation, we propose VDR signaling to be critical for maintenance of the barrier integrity.

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Prospective clinical application of the Bethesda System on thyroid cytology

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Introduction
The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) is used to categorize thyroid fine needle aspiration biopsy (FNAB). The aim of this study was to evaluate the distribution of BSRTC categories and associated risk of malignancy in an unselected cohort, and to assess the derived management in terms of performing repetition biopsy or surgery.

Material and Methods
Thyroid FNABs assessed at the Department of Pathology, AUH, in the period 2016-2019 were retrieved from The Danish Pathology Data Bank. Prospectively applied BSRTC category was available for all biopsies. In addition, the number of biopsies and histological diagnosis (if available) were retrieved.

Results
2,873 thyroid nodules in 2,547 patients were included, resulting in 3,669 biopsies. The majority were BSRTC II (52.4%), while BSRTC I was found in 26.3% of the first available FNAB. BSRTC III-VI were less frequent (3.6-7.5 %). Repetition biopsy was performed in 23.6% of nodules. The frequency of BSRTC II increased (61.3%) while BSRTC I decreased (14.8%) from first to last biopsy. Surgery was performed in 38.2% of nodules. The malignancy rate correlated positively with the BSRTC category, being 2.8% in BSRTC II and 95.3% in BSRTC VI.

Discussion and conclusion
The BSRTC proved effective for communicating thyroid cytology in a standardized and concise way. The malignancy rates of BSRTC categories were in accordance with previous results. Repetition biopsy resulted in a higher rate of conclusive results. The indeterminate BSRTC categories (III-V) are challenging from a pathological and clinical perspective, but these were relatively rare in our series.

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Immunohistochemical staining for c-myc and Ki-67 may aid in distinguishing highly differentiated radiation-associated angiosarcomas from atypical, but benign vascular structures.

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Introduction:
Angiosarcomas of the breast are aggressive and rare tumors often displaying heterogenicity in differentiation. Distinguishing highly differentiated components of angiosarcoma from reactive, atypical vessels is challenging. c-myc is activated by radiotherapy and may be overexpressed in radiation-induced angiosarcomas. The aim was to investigate combination of c-myc and Ki-67 to distinguish highly differentiated components of radiation-induced angiosarcomas from benign, atypical vessels.

Material and methods:
From 25 patients surgically treated for angiosarcoma of the breast at Aarhus University Hospital (2010-2020), formalin-fixated, paraffin-embedded blocks encompassing 1) obvious angiosarcoma, 2) normal tissue, and 3) atypical vessels was selected from each specimen. Immunohistochemical staining for c-myc, Ki-67, and ERG were performed on all 75 blocks.

Results:
24 of 25 angiosarcomas showed c-myc expression with varying intensity (4 weak, 13 moderate, 7 strong) and varying Ki-67 index (range: 5-70%, median: 40%). In samples containing atypical vessels, 24 showed c-myc expression (11 weak, 11 moderate, 2 strong intensity). In 20 of the 24 samples with atypical vessels, the Ki-67 index was higher than observed in normal vessels (>1 Ki-67 positive cell/10 endothelial cells). In 16/25 normal tissue samples, numerous normal vessels had weak c-myc expression with no/low Ki-67 expression.

Discussion and conclusion:
The combination of c-myc and Ki-67 may be useful in identifying and distinguishing highly differentiated areas of angiosarcoma from reactive, atypical vessels, but is not specific enough to rule out angiosarcoma in e.g., resection margins. The utility relies on strong c-myc intensity, since weak, but widespread c-myc expression was observed in normal vessels.

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Real-world data on correlation between genetic profiling and response to Crizotinib in Danish ALK-positive metastatic NSCLC patients

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Introduction: Crizotinib was the first approved tyrosine kinase inhibitor (TKI) to treat metastatic anaplastic lymphoma-kinase-positive (ALK+) non-small cell lung cancer (NSCLC). Given the observed variable clinical courses, genomic profiling was performed and correlated to treatment response.
Materials and methods: Retrospective analysis of diagnostic biopsies from 29 consecutive patients receiving Crizotinib as 1st/2nd line between 2011 and 2021, was performed. Genomic profiling was accomplished using targeted DNA next-generation sequencing (NGS) and Archer® Solid Tumor (ArcherDx) RNA NGS assays. Genetic information was correlated with progression free survival (PFS) and overall survival (OS).
Results: Crizotinib-responders represented 71% and -non responders 29%. Median PFS was 5.5 months (confidence interval [CI] 2.9-10.2), median OS was 17.1 months (CI 8.8-33.2). The most common ALK-fusion-partner was EML4 (n=19). In 7 cases (25%) the FISH-detected ALK-rearrangement was not confirmed by IHC and NGS, 4 of them did not respond, while 1 with pre-existing compound ALK-mutation R1275Q+F1174L has responded for 30 months. Also 1 FISH-/IHC-positive but NGS-negative case responded. 4 of 8 non-responders showed concomitant de novo cancer-driver gene-alterations, while one with KRAS co-mutation surprisingly responded. Two patients with non-EML4 fusions, KIF5B(17)-ALK(20) and TEMP3(6)-ALK(20), had an aggressive clinical course. Longest response to first line Crizotinib was 41 months.
Conclusions: ALK+ NSCLC represents a clinically and molecularly heterogenous disease. Although this small cohort does not allow unambiguous conclusions, it does indicate that efficacy of treatment varies with different ALK-fusion-partners and possible co-mutations. For optimal treatment, ALK-positive NSCLC should be validated and classified by RNA- and DNA NGS-testing at baseline.

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Experiences from the general implementation of HPV self-sampling to Danish screening non-attenders

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HPV self-sampling to screening non-attenders is a novel modality to increase participation in Danish Cervical Screening, but what should we expect from this nationwide initiative? Here, we evaluated participation after invitation to HPV self-sampling amongst screening non-attenders in a Region Hovedstaden study from 2017–2020. A total of 57,717 women, 23-64 years, unscreened for 4 years or more, were invited for HPV self-sampling in an opt-in program. Of all invited, 27% actively accepted the screening invitation with 63% returning the self-sample for analysis (17% of all invited). In addition, 14% chose a clinician collected screening sample resulting in a total screening uptake of 31% after invitation for self-sampling. Women aged 40-49 years were more likely to use HPV self-sampling (19.2%, $p<0.001$) compared to other age groups. A larger proportion of women aged 27-29 years (20.0%) and 30-39 years (19.9%) chose screening by clinician collected sample after self-sample invitation compared to older women ($p<0.001$). Low participation rate correlated with number of years unscreened ($p<0.0001$), with a larger proportion of women with last screening participation >10 years ago remaining unscreened. HPV prevalence was 15% amongst all participants and decreased with age. HPV 16 was the most frequent genotype followed by HPV 31, 51, 52, 45, and HPV 18 when considering individually detected HPV genotypes only. Of women positive for HPV, 92% heeded the follow-up recommendation, with no association between age and adherence to follow-up. In conclusion, HPV self-sampling will increase screening participation, but long-term unscreened women remain difficult to reach.

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CAN HPV GENOTYPING AND CYTOLOGY TRIAGE FACILITATE HPV SCREENING OF YOUNG WOMEN 23-29 YEARS OLD?

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Introduction
Primary HPV screening of young women have been advised against on the premise that high HPV prevalence in this group would lead to unnecessary referrals and treatment. Here we present HPV prevalence and genotype frequencies in women age 23-29 attending the organized cervical cancer screening program in Denmark.
Material and Methods
1000 SurePath cervical screening samples from women age 23-29 from the Capital Region of Denmark were collected in Q3, 2021, tested by the BD Onclarity HPV assay, and combined with routine cytology outcomes. Referral rates for the 23-29 years old were modelled upon the Danish algorithm for primary HPV screening for women 30-59 years old.
Results
Overall HPV positivity was 25%(N=252) for women age 23-29. Genotypes ranked by frequency was HPV18(0.8%), HPV16(2%), HPV31(5%), HPV45(6%), HPV33/58(15%), HPV51(16%), HPV52(25%), HPV35/39/68(32%), and HPV56/59/66(35%). Applying the algorithm, direct referral rate to colposcopy would be 3.5% compared to 1.9% with the current cytology algorithm for this age group. Overall, 4.3% of all tested would be referred to a re-test in 6 months.
Discussion and conclusion
HPV16 and 18 is close to be eradicated amongst 23-29 year old women due to vaccination, and the majority of HPV positive screening findings are one or more of HPV 35,39,56,59,66 and 68. Compared to current cytology screening, we conclude that difference in referral rate is limited when utilizing a HPV screening with genotyping and cytology triage and thus could make primary HPV screening feasible, offering the general benefit of improved CIN2/3 detection by HPV screening over cytology.

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Adrenal pseudo-oncocytic pheochromocytoma in a male with Li-Fraumeni syndrome: the first documented case in the litterature

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Introduction: The oncocytic variant of pheochromocytomas is an exceedingly rare subtype, in counting so far 6 cases described in the literature. Oncocytic changes is usually considered as a cellular degenerative phenomenon, but these tumors have a relatively high malignant potential. Materials and methods: Clinical examination, blood sampling, computed tomography (CT) scan, I-metaiodobenzylguanidine (123I-MIBG) scintigraphy and surgery were performed. Macroscopy, microscopy (conventional hematoxylin and eosin staining and immunohistochemistry) and transmission electron microscopy were applied. The Pheochromocytoma of the Adrenal gland Scaled Score was used to evaluate the malignant potential of the tumor. Results: We present a 33-year old male patient, known with Li-Fraumeni syndrome. A right-sided adrenal mass was found on CT in 2019. Clinical and biochemical assessment indicated a pheochomocytoma, which was supported by pathological increased tumor uptake on 123I-MIBG scintigraphy. Histology showed a pheochromocytoma with morphological oncocytic features but without increased number of mitochondria confirmed by immunohistochemistry and transmission electron microscopy indicating a pseudo-oncocytic subtype. Discussion / conclusion: So far in the literature this subtype has never, to our knowledge, been described. The exact incidence and nature of this variant is unknown, and further explorations are needed in the future.

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Association of tumor-infiltrating lymphocytes and the tumor microenvironment in patients with colorectal cancer undergoing curative intended surgery.

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Introduction: Characterizing tumors as “hot” or “cold” based upon assessment of the tumor-infiltrating lymphocytes is gaining attention as a prognostic marker of recurrence and mortality. However, this quantification does not describe the function or activity of the immune cells or assess the remaining part of the tumor microenvironment. Phenotyping the tumor microenvironment via mRNA gene expression analysis could provide more insight. In this study, we performed quantification of tumor-infiltrating lymphocytes and mRNA gene expression analysis to ascertain the immunogenicity of tumors in patients with colorectal cancer (CRC).

Material and methods: The study cohort consisted of 160 consecutive patients diagnosed with CRC. Quantification of tumor-infiltrating CD3 and CD8 T-cells was performed with Visiopharm Quantitative Digital Pathology software. mRNA gene expression was measured using the PanCancer IO 360 panel with the nCounter platform. A combined immune score from 0 to 5 was based upon the mean density of tumor-infiltrating CD3 and CD8 T-cells at the invasive margin and central tumor with 0 representing a low score and 5 being a high score in all four compartments. Results: 66 (41.3%) patients were scored as 0, 24 (15.0%) scored as 1, 25 (15.6%) as 2, 14(8.8%) as 3, and 31(19.4%) as 4. Differential gene expression analysis revealed distinct immune phenotypes within each of the immune-score subgroups.

Discussion and conclusion: We discovered distinct immune phenotypes within each of the immune-score subgroup. Further analysis will reveal if combining immune score of tumor-infiltrating lymphocytes and mRNA gene expression analysis is a more precise prognostic marker of recurrence.

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Deep Visual Proteomics: Ultra-sensitive mass spectrometry for molecular mapping of human cancer tissue

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Understanding intratumoral heterogeneity in cancer biology to improve the diagnosis and treatment of specific cancer subtypes is key. Single-cell RNA sequencing has revealed novel molecular regulators associated with tumor growth, metastasis and drug resistance. However, at the protein level, arguably the closest proxy for biological function or dysfunction, single-cell variations are particularly challenging to study due to limited sensitivity and robustness of current instrumentation. Combining the visual information with the molecular phenotype using antibody-based bioimaging, and the unbiased characterization of proteomes that integrates single-cell and spatial data, remains elusive. Here we combine artificial intelligence (AI)-powered image-based analysis of cellular phenotypes with ultra-high sensitivity (single-cell) mass spectrometry (MS)-based proteomics. This concept, called Deep Visual Proteomics (DVP), ties together the visual information defining cellular identity and heterogeneity with cellular neighbourhoods and the underlying proteomic signatures in an unbiased and systems-wide way. Applied to biobank tissue samples of cutaneous melanoma, DVP captured the spatial proteome during disease progression from normal melanocytes, over pre-cancerous in-situ lesions to fully invasive melanoma. We described key pathways, e.g. a metabolic switch and RNA splicing, dysregulated in cancer progressing. This highlights the sensitivity of DVP to retain the spatial information of protein expression in the tissue context essential for a true mechanistic understanding of tissue organization, development, and pathogenesis.

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Assessing Tissue Tek Genie ® IHC for Breast Needle Biopsy diagnosis

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Introduction
We have assessed Tissue Tek Genie ® for immunohistocemical (IHC) staining with ER, Ki67, HER2, Ecadherin, CK5/6, Actin smms1, Mammaglobin and GCDFP15.
Aim: To assess a quick and easy IHC staining system for fast track Breast Needle Biopsy diagnosis
Materials and Methods
Samples: Breast cancer tissue from 19 tumors.
Stains: HE, ER, Ki67, HER2, HE-FISH and FISH-HER2.
IHC platforms: Tissue Tek Genie ®, DAKO Omnis and Benchmark Ultra.
Assessment: Scoring 0-3 for poor to optimal staining according to NordiQC scoring system
Results and Discussion
IHC stains on Genie have a darker reaction because of an amplifier Cu2+. Which gives a black brown reaction in contrast to our standard orange brown reaction.
IHC stains on Genie have more reactions that score 1 and 0 in comparison to Dako Omnis and Benchmark Ultra.
ER and Actin SMM1 are marginally better on Genie. Ki67, ECAD, GCDFP15, Mammaglobin and CK5 are better on Dako Omnis, and HER2 is better on Benchmark Ultra.
Conclusion
Tissue Tek Genie ® performs as well as DAKO Omnis on several of the tested antibodies, and some of the differences are marginally.
HER2, ECAD and CK5 does not perform to the required standards in our laboratory on Tissue Tek Genie ®

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Validation of Magnus tissue processing platform

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Introduction
Validation is carried out to define the best processing protocol for the different tissue types.
Materials and Methods
Organs: Biopsies and surgical specimens from skin, intestine and uterus. Needle biopsies from breast and prostate. 5-10 patients in each group.
Platform standard: Tissue-Tek VIP® from Sakura
Platform tested: Magnus from Milestone
Stains: HE, Immunohistochemistry (IHC) and Flourescens In Situ Hybridisation (FISH)
DNA and RNA concentration measured by Qubit.
DNA Fragment analysis with GeneScan™ 400HD ROX: Sizing DNA fragments.
RNA purity measured by Nanodrop
Assessment: the project manager and 2 pathologists specialized in the relevant tissue.
Score from 0=poor to 3=optimal based on the scoring system from NordiQC. A percentage for all scores and a correlation coefficient for all assessors were calculated.
Results and Discussion
Morphology shows similar results on both platforms. The tissue which scores 1 on Magnus is the consequence of a misfortunate change in processing program from 3 mm to 2 mm for skin and intestine biopsies mid project.
Most IHC stains were similar on both platforms with scores of 3-2, both acceptable for diagnosis.
Some IHC stains scores 1 for different reasons: missing epithelium, non-cancerous tissue, stains with known fluctuating results and a misfortunate processing program change mid project.
The results for FISH, DNA concentration and fragments are similar for both processing platforms with minor fluctuations.
Conclusion
All tested tissue types can be processed on both VIP and Magnus with no significant difference in morphology, histochemistry, IHC, FISH or molecular analysis.

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Variation i vurderingen af svag HER2-ekspression ved brystkræft i perioden 2007-2019 - et nationalt registerstudie

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Introduktion: Efterhånden som nye, mere effektive HER2-rettede behandlingstilbud udvikles, er det sandsynligt, at brystkræftpatienter med svag HER2-ekspression kan få gavn af HER2-targeteret behandling. Formålet med dette studium var at opgøre, hvordan HER2-status er blevet bedømt i Danmark og at undersøge variationen i vurderingen af svag HER2-ekspression (HER2 Low), defineret som HER2-score 1+ og 2+ uden genamplifikation.
Materiale og metoder: Fra Dansk Brystcancergruppe indhentede vi data for alle registrerede patienter med operabel brystkræft, som indgik i et protokolleret behandlingsforløb i perioden 2007-2019.
Resultater: Af 50.708 registrerede patienter var HER2-score og -status rapporteret for 95,4%, hvoraf 14,0% var positive for HER2. Denne andel varierede fra 12,7%-15,7% fra år til år (p=0,007), fra 13,1%-14,6% fra region til region (p=0,005) og fra 11,8%-17,2% fra afdeling til afdeling (p<0,0001). Større variation sås for den indbyrdes fordeling af de immunhistokemiske scorer (0, 1+, 2+ og 3+), såvel geografisk fra region til region og fra afdeling til afdeling, som over tid, hvor der på flere afdelinger sås klare udviklingstendenser, som gik i forskellige retninger på forskellige afdelinger. Ved sammenligning af de otte største patologiafdelinger sås en variation på 16,7%-36,4% på andelen af patienter bedømt til HER2-score 0 og en variation på 46,9%-65,5% på andelen af patienter bedømt til svag HER2-ekspression.
Diskussion: De aktuelle resultater viser stor variation i differentieringen mellem ingen og svag HER2-ekspression og sår tvivl om, hvorvidt den nuværende immunhistokemiske scoringsmetode kan anvendes til allokering af patienter med svag HER2-ekspression til HER2-targeteret behandling.

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Single-cell analysis of tumor-associated microglia and macrophages from human glioblastoma

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INTRODUCTION

Patients with glioblastoma have a very poor prognosis. Tumor-associated microglia and macrophages (TAMs) constitute up to 30 % of the cells in glioblastoma, and they secrete cytokines, chemokines and growth factors that influence the microenvironment. The existence of different TAM subtypes appears to be more complex than the established M1 and M2 phenotypes, but their role in glioblastoma is not fully understood and rarely considered therapeutically. This could explain why many clinical trials fail despite of promising preclinical results. This project aims to interrogate the existence and characteristics of different TAM subtypes in human glioblastoma in order to identify novel subpopulations and therapeutic targets.

MATERIALS AND METHODS

CD11b+ TAMs were isolated from patient glioblastoma tissue, and single-cell RNA sequencing (scRNA-seq) was performed using the 10X Genomics Chromium platform. The data was processed and analyzed with the R-package Seurat.

RESULTS

We have sequenced 74,000 TAMs/microglia from 3 glioblastomas and 2 control brain biopsies. In the controls, we detected mostly microglia, while the primary glioblastomas showed a predominance of monocyte-derived TAMs. We identified 11 TAM subtypes, such as hypoxic, proliferating, interferon-induced, chemokine-producing and TNF-producing TAMs, as well as a novel subtype potentially involved in tumor progression.

DISCUSSION AND CONCLUSION

We have detected a spectrum of TAM subtypes, which is more complex than the established M1 and M2 phenotypes. Our findings confirm a recent TAM scRNA-seq study, and in addition, we identify a novel subpopulation, which express known tumor-promoting genes, normally expressed by cancer cells.

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ciRS-7 ekpression i cervixcancer og forstadier hertil

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Introduktion:
Cirkulære RNA, herunder ciRS-7, har betydning for progression af mange forskellige cancertyper. Høj ekspression af ciRS-7 er bl.a. beskrevet ved cervixcancer. Den gængse opfattelse har været at ciRS-7 fungerer som tumorsupressorgen ved at binde miR-7, som er et microRNA, i tumorcellerne. Denne teori er dog for nyligt blevet udfordret af, at man ved RNA chromogen in situ hybridisering (CISH) har vist at ciRS-7 i en række adenokarcinomer ikke er lokaliseret i tumorcellerne, men i tumorstromaet.
Formålet med projektet er at undersøge ekpression og lokalisation af ciRS-7 ved cervixcancer og forstadier, med henblik på at afgøre om ciRS-7 kan have potentiale som markør for udvikling af cervixcancer.
Metode:
20 portiobiopsier med diagnoserne: ingen tegn på malignitet, CIN1, CIN3, planocellulært karcinom, adenokarcinom in situ (AIS) og adenokarcinom, undersøges med CISH for ciRS-7. Ekspression og lokalisation evalueres. Der udføres laser-mikrodissektion på et planocellulært carcinom og ciRS-7 expressionen undersøges vha. NanoString på både tumorcelle- og stromafraktionen.
Resultater:
CISH viser moderat til kraftig ekspression af ciRS-7 i stromaet i alle 20 prøver, samt svag til moderat reaktion i benignt endocervikalt cylinderepitel. Der ses ingen eller meget sparsom ekspression i epitelet ved AIS og adenokarcinom. Benignt, dysplastisk og malignt pladeepitel er uden eller med meget sparsom ekspression. In-situ resultater vil blive suppleret med resultaterne fra NanoString analysen.
Diskussion:
De foreløbige resultater viser at ciRS-7, både ved cervixcancer og forstadier hertil, langt overvejende er lokaliseret i stromaet og ikke i de epiteliale celler. Dette udfordrer den gængse teori bag ciRS-7 men understøtter nylig publikation.

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High expression of circRNAs in muscle and stromal cells confound bulk tissue analyses in cancer research

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Introduction
Circular RNAs (circRNAs) are covalently closed molecules with diverse mechanisms of action and functional roles in cancer and possible biomarker potential. Many studies have recently compared endogenous circRNA expression profiles in solid tumors and adjacent normal tissue in a search for differentially expressed circRNAs. However, if circRNA expression profiles within sub compartments of tumors and adjacent normal tissues are systematically different, something that has not been investigated previously, analyses of bulk tissues may lead to misinterpretations of the data. Thus, our aim was to investigate the expression of circRNA in different tissue compartments in colon cancer.

Material and methods
Samples from five patients with stage II colon and available formalin fixed paraffin embedded (FFPE) tumor and adjacent normal tissue were laser micro dissected to separate tissue into compartments of epithelium, stroma and smooth muscle. To analyze the expression of circRNA we designed and used a custom NanoString nCounter panel of 50 probes targeting previously validated circRNAs.

Results
Our data showed that circRNAs are generally more abundant in smooth muscle cells and in the tumor stromal cells compared to normal epithelium and tumor epithelium.

Discussion and conclusion
Our data emphasize the limitations of using bulk tissues for differential circRNA expression studies in cancer. In particular, high expression of circRNAs in muscle cells and tumor stromal cells may confound differential expression analyses. Indeed, very few circRNAs were more abundant in cancer cells compared to normal epithelial cells suggesting that functional studies of most circRNAs should also consider the tumor microenvironment.

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Automated annotation of virtual dual stains generates high-performing convolutional neural network for detecting colorectal cancer metastases in H&E-stained lymph nodes

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The technology of visual recognition is improving rapidly. It is used in various technologies, like self-driving cars, but can also assist pathologists in making faster and more precise diagnoses. Various studies have already created algorithms that identify tumor cells; however, these algorithms are often trained by manually annotating whole slide images (WSI), a labor-intensive and cumbersome task that often requires highly skilled pathologists. This proof-of-concept study aims to train a convolutional neural network (CNN) that detects lymph-node metastases using only an immunohistochemical WSI as the ground-truth-mask. From 10 patients diagnosed with lymph-node metastasis, an H&E slide with and one without metastases were scanned. The same slides were subsequently stained with pan-cytokeratin and rescanned. A simple algorithm identified pan-cytokeratin-positive cells and the remaining tissue using color thresholding. Based on these automated annotations, a CNN (U-net) was trained. The CNN was tested on H&E stains from 10 different patients with both a metastasis negative and positive lymph node. The trained CNN recognized most metastatic areas and even found one micrometastasis, initially unnoticed by the pathologist. By setting a cut-off point for malignancy at 700 µm2, the sensitivity of the CNN was 100% and the specificity 90% (one false-positive node). The area under the receiver-operating curve was 0.96. The study showed that this technique has promising potential and should be tested on a larger scale. Importantly, it seems manageable to create large, annotated H&E training sets with high quality within a reasonable timeframe. Accordingly, the performance of the final CNN may be optimized.

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Morfologisk analyse af lever med centrilobulær nekrose med henblik på at fastslå ætiologi

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Introduktion: Centrilobulær nekrose i leveren har oftest en medikamentel eller en cirkulatorisk ætiologi. Der er i litteraturen ikke beskrevet, hvorvidt man på bioptisk materiale kan skelne mellem disse to ætiologier. Der er tidligere vist sammenhæng mellem graden af nekrose og mortalitet, men ikke mellem andre morfologiske fund og mortalitet. Det primære mål med studiet var at undersøge hvorvidt patoanatomisk vurdering kan bruges til at fastslå ætiologi. Materialer og metoder: Der blev inkluderet 49 leverbiopsier fra patienter med biopsier med SNOMED-koder for konfluerende nekrose og zonal nekrose udført mellem 1989 og 2010. Følgende fem parametre blev vurderet på en semikvantitativ skala fra 0-3: nekrose, blødning, sinusoidal ektasi, jernophobning og fibrose. Ved journalgennemgang var det muligt at fastslå 21 sager med medikamentel og 23 med cirkulatorisk ætiologi. Der blev udført en regressionsanalyse mellem alle fem parametre og mortalitet. Resultater: For sinusoidal ektasi score 2-3 findes 9 patienter med cirkulatorisk ætiologi og 0 med medikamentel ($p = 0,002$). Derudover findes en mindre udtalt sammenhæng mellem højere nekrosegrad og medikamentel ætiologi. Der blev ikke observeret signifikant sammenhæng mellem blødning, fibrose eller jernophobing og ætiologi. Ved Cox-regression ses ved lavere fibrosegrad en højere mortalitet, mens de øvrige morfologiske parametre ikke kunne vises at have en sammenhæng med mortaliteten. Diskussion og konklusion: Overordnet viser studiet ikke er muligt at sikkert kan fastslå ætiologi af centrilobulær nekrose ud fra morfologi. Dog findes en stærk sammenhæng mellem moderat til svær sinusoidal ektasi og cirkulatorisk ætiologi. Der blev ikke vist nogen stærk sammenhæng mellem morfologi og mortalitet.

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IS PRE-ANALYTICAL STABILITY OF DNA FROM HPV SELF-SAMPLING DEPENDENT ON THE SAMPLING BRUSH TYPE?

A COMPARATIVE STUDY WITH POSSIBLE IMPLICATIONS.

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Introduction

HPV self-sampling facilitate increased participation in cervical cancer screening programs. Self-sampling kits are mailed to women and are returned by regular mail service to the analysis laboratory. Here, we evaluate the pre-analytical stability of the self-sampled DNA on two different brush types under different time and temperature conditions.

Material and Methods

COPAN FLOQSwab and Rovers Evalyn brushes were inoculated in a homogeneously pooled, non-fixated, HPV16 positive cervical sample and analyzed at day 0 at room temperature (RT) and kept at either 4°C, RT (only FLOQSwab) or 40°C for either 4, 8 or 16 weeks. The pre-analytical quality was assessed using the human beta-globin (HBB) and HPV16 Ct scores of the BD Onclarity HPV-test. As endpoints we used changes in Ct scores and % of analytically invalid self-sampling brushes.

Results

Time and temperature affected the analytical quality of the samples. For FLOQSwab, 20% were analytically invalid at RT at 4 weeks and 70% at 16 weeks. At 40°C analytically invalids increased to 73% at 4 weeks and 100% at both 8 and 16 weeks. For the Evalyn brush, 3.3% were analytically invalid at RT and 57% at 40°C for timepoints 4 to 16 weeks.

Discussion and conclusion

The results imply that the Evalyn brush can be safely used with prolonged transport at RT, whereas FLOQSwab DNA-stability at RT is limited to 4 weeks. At 40°C, both brush types showed high invalid rates. In conclusion, the FLOQSwab was less analytically stable over time than the Evalyn brush.

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Invasive ductal carcinoma within a fibroadenoma - a case report

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Fibroadenoma of the breast is the most common benign neoplasia in women, typically consisting of heterogenic changes with both an epithelial and a stromal component. The condition typically arises in young women in their 20's and 30's. Carcinoma developing from a fibroadenoma is seen in less than 0,5 % of fibroadenomas, and typically is found in women older than 40 years old.
A 71-year-old woman presents with a history of skin redness, skin swelling and pain of the right breast lasting 2 days. Mammography identifies a 28 mm benign mass (BIRADS 2). Likewise, ultrasound reveals a well-defined tumor measuring 20 mm. Core needle biopsy demonstrates changes suspicious for malignancy . The macroscopic and histopathological analysis show a complex fibroadenoma. In addition to the fibroadenoma, an area with hyperplastic epithelium and nuclear atypia corresponding to an in-situ component is seen. Furthermore, there are three areas measuring 1-2 mm without myoepithelium around the ductal structures morphologically. Immunohistochemical staining for myoepithelial markers are all negative, suggesting that the changes represent three small foci of highly differentiated IDCs.
This report presents a rare case of invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS) arising in a fibroadenoma in a 71-year-old woman.

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BRAF mutationsstatus og MLH1-promotor-methyleringsanalyse som indikator for Lynch syndrom i kolorektal cancer

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Introduktion: BRAF mutation har været anvendt til at udelukke Lynch syndrom relateret kolorektal cancer (KRC) i tilfælde med MLH1/PMS2 tab, således at man kun har udført methyleringsanalyse på BRAF vildtype cases. Et nyere studie har skabt tvivl om rigtigheden heraf, særligt i yngre aldersgrupper, hvorfor man på Herlev Hospital, har ændret praksis. Således udføres nu methyleringsanalyse på alle KRC med MLH1/PMS2 tab. Formålet var at undersøge om der fandtes cases med samtidig BRAF mutation og manglende MLH1-promotor-methylering. Derudover om ovenstående nye strategi var implementeret.
Materialer og metoder: KRC, diagnosticeret på Herlev Hospital i perioden 15.06.2020-15.12.2021, med tab af MLH1/PMS2 blev identificeret. Data vedrørende alder, BRAF mutationsstatus og methyleringsstatus blev trukket ud.
Resultater: 188 cases blev inkluderet, heraf var 3 patienter < 50 år. I 171 cases var der udført MLH1-promotor-methyleringsundersøgelse. I 8 tilfælde var det ikke teknisk muligt at lave methyleringsundersøgelsen, mens det i 9 tilfælde (4,8%) ikke var iværksat. Vi fandt ingen cases med samtidig BRAF mutation og manglende MLH1-promotor-methylering. 6 havde manglende MLH1-promotor-methylering, hvoraf 4 havde BRAF vildtype og hos de resterende 2 var BRAF status ej undersøgt. 72 havde BRAF mutation og MLH1-promotor-methylering, mens 27 havde BRAF vildtype og MLH1-promotor-methylering. Hos 66 var der påvist MLH1-promotor-methylering men ikke undersøgt BRAF status.
Diskussion og konklusion: I vores materiale ville man ikke have overset potentielle Lynch syndrom cases ved at have anvendt BRAF mutationsstatus som vejledning. Dog er der tale om et relativt lille materiale med få yngre patienter. Den nye strategi vurderes implementeret efter hensigten.