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01

Detection of predictive genetic alterations in lung adenocarcinomas by next-generation sequencing

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Background. Predictive testing of lung adenocarcinomas (ADCs) in molecular pathology is crucial for therapy selection. EGFR mutations and gene rearrangements are routinely tested in advanced stage lung ADCs. Because of the multitude of predictive markers known so far and the often limited material of lung ADCs parallel testing is preferable. We aimed to establish the simultaneous detection of clinically relevant gene mutations and gene rearrangements by next-generation sequencing (NGS).
Methods. Sixteen lung ADCs (13 FFPE tissue blocks, 3 cytological FFPE cell blocks, and 2 corresponding cytological smears matching for 2 out of 13 FFPE tissue cases) were selected as a first test cohort which were previously molecularly characterized by Sanger sequencing and FISH. For NGS library amplification the Ion AmpliSeq Colon and Lung Cancer panel v2 (CLP2) and the Ion AmpliSeq RNA Fusion Lung Cancer Research panel (LFP) was applied with subsequent sequencing on the Ion Torrent Personal Genome Machine (PGM).
Results. All 16 FFPE samples produced acceptable quality metrics for the CLP2 (mean coverage: 2265x, mean uniformity: 98 %). Of note, the two cytological smear samples resulted in a mean coverage of 1508x and mean uniformity of 93 %. NGS of the LFP failed in one of 16 ADCs by not reaching enough mapped reads for sensitive detection of potential translocations. Importantly, all previously detected EGFR and KRAS mutations as well as ALK and ROS1 gene rearrangements were confirmed.
Conclusions. In conclusion, we established in a retrospective clinical setting simultaneous NGS-based testing using the CLP2 and the LFP. This combined assay allows the input of different types of specimen with limited material, proves to be highly accurate for therapeutically significant mutations and gene rearrangements, and will hopefully reduce time until oncological treatment decision.

02

BAP1 immunohistochemistry for diagnosis of diffuse malignant mesothelioma in effusion cytology

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Background. We hypothesized that immunostaining for BRCA1-associated protein 1 (BAP1) might be of use to support a diagnosis of diffuse malignant mesothelioma (DMM) in effusions of serous cavities.
Methods. We analyzed a series of cytological DMM samples and reactive effusions for loss of BAP1 immunoreactivity—a surrogate of BAP1 mutations—in mesothelial cells.
Results. BAP1 expression was lost in 16 out of 30 (53 %) DMM specimens, while it was retained in all samples of reactive/non-neoplastic mesothelial cells analyzed ($n = 108$). BAP1 loss was variably associated with p16 deletion by fluoresence in-situ hybridziation (FISH). BAP1 status was highly concordant between corresponding cytological and histological DMM specimens.
Conclusions. Our findings indicate that loss of BAP1 as detected by immunohistochemistry is a moderately sensitive (53 %) and highly specific (100 %) marker of malignancy in shedded mesothelial cells in serous cavity effusions. Combined analysis of BAP1 and p16 results in an increased diagnostic yield as compared to each marker individually. BAP1 status of neoplastic mesothelial cells in effusions reflects that of the corresponding histological samples.

03

Genetic and phenotypic diversity of BRAF mutations in lung cancer

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Background. BRAF mutations have been identified as a potential therapeutic target in a variety of human cancers. Our aim was to determine clinical, morphologic and molecular characteristics of BRAF mutated lung cancer samples.
Methods. 2529 consecutive lung cancers were tested for BRAF mutations as part of a comprehensive genetic characterization. Immunohistochemical staining for the V600E mutation was carried out in a subset of cases.

Results. 56 tumors harbored a BRAF mutation, among them 54 (96.4 %) adenocarcinomas, one (1.8 %) squamous and one sarcomatoid carcinoma (1.8 %). We identified five different mutational subtypes in exon 11, including one newly described in this report. Among thirteen different mutational subtypes in exon 15 five are here reported for the first time.
Conclusions. Having evaluated the so far largest series of unselected lung cancers we could determine the frequency of BRAF mutations at 2.2 % and show that BRAF mutations occur predominantly in solid and acinar subtypes of adenocarcinomas. The spectrum of BRAF mutations in pulmonary carcinomas is broader than previously thought with only 39.3 % accounting for the V600E subtype (1 % of all cancers). We provide first evidence that BRAF mutations can also occur in squamous and sarcomatoid carcinomas.

04

Molecular profiling of lung adenosquamous carcinoma: hybrid or genuine type?

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Background. Lung adenosquamous carcinoma is a particular subtype of non-small cell lung carcinoma that is defined by the coexistence of adenocarcinoma and squamous cell carcinoma components. The aim of this study was to assess the mutational profile in each component of 16 adenosquamous carcinoma samples from a Caucasian population.
Methods. The mutational profile was assessed by a combination of next generation sequencing using the cancer hotspot panel as well as the colon and lung cancer panel and FISH. Identified mutations were confirmed by Sanger sequencing of DNA from cancer cells of each component collected by Laser Capture microdissection. Expression of adenocarcinoma and squamous cell carcinoma-specific markers was assessed by immunohistochemistry. Expression of classifier miR-205 was assessed by real-time PCR.
Results. Mutations typical for adenocarcinoma as well as squamous cell carcinoma were identified. Driver mutations were predominantly in the trunk suggesting a monoclonal origin of adenosquamous carcinoma. Most remarkably, EGFR mutations and mutations in the PI3K signaling pathway, which accounted for 30 % and 25 % of tumors respectively, were more prevalent while KRAS mutations were less prevalent than expected for a Caucasian population. Surprisingly, expression of classifier miR-205 was intermediate between that of classical adenocarcinoma and squamous cell carcinoma suggesting that adenosquamous carcinoma is a transitional stage between these tumor types.
Conclusions. Our findings that both components share the same driver mutations and express intermediate levels of classifier miR-205 suggest that adenosquamous carcinoma is not a hybrid cancer but rather constitute an own entity. Owing to the high prevalence of therapy relevant driver mutations, a higher proportion of patients may benefit from targeted therapy than expected for a Caucasian population.

05

Genome abstraction analyses identify deficiencies in homologous recombination repair as a common trait in osteosarcoma

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Background. Osteosarcomas are aggressive bone tumors with a high degree of genetic heterogeneity which complicates the identification of driver genes and the development of more individualized treatment approaches.
Methods. We inferred signatures of the underlying mutation processes in 31 exome-sequenced osteosarcomas and validated these findings by SNP array and re-sequencing data in a replication set of 92 tumors. Since the vast majority of tumors revealed mutations in the BRCA-associated pathway of homologous recombination repair, we complemented in vitro cell viability tests using the phase-3 PARP inhibitor Talazoparib and the osteosarcoma cell line MNNG/HOS.
Results. We identified 14 distinct driver genes in our set of tumors, of which five were formerly unknown in the context of osteosarcoma. None of the drivers was clearly responsible for the majority of tumors and even TP53 mutations were frequently mapped into subclones. However, > 80 % of osteosarcomas exhibited a specific combination of single base substitutions, LOH, or large-scale genome instability signatures characteristic for BRCA1/2-deficient tumors that are known to respond to treatment with PARP inhibitors. Since osteosarcoma cell lines with BRCA1/2 deficiency do not exist, we carried out testing of MNNG/HOS osteosarcoma cells carrying ATM and PTEN mutations, which inactivate the BRCA pathway upstream from BRCA1. Indeed, the in vitro tests showed good evidence of cell viability reduction with standalone Talazoparib treatment and an even increased response in combination with the alkylating agent Temozolomide and the topoisomerase I inhibitor SN-38.
Conclusions. Our findings imply that multiple oncogenic pathways can drive chromosomal instability during osteosarcoma evolution and result in the acquisition of BRCA-like traits in the majority of tumors, which could be therapeutically exploited.

06

Phase II explorative trial to prospectively investigate predictive molecular biomarkers for efficacy of panitumumab (P) in platinum-pretreated head and neck squamous cell cancer (HNSCC) S10PANI01)

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Background. Monoclonal antibodies directed against EGFR (such as cetuximab -C- or panitumumab -P) were extensively investigated in Head and Neck Squamous Cell Carcinoma (HNSCC). Even if clinical efficacy in terms of survival was shown to be marginal, there seems to be a subgroup of patients with clear benefit and durable response. So far, no single biomarkers or biomarker combination pattern was found to identify this subgroup. The aim of this study was to analyze pre-specified biomarkers in a prospective phase II trial with P in HNSCC patients.
Methods. Patients with platinum-pretreated HNSCC included in a prospective phase II multicenter trial examining P as second line treatment consented for molecular biomarker analysis on available tumor tissue. As P is not active through indirect mechanisms as ADCC (antibody dependent cellular cytotoxicity), it seems to be an ideal candidate to examine the pure effect on EGFR pathway, held responsible for good and durable responses. To find a predictive marker pattern for response, a central laboratory investigated the following markers: KRAS, NRAS, HRAS, PI3KCA, BRAF gene mutations by Sanger sequencing; EGFR gene status by FISH; HPV genotyping.
Results. 25 pts were included in the phase II trial and consented for the biomarker sub-study. Tumor tissue was available in all patients. One case was excluded for bad quality of DNA. Two uncommon KRAS mutations (G48E, T50I, 8 %) and 3 (12.5 %) canonical PIK3CA mutations (E545K in all the cases) were detected; all the other genes were wt. HPV high-risk 16 was found in 9 (37.5 %) and EGFR gene copy number gain (CNG) in 12 patients (50 %), due to either EGFR gene amplification or chromosome 7 polysomy. No correlation between response and molecular alterations was observed, with the exception of EGFR: all the 3 patients experiencing partial response showed EGFR CNG, a feature not identified in patients with stable or progressive disease.
Conclusions. EGFR CNG by FISH may identify HNSCC patients who will profit from P administration and therefore may be considered a useful marker for the prediction of P efficacy. This preliminary observation needs to be confirmed in a larger series. The cascade of MAP kinases (RAS, BRAF) seems not to be prognostically relevant. The role of PIK3CA mutations remains to be elucidated.

07

Are patients with non-invasive papillary thyroid carcinoma, follicular variant (niFV-PTC) and minimally invasive follicular thyroid carcinoma with capsular invasion only (miFTC-CIO) overtreated? An institutional experience

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Background. Recently several meetings and position papers advocate a change in terminology regarding thyroid neoplasms with indolent behavior, proposing the term "tumor" instead of "carcinoma". This change concerns non-invasive papillary thyroid carcinoma, follicular variant (niFV-PTC) and minimally invasive follicular thyroid carcinoma with capsular invasion only (miFTC-CIO). The aim of our study was to evaluate the impact of considering niFV-PTC and miFTC-CIO as lesions of low malignant potential, and to see how this change would influence patient management at our institution.
Methods. A 32 months retrospective review of all well differentiated thyroid carcinomas (WDTC) (papillary and follicular carcinomas) diagnosed at our institution was performed, excluding tumors of uncertain malignant potential as well as poorly differentiated and anaplastic carcinomas. We retrieved cases of niFV-PTC and miFTC-CIO, reviewed histological slides to confirm diagnosis and recorded patient treatment.
Results. A total of 9 (7.3 %) niFV-PTC (4 males and 5 females, aged between 30 and 68 years, mean: 50.8 years old) and 2 (1.6 %) cases of miFTC-CIO (2 females, 31 and 51 years old) were identified out of 122 WDTC diagnosed in the study period. The initial treatment consisted in 5 lobectomies and 6 total thyroidectomies (3 because of a compressive goiter, 2 because of a fine-needle aspiration diagnosis of suspicious for papillary thyroid carcinoma and 1 because of a fine-needle aspiration diagnosis of papillary carcinoma). The treatment following the histological diagnosis consisted in 4 thyroidectomy completions among patients who underwent simple lobectomy (4/5, 80 %) and 9/11 (82 %) radioablations with I131.
Conclusions. The incidence of niFV-PTC is low at our institution, probably because we apply strict diagnostic criteria for this lesion. Simple lobectomy with negative margins is the treatment of choice in cases diagnosed as niFV-PTC and miFTC-CIO, due to the indolent course of these neoplasms. All cases with thyroidectomy completions and radioablations could have been avoided. As a consequence, the change of such terminology heavily impacts the malignancy risk evaluated cytologically as well as patients' management.

08

The role of Phosphoprotein Enriched in Diabetes (PED, PEA15) in non-alcoholic steatohepatitis and liver cancer

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Background. Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality worldwide. Risk factors for HCC development include chronic viral infection, alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH). As the incidence of NASH is growing at an alarming rate, it is predicted that HCC prevalence will further increase and that NASH will become a frequent cause of HCC in western countries. NASH is a complex disease and has been associated with the metabolic syndrome and type 2 diabetes mellitus. However, the underlying mechanisms, which link NASH with HCC development, are poorly understood.
Methods. To further depict the molecular link between NASH and HCC development we analyzed expression levels and function of Phosphoprotein Enriched in Diabetes (PED, PEA15), a well-known protein that regulates glucose metabolism through the modulation of the insulin regulatory mechanism. PED expression was measured in human NASH and HCC samples, but also in two different mouse models, which recapitulate human NASH. In addition, in vitro experiments with human HCC cell lines were performed to evaluate the involvement of PED in lipid accumulation, cell growth, migration and response to treatment with sorafenib, a drug used for HCC treatment.
Results. PED expression in healthy liver was low. Yet, we observed a step-wise increase of its expression from steatosis, to steatohepatitis (NASH) and finally HCC in human and murine samples. PED expression in human HCC was further associated with higher Edmondson grade and metastasis. In vitro experiments revealed that PED was able to regulate the accumulation of lipid droplets. In addition, PED affected cell migration and response to sorafenib therapy. In contrast, no influence on cell proliferation was noted.
Conclusions. Our data indicate that PED is involved in the pathogenesis of NASH. It has further a functional role in HCC and therefore may represent one of the players linking NASH to HCC development.

09

Comparison of EndoPredict and MammaPrint test results in hormone receptor positive, HER2 negative invasive breast cancer

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Background. The assessment of the risk of distant metastasis in patients with breast cancer is critically important for the choice of adjuvant treatment.
Methods. In the present study 48 breast cancer samples (grade 1: 4.2 % (2/48), grade 2: 45.8 % (22/48), grade 3: 50 % (24/48)) were analysed to compare the quantitative reverse transcription polymerase chain reaction (RT-qPCR)-based multigene assay EndoPredict and the multigenomic assay MammaPrint.
Results. Based on the molecular EP-Score of the EndoPredict test 18.2 % (8/44) of the tumours were assigned to the low risk group and 81.8 % (36/44) to the high risk group. The EPclin-Score of the EndoPredict test integrates the molecular data as well as tumour diameter and nodal status. Based on the EPclin-Score 38.64 % (15/41) of the tumours were classified as low risk and 61.36 % (27/41) as high risk. There was a significant correlation between the EP-Score and EPclin-Score (Fisher's Exact Test $p < 0.001$). However, there was no significant correlation between the EndoPredict test results and the MammaPrint (Spearman's rho: EP-Score: $\rho < 0.212$; EPclin-Score: $p < 0.284$). There was a correlation between the tumour cell proliferation detected by Ki-67 and the EP-Score (Spearman's rho $\rho = 0.345$) as well as the MammaPrint (Fisher's Exact Test $p = 0.499$*10–2). No correlation was found between the Ki-67 index and EPclin-Score. In a virtual tumour board the choice of therapy based on classic clinical-pathologic prognostic markers and on the EndoPredict as well as on the MammaPrint test results were compared. In comparison to the clinical-pathological prognostic markers including MammaPrint data the EndoPredict would lead to a change of therapy in 36.4 % (16/44). It was noticeable that the amount of recommendations for chemotherapy based on the EndoPredict test grew by one-third, which is in contrast to all studies published so far.
Conclusions. One reason could be a selection bias of the sample cohort because of the high amount of tumours with a grading of 3. If the choice of therapy would base solely on the MammaPrint result (without considering classical clinical pathological markers) the EndoPredict would lead to a change of therapy in 37.5 % (15/40). Both tests lead to different risk classification in 34.2 % of patients. Our results shed doubt on the reliability of the current gene expression tests. Follow-up of our patients for extended time periods will be necessary to properly assess the value of the assays.

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Detection of tumour-specific mutations in blood-derived cell-free circulating tumour DNA from metastatic and non-metastatic breast cancer patients

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Background. The aim of this pilot study was to assess the biological relevance of plasma derived circulating cell free tumour DNA (ct-DNA) in tracing the mutational changes and tumour load during the follow-up period.
Methods. This retrospective study included 12 patients with invasive ductal, lobular or ductal-lobular breast cancer. Patients were stratified into three subgroups depending on metastatic state at the time of the blood withdrawal. The "MET" subgroup contains patients with metastases, whereas "LATE_MET" patients were metastasis-free at the time of blood sampling but developed metastatic disease during the follow-up period of at least five years. "NO_MET" patients did not have metastases during entire documented follow-up period. Our approach is based on next-generation sequencing (NGS) of tumour DNA isolated from diagnostic formalin-fixed, paraffin embedded specimens (FFPE) and plasma ct-DNA. We used the commercial "Ion AmpliSeq Cancer Hotspot Panel v2" and "Ion AmpliSeq Comprehensive Cancer Panel" (Life Technologies) targeting genes frequently mutated in various neoplasms. The validation of detected somatic mutation of low allelic frequency < 2 % was performed with droplet digital PCR.
Results. To test the reliability of measurements in ct-DNA we analysed four primary metastatic lung cancer FFPE samples with known mutation status based on routine diagnostics and their accompanying plasma/ serum ct-DNA. The identified KRAS and EGFR mutations from the primaries were detected in the plasma of all four patients, but only in the serum of two patients. Analysis of NGS data from the primary breast tumours revealed mutations in frequently mutated genes (PIK-3CA, KAT6B, NOTCH4, MLL3, TP53) at more than 20 % allelic frequency in all of the 12 examined patients. So far, plasma ct-DNA of four patients has been analysed. We detected somatic mutations in the ct-DNA of patients of the LATE_MET group (TP53:c.833C > G), as well as in three patients without metastasis during the follow-up period (NO_MET group, mutation PIK3CA:c.3140A > G). Analysis of the other patient's specimens is currently ongoing.
Conclusions. The sensitivity of ct-DNA sequencing for somatic mutation content is higher in plasma than in serum samples. Furthermore, we have demonstrated that mutations of the primary tumour can already be detected in plasma ct-DNA of early-stage breast cancer patients. Ongoing analysis will elucidate the potential of plasma ct-DNA in identification patients at risk of possible relapse.

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Primary breast cancer culture in a perfusion-based bioreactor – a suitable alternative for cell lines and mouse models

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Background. Interaction between cancer cells and the immune system critically affects development, progression and treatment of human malignancies. Two-dimensional in vitro culture systems and in vivo animal models are the primary tools used to test cancer cell response to drugs, but are not suited for the development of immune-mediated therapies. We present an innovative method to culture breast cancer tissue in porous 3D scaffolds by using a perfusion-based bioreactor system that allows the maintenance and expansion of tumor microenvironment.
Methods. Freshly excised breast cancer specimens were fragmented and cultured in a 3D "sandwich-like format" between two layers of porous collagen scaffold under perfusion flow (U-CUP). DMEM/F12, supplemented with 10 % autologous human serum, was used as a culture medium. We assessed the ability of tumor and non-malignant cells to survive and expand into the scaffold, as well as their capacity to recapitulate features of the original breast cancer tissue. The maintenance of immune-infiltrating cells allowed testing of immune blockade therapy using anti-PD-L1 and anti-CTLA-4 antibodies alone or in combination in triple negative breast cancer.

Results. The U-CUP culture system preserved tissue viability and promoted the expansion of breast cancer cells from surgical specimens together with accompanying stromal and immune cells into the porous scaffold. Tumor tissues were viable up to 21 days and recapitulated the initial histology. Administration of anti-PDL1 antibody, alone or in combination with anti-CTLA4, was associated with increased expression of the immune-activation marker IFNγ and decreased expression of the immunosuppressive cytokine IL-10. In triple negative breast cancers, viable tumor cells were markedly reduced or absent after treatment with anti-PD-L1 and anti-CTLA-4 alone or in combination for 7 days, while the number of lymphocytes remained roughly stable, and normal breast cancer tissue was unaffected.
Conclusions. Culture of breast cancer tissue in a 3D perfusion-based bioreactor represents a promising system for the pre-clinical evaluation of immune-mediated therapies. Preserving malignant, interstitial and immunocompetent cells allows direct evaluation of the effects of various treatments on the complex tumor microenvironment. This engineered ex vivo in vitro model could be extended as a platform allowing testing of innovative approaches for the treatment of human malignancies.

12
Generation of patient derived tumor spheres to guide precision medicine

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Background. Precision oncology is a clinical approach aimed towards tailoring treatment strategies for patients based on the genetic profile of each patient's cancer. Available cell line models alone often do not accurately recapitulate the genetic profile of individual patients tumors and therefore limit preclinical evaluation of new targeted agents. Furthermore, a high failure rate of drug candidates can be attributed in part to use of monolayer cultures as the initial screening method that is associated with highly variable responses and does not predict clinically observed chemoresistance. In our Institute for Precision Medicine we developed a program utilizing patient derived 3D tumor spheres, in combination with individualized genomic sequencing, to nonimate drug candidates and drug resistance in a precision patient care setting. Utilizing these various genomic and biological platforms for pharmacological screenings, we can more closely recapitulate the in vivo tumor microenvironment of individual patient tumors and can more accurately model personalized therapeutic response and resistance in vitro and in vivo.
Methods. Fresh tissue samples were collected, washed and mechanically or enzymatically dissociated and then plated in a Matrigel (BD) scaffold with primary culture media. Primary spheres were characterized according to our cytology, histology and genomic platforms. Established and characterized tumor spheres were expanded, cryopreserved for banking, used for in vitro studies and implanted in nude mice for patient derived xenografts (PDX).
Results. Our success rate in generating patient derived tumor spheres ranges from 20–30 % depending on the tumor type. Morphology and molecular profiles show good concordance among in vitro spheres and native tumor tissues. In vitro studies show tumor specific drug sensitivity that can be further characterized in PDX models.
Conclusions. We have developed platforms for the generation and characterization of individual patient-derived 3D tumor spheres. Tumor sphere characterization, patient's tumor specific pharmacological screening and drug validation in PDX models are effective models which can be used to tailor standard of care treatment, study drug resistance, and nominate novel therapeutic targets unique to the individual genomic landscape of each tumor.

13
Does vaccination change HPV genotype distribution in gynaecological smears?

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Background. Cervical cancer is induced by human papilloma virus (HPV) and develops throughout epithelial preneoplastic lesions. Prerequisite for the development of the tumour is the persistence of high risk HPV. In 2008, a quadruple vaccine, covering HPV 6, 11, 16 and 18 has been introduced in Switzerland. Aim of the study was the analyses of the development of HPV genotypes in Switzerland prior and after the implementation of the HPV vaccination.
Methods. Liquid based gynaecological smears were analysed by PCR using GP and MY primers of the L1 capsid protein gene. Positive samples by either reaction were further characterized by DNA sequencing.
Results. Between 1.1.2002 to 31.12.2014, a total of 4795 samples were analysed and 2773 (57.8 %) were positive by either PCR reaction. The cytological diagnosis of the samples include ASCUS ($n = 657$), Low SIL (873), High SIL ($n = 297$), mixed diagnosis ($n = 274$) and in 672 samples the cytological diagnosis was unknown. 50 genotypes were recovered, the most common HPV 16 (24.8 %). The HPV genotypes included in the vaccine (HPV 6, 11, 16 and 18) were detected in 35.2 %, no difference of the prevalence was observed before (2002–2007) and after (2008–2014) the introduction of the vaccination (38.1 vs. 36.5 %; not significant). In addition, no difference was observed in the youngest age group (< 23 years), before and after the introduction of the vaccination (46.7 vs. 44.0 %; not significant).
Conclusions. Using broad spectrum PCR with consecutive DNA sequencing, a broad range of HPV genotypes were detected in the various gynaecological lesions. However, within the past 13 years, no significant shift of the genotypes was observed including seven years after the introduction of the vaccination program. In the population analysed the introduction of the HPV vaccination has not yet changed the distribution of HPV genotypes in cervical lesions.

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IL-33 signaling contributes to the pathogenesis of myeloproliferative neoplasms

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Background. Myeloproliferative neoplasms (MPNs) are characterized by the clonal expansion of one or more myeloid cell lineages. In most cases, proliferation of the malignant clone is ascribed to defined genetic alterations. MPNs are also associated with aberrant expression and activity of multiple cytokines; however, the mechanisms by which these cytokines contribute to the pathogenesis of disease are currently poorly understood. Here, we have investigated the role of IL-33 in MPN.
Methods. We applied a genetic approach to dissect the molecular triggers underlying spontaneous MPN-like disease in mice deficient in the inositol phosphatase SHIP. To validate the clinical relevance of our findings, we performed experiments using transgenic JAK2V617F mice and samples from MPN patients.
Results. Genetic ablation of the IL-33 signaling pathway was sufficient and necessary to restore normal hematopoiesis and abrogate MPN-like disease in animals lacking the inositol phosphatase SHIP. Stromal cell–derived IL-33 stimulated the secretion of cytokines and growth factors by myeloid and non-hematopoietic cells of the bone marrow (BM), resulting in myeloproliferation in SHIP-deficient animals. Additionally, in the transgenic JAK2V617F model, the onset of MPN was delayed in animals lacking IL-33 in radio-resistant cells. In human BM, increased numbers of IL-33–expressing cells were detected, specifically in biopsies from MPN patients. Exogenous IL-33 also promoted cytokine production and colony formation by primary CD34+ MPN stem/progenitor cells from patients. Moreover, IL-33 improved the survival of JAK2V617F-positive cell lines.
Conclusions. Together, these data indicate a central role for IL-33 signaling in the early pathogenesis of MPNs. Further investigation is warranted to assess the contribution of this pathway to MPN progression.

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EGFR mutation detection in cytology samples with very low DNA content using Therascreen® EGFR Pyro (Qiagen)

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Background. Cytology samples like fine needle aspirates and pleural fluid is a non-invasive method for lung cancer diagnosis. The Therascreen EGFR Kit enables quantitative measurement of EGFR mutation in blood or FFPE tissue. Since half of our routine diagnostic samples are cytology preparations, we tested the capacity of Therascreen EGFR Pyro to detect known mutation in very low DNA content samples.
Methods. DNA was extracted from enriched tumour cells of 71 preselected cytology preparations for routine diagnosis by scratching or micro laser capture. 28 cases were wild type and 43 cases harboured at least one mutation in the EGFR gene (exon 18–21). The DNA content was measured and 10 times diluted in order to have enough material for comparing Therascreen EGFR Pyro and Sanger Sequencing methods.
Results. The median DNA content of all samples was 0.034 ng. With the Therascreen method 33 cases showed suitable results with a median DNA content of 0.043 ng (including 2 false negative cases (median DNA content: 0.030 ng), and with the Sanger Sequencing method 29 cases were successful with a median DNA content of 0.046 ng (including 4 false negative cases with a median DNA content of 0.023 ng).
Conclusions. Both methods produced suitable and reliable results with very low DNA contents, despite the Therascreen EGFR Pyro kit is only recommended for specimens with a DNA content of 2–10 ng. These results raise the question if the Therascreen EGFR pyro is suitable for verification of questionable next generation sequencing results as well as for high DNA containing "liquid biopsies".

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Phase tomography as a three-dimensional complement to histology for nervous tissue visualization

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Background. X-ray phase tomography provides superior contrast in soft tissue visualization and micrometer resolution in the three orthogonal directions. Being a non-destructive method that does not require any contrast agent, it can be performed before the standard histological processing of any sample, regardless of its preparation.
Methods. Cortex samples of stroke tissue and cerebellum samples of healthy tissue have been cut from donors post mortem. After formalin fixation and paraffin embedding, punches 6 to 9 mm in diameter were extracted from the tissue blocks. The specimens were scanned at the beamline ID 19 (ESRF, Grenoble, FR) with a photon energy of 19.5 keV and an effective pixel size of 5 µm.
Results. The phase tomography data differentiated well between white and gray matter, both in the case of the cortex as well as in the cerebellum, where the molecular layer, the granular layer and the white matter were clearly visible. The volume of each layer can be accurately measured automatically improving the standard stereological estimation. For the case of the neocortex stroke sample, the phase tomography data also allowed for the distinction between healthy and necrotic tissue, as demonstrated in a virtual slice in **Fig. 1,** correlated with and validated by histology.

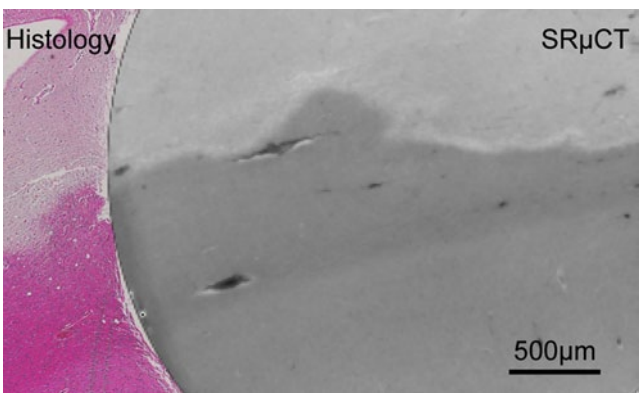

Fig. 1 ▲ Phase tomography distinguishes between white matter, gray matter, healthy and necrotic tissue, as well as the boundary between them. Comparison with histology is used for validation of findings.

Conclusions. Phase contrast tomography can be used as a first-step visualization for prioritizing sample processing and indicating the region of choice for histological slices. Tomography data can also be correlated to histology in order to extrapolate functional histological information based on a number of slices to an entire three-dimensional dataset, offering an attractive alternative to serial sectioning. Lastly, computationally combining the two modalities could allow for the quantitative extraction of additional, previously non-obtainable information.

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Tularemia – an underdiagnosed disease?

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Background. Tularemia is a rare zoonosis caused by the Gram-negative rod-shaped bacterium Francisella tularensis. The Swiss Federal Office of Public Health (BAG) reports an average of 8 cases per year (data from 2004 to 2011). Reported mortality rate with appropriate antibiotic therapy is about 1 %.
Methods. Cases with confirmed diagnosis of tularemia either by PCR or bacterial culture were retrieved from the archives of the Institute of Surgical Pathology ($n = 10$) in order to analyze morphological characteristics and to correlate with the clinical presentation.
Results. 5/10 cases where fine needle aspirations, 3/10 cases where lymph node biopsies, 1/10 cases was a soft tissue biopsy and 1/10 cases was a lymph node from an autopsy. The typical clinical presentation was a cervical lymphadenopathy partially with grotesque enlarged nodes. One patient was referred to EBUS guided FNA with a suspicion of lung cancer due to FDG active mediastinal lymph nodes on PET scan. A very unusual presentation was a prosthetic joint infection with Francisella tularensis. Almost all patients had a history with animal contact (especially rodents like rabbits, rats or mice). Morphologically the specimens showed a broad range of patterns from acute purulent to granulomatous inflammation. Bacteria were morphologically not detectable in special stains. The diagnosis was established by PCR or bacterial culture.
Conclusions. Tularemia is a rare but serious illness which can be cured by appropriate antibiotic therapy. The clinical presentation might raise suspicion of malignancy because of fast growing tumor masses and enlarged lymph nodes. Although malignant neoplasms can be easily ruled out morphologically in adequate specimen the inflammatory pattern is not pathognomonic which might lead to under-diagnosis. Therefore awareness of this differential diagnosis and clinicopathological correlation are crucial to initiate specific tests (PCR, serology or bacterial culture) in order to confirm the diagnosis of tularemia.

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VEGFA amplification in colorectal cancer: consequences for the tumor microenvironment

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Background. Colorectal cancer (CRC) is one of the most common lethal malignancies with around 694'000 deaths worldwide each year (WHO, 2012). Recently, we showed that a small subgroup (circa 7 %) of highly aggressive CRC harbors the amplification of vascular endothelial growth factor A (VEGFA). Although the impact of VEGFA amplification on cancer cell behavior has been already addressed in several tumor types, our understanding of its consequences on the tumor microenvironment is very limited. This study is aimed to assess the influence of VEGFA amplification on the tumor microenvironment in CRC.
Methods. FISH analysis were performed on two tissue microarrays comprising a total of 124 CRC samples, of which amplification of VEGFA gene locus was found in 9 % of all tissue samples. Whole tissue slides of human colorectal cancer ($n = 38$) were stained with CD68, CD163, PD-1 and PD-L1. The following parameters were tested at the tumorfront: distribution of macrophages (CD68 and CD163) and expression of programmed death 1 (PD-1) and its ligand PD-L1. For PD-L1 staining a scoring system as described by Jung Ryul Kim et al. (2013) was used for the evaluation.
Results. An increase in total macrophage (CD68) count was detected in non-amplified VEGFA cases compared to polysomic ($p < 0.05$) and amplified VEGFA cases ($p < 0.0001$). Similarly, M2 macrophages (CD163) were also decreased in VEGFA amplified CRC samples compared to polysomic ($p < 0.05$) and normal cases ($p < 0.0001$). Furthermore, we observed an increased number of PD-1 positive lymphocytes ($p < 0.05$) and a higher expression of PD-L1 ($p < 0.05$) in non-amplified VEGFA cases compared to the other analyzed groups. PD-L1 expression was significantly associated with higher tumor grade ($p < 0.05$) and size ($p < 0.05$).
Conclusions. Our results suggest that VEGFA amplification in CRC correlates with the distribution of macrophages and the expression of PD-1 and PD-L1 at the tumorfront. Based on these observations, we hypothesize that VEGFA amplification in CRC might modify the tumor microenvironment by altering the number and the distribution of both macrophages and lymphocytes expressing the PD-1. Our data provide a solid base for additional functional studies aiming to further clarify the impact of VEGFA amplification on the tumor microenvironment in CRC.

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Perivascular epithelioid cell tumor (PEComa) of the uterus: A case report

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Background. Perivascular epithelioid cell tumors (PEComas) are a rare family of mesenchymal tumors arising in a wide array of anatomic locations and characterized by coexpression of melanocytic and muscle markers. The uterus accounts for around one-fourth of the overall PEComa cases reported in the literature.
Methods. We report a case of PEComa of the uterus with multiple malignancy features.
Results. A uterine mass suspect for leiomyosarcoma was found in a 53-year-old woman with post-menopausal bleeding. Total hysterectomy and bilateral adnexectomy was performed. The tumor measured 7 cm in diameter, was unique, well-circumscribed, nodular, and white-yellow without haemorrhage or necrosis. Microscopically, two populations of cells could be seen: small fusiform cells growing in fascicles resembling a smooth muscle tumor, and large epithelioid cells with abundant pale vacuolated cytoplasm growing in a diffuse pattern. Cytologic atypias were marked and mitoses numerous and often atypical in the second component. The tumor infiltrated into the myometrium with lymphovascular invasion. Immunostains showed positivity for Melan-A, HMB45, smooth muscle actin, CD10, TFE3 and cathepsin K.
Conclusions. This PEComa case presents several of the recently precised criteria for malignancy (Schoolmeester JK et al. Perivascular epithelioid cell neoplasm (PEComa) of the gynecologic tract: Clinicopathologic and immunohistochemical characterization of 16 cases. Am J Surg Pathol 2014; 38:176–188).

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PTEN is increased in the matricellular stroma of lung squamous cell carcinoma

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Background. Phosphatase and tensin homolog (PTEN) is an important tumor suppressor in malignant tumor epithelia but few is known about its function in the surrounding stroma. Recently, Trimboli et al. demonstrated that PTEN inactivation in stromal fibroblasts of mouse mammary glands accelerated malignant transformation of mammary epithelial tumors. We investigated the PTEN protein expression in the immediate peritumoral stroma, called matricellular space, and the corresponding tumor epithelia of non-small cell lung carcinoma in both cell cytoplasm and nucleus.
Methods. Cytoplasmic and nuclear PTEN protein expression was immunohistochemically determined in the matricellular stroma and the tumor epithelia on a tumor tissue microarray of 468 patients suffering from non-small cell lung carcinoma (NSCLC) of all pT stages (pT1-pT4). The H-score (intensity of immunoreactivity multiplied by the frequency of stained cells, range 0 to 300) was correlated with histological subtype and pTNM.
Results. Our study included 233 (51.1 %) squamous cell carcinomas (SCC) and 223 (48.9 %) adenocarcinomas (ADC). For all NSCLC, PTEN expression in both cytoplasm and nucleus was higher in the matricellular cancer-associated fibroblasts (CAFs) than in the carcinoma epithelia: mean H-score 145 for cytoplasm and 150 for nucleus versus 103 and 69, respectively. A complete loss of stromal PTEN was infrequent (< 0.5 % of tumors). High PTEN in both stroma and tumor was associated with lower pT and smaller tumor size. Stratification for histology indicated that ADC displayed higher nuclear PTEN (H-score 110 vs. 90) than SCC. Further, complete loss (H-score 0) of epithelial PTEN occurred more frequently in SCC compared to ADC (cytoplasmic 15.2 vs. 11.8 %, nuclear 28.3 vs. 15.8 %). Among SCC, high PTEN in both cytoplasm and nucleus of CAFs as well as in the cytoplasm of tumor epithelia correlated again with smaller tumor size. No correlation was observed with lymph node or distant metastasis.
Conclusions. PTEN loss in both cytoplasm and nucleus occurs more frequently in lung squamous cell than adenocarcinoma. Peritumoral cancer-associated fibroblasts have a higher PTEN protein expression than the adjacent malignant epithelia. However, protein synthesis decreases with increasing tumor size, probably via alteration of the extracellular matrix composition.

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Intraoperative frozen section in congenital hyperinsulinism

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Background. In the differential diagnosis between focal versus diffuse congenital hyperinsulinism (CHI), intraoperative frozen sections may guide the extent of pancreas resection. We present the case of a newborn boy, who presented with recurrent hyperinsulinic hypoglycemia. Enteral feeding was started, as well as treatment with diazoxide, with low response, followed by subcutaneous octreotide. A F-DOPA PET scan was then performed, which revealed increased focal activity in the uncus of the pancreas, leading to surgical resection. CHI is characterized by inappropriate secretion of insulin by pancreatic β-cell mainly due to mutations in genes (KCNJ11, ABCC8) encoding KATP channels protein (40–45 % of all cases).
Methods. Initial studies consisted of frozen sections of the uncus of the pancreas, followed by biopsies of the body and the tail, with immunohistochemistry analysis. Genetic analyses looking for mutations in the different genes described in CHI, especially ABCC8, KCNJ11 were done.
Results. Intraoperative frozen sections from the uncus showed endocrine cells with enlarged and irregular nuclei, in non confluent islets, and in only a small proportion of the lobules. At this point, diffuse versus equivocal CHI was suspected. Further biopsies from the tail and the body of the pancreas displayed similar foci throughout the sampling. Immunohistochemistry showed diffuse insulin and glucagon staining. Genetic analysis revealed compound heterozygous mutations in the KCNJ11 gene, compatible with the histological diagnosis of diffuse congenital hyperinsulinism.
Conclusions. The two main histologic subtypes of diffuse versus focal hyperinsulinism are important to recognize, since treatment and outcome are different. Focal hyperinsulinism may be completely cured by the limited resection of the abnormal β-cell foci. In contrast, diffuse hyperinsulinism responds in part to medication, although subtotal pancreatectomy, with severe long term consequences, may be required. Radiological and biological assessment is required before surgery, but intraoperative frozen sections are necessary to ascertain the diagnosis, and to guide selective resection.

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Characterization of NRAS in CRC patients: a faster and more sensitive Real-time PCR-based technology

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Background. KRAS and NRAS gene mutations have been demonstrated to lead to resistance to anti-epidermal growth factor (EGFR) targeted therapies in colorectal cancer (CRC) patients. Although there are several methods for KRAS exon 2 analysis, only few tests are available for NRAS, none of them with a multiplex approach. Our aim was therefore to develop a new Real-time PCR-based assay for the analysis of NRAS exons 2–3 (in close collaboration with Danish Company, PentaBase) and to compare it with direct sequencing (DS), the gold standard for these exons.
Methods. Sensitivity tests were performed on the DNA from cell lines carrying several NRAS mutations in exon 2 (codons 12–13) and 3 (codon 61): G12C, G12D, G12V, G13V, Q61K, Q61L, Q61R and Q61H. In this tests, we evaluated seven percentage of mutated DNA in fixed amounts of wild type (wt) DNA (10 %, 1 %, 0.5 %, 0.1 %, 0.05 %, 0.01 % and 0 %) by DS and Pentabase kit. Then we applied the new PentaBase assays and DS on a series of 155 consecutive CRC patients. PentaBase assays are based on Real-time PCR using SuPrimers™ (DNA primers with increased specificity), BaseBlockers™ (oligos suppressing amplification of wt genes) and HydrolEasy™ probes (hydrolysis probe with increased signal-to-noise ratio and sensitivity). In particular we used a multiplex Real-time PCR approach, 2 reactions for exon 2 and 1 for exon 3.
Results. In cell lines experiments sensitivity was 10 % for DS and < 0.1 % for PentaBase assays. Concerning NRAS exon 2, 1/155 (0.6 %) CRC patient was mutated by DS and 3/155 (1.9 %) by PentaBase kit, whereas 4/155 (2.6 %) patients were mutated in NRAS exon 3 by DS and 10/155

(6.4%) by PentaBase kit. PentaBase multiplex kit found the same mutations than DS plus 2 additional cases in NRAS exon 2 and 6 additional cases in NRAS exon 3. These mutations were confirmed with a simplex test developed by PentaBase. All NRAS mutated patients were also KRAS/BRAF wt.

Conclusions. Our results underline that PentaBase multiplex assays are a new faster, easy to use and more sensitive methodology enabling the identification of more mutations than DS and permitting a better characterization of CRC for NRAS exons 2–3. In particular, our approach is able to investigate all NRAS codons 12–13 changes (at odds with other Real-time based assays such as Entrogen test) and is the first available with a multiplex approach. These assays can also be applied to specimens of other cancer types (e.g.: follicular thyroid cancer, melanoma).

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A new Real-time PCR-based technology for a better identification of KRAS exon 3 mutations in colorectal cancer

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Background. 80–90% of patients affected by colorectal cancer (CRC) do not show any benefit from anti-epidermal growth factor receptor (EGFR) targeted therapies. This lack of response is mainly due to mutations in KRAS and BRAF genes. KRAS exon 2 has been the first test for the stratification of CRC patients and several tests have been developed. Recently, also KRAS mutations in exon 3 have been demonstrated to play the same clinical role. However, only few tests are available for such exon. The aim of the present study was to compare a new assay, which was developed by our institute in collaboration with Danish company (PentaBase), with direct sequencing (DS), the gold standard method for KRAS exon 3 analysis.

Methods. NCI-H460, SNU-387 and Calu-6 cell lines, carrying respectively Q61H, Q61L and Q61K mutations in KRAS, were used to perform sensitivity assays to test PentaBase kit before the validation on tissue samples. Seven percentages of mutated DNA in fixed amounts of wild-type (wt) DNA (10%, 1%, 0.5%, 0.1%, 0.05%, 0.01% and 0%) were tested by PentaBase kit and DS. KRAS mutations in exon 3 were evaluated by DS and multiplex PentaBase kit in sensitivity experiments and in 154 consecutive CRC patients. PentaBase kit is based on Real-time PCR using SuPrimers™ (DNA primers with increased specificity), BaseBlockers™ (oligos suppressing amplification of wt genes) and HydrolEasy™ probes (hydrolysis probe with increased signal-to-noise ratio and sensitivity). In particular we used a multiplex Real-time PCR approach.

Results. In cell lines, DS had a sensitivity of 10% and PentaBase multiplex kit of < 0.1%. In CRC cases, we found 4/154 (2.6%) KRAS exon 3 mutated patients by DS and 7/154 (4.5%) by PentaBase multiplex real-time. Mutations were then characterized and confirmed with a simplex assay. PentaBase multiplex kit found the same mutations than DS and 3 additional cases. All the new mutated cases were KRAS exon 2 and BRAF wild-type.

Conclusions. Through the application of a new highly sensitive methodology based on a multiplex Real-time PCR we were able to double the number of patients with KRAS exon 3 mutations. Our data indicate that PentaBase multiplex kit represents a new, faster and more sensitive method for the analysis of KRAS exon 3. Furthermore, the new kit represents the first multiplex assay for such exon, leading this test to be used also when only few DNA is available.

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Intralymphatic histiocytosis: clinicopathologic correlation of two cases

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Background. Intralymphatic histiocytosis is a rare disease with less than 100 reported cases since its first description in 1994. It is characterized by CD68-positive histiocytes in the lumina of lymphatic vessels and may occur in the context of autoimmune diseases as rheumatoid arthritis, after implantation of metal or idiopathic. Up to now no effective therapy is known.

Methods. We describe our experience with two cases.

Results. The first patient was a 83-year-old woman with an infiltrated erythematous lesion on the left leg. The second patient was a 78-year-old man with an erythematous, partially brownish lesion on the chest. The lesions were asymptomatic, but with a clinically worrying aspect, suspicious for erysipelas, phlebothrombosis, borreliosis, morphea, lymphoma or angiosarcoma. Both patients had a history of joint replacement with metal implants adjacent to the skin lesions. Histologically the lesions showed dilated lymphatic vessels in the reticular dermis, some of them containing aggregates of mononuclear cells (histiocytes and endothelial cells). An inflammatory infiltrate consisting of lymphocytes, granulocytes and histiocytes, in the second case with formation of epitheloid granulomas, was present in both cases. The histological differential diagnoses comprised an infectious process due to the inflammatory infiltrate and (intra-) vascular neoplasms such as intravasular lymphoma, leukemia and angiosarcoma. In special and immunohistochemical stains neither pathogenic agents nor evidence for lymphoma, leukemia or angiosarcoma could be detected.

Conclusions. Intralymphatic histiocytosis is an important differential diagnosis. It is a benign lesion that can clinically and histologically mimic a variety of other diseases.

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Telangiectatic oncocytoma: a rare variant of classic oncocytomas

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Background. Telangiectatic oncocytomas are rare epithelial tumors of the kidney. Unlike classic oncocytomas they present with a spongy appearance without the typical central scar.Therefore, they are often highly suspicious for malignancy in preoperative medical imaging. Differentiation against renal cell carcinomas with cystic growth can be difficult.

Methods. A 54 years old male patient underwent total nephrectomy because of a large tumor of the left kidney. Macroscopic workup and histopathological reprocessing followed as well as HE stain and immunohistochemistry stainings against CK7, vimentin, CD10 and CD31 were performed.

Results. During the pathological workup, the tumor presented with a multicystic, hemorrhagic cut surface with clear margins. Microscopic examination of the tumor revealed telangiectatic elements surrounded by eosinophilic oncocytes. Hedging renal tissue was without pathological findings. In absence of cytological atypia and lacking reactivity with markers for CK7, vimentin, CD10 and CD31, a telangiectatic oncocytoma was diagnosed.

Conclusions. Telangiectatic oncocytomas are rare variants of classic oncocytomas, which should be kept in mind when being faced with renal lesions with hemorrhagic spongy or multicystic cut surface. Particular-

ly, the distinction against malignant renal neoplasias by means of morphological analysis and immunohistochemistry stainings is indispensable in order to ensure appropriate subsequent clinical management.

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The phenotype of cancer associated myofibroblasts is reproduced by epigenetic modification of normal myofibroblasts

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Background. Cancer associated myofibroblasts (CAMs) are stromal cells that can be distinguished from their normal counterparts by cancer growth promoting properties. Relative DNA hypo-methylation has been reported in CAMs of upper gastrointestinal cancers. Here we report that epigenetic modification of normal myofibroblasts produces a CAM-like phenotype.
Methods. Myofibroblasts from tumour-free organ donors were treated with 5-aza-2′-deoxycytidine (DAC), a DNA methyltransferase inhibitor to induce global DNA hypo-methylation which was monitored by pyrosequencing. We studied the effects of DAC treatment on myofibroblasts proliferation using expansion- and EdU incorporation assays, on migration using Boyden chambers and on function using gel contraction assays; treated myofibroblasts (MFDAC) were also studied in co-culture models with oesophageal squamous cancer cells (OE21) and in vivo in a mixed human OE21 cell/MFDAC xenograft model.
Results. DAC-treated myofibroblasts exhibited reduced global DNA methylation as expected, and persistently decreased cell expansion and proliferation; the changes were dose dependent. DAC increased myofibroblast contractibility and migration. MFDAC stimulated oesophageal cancer cell migration and invasion in vitro and accelerated tumour growth in vivo.
Conclusions. DAC treatment persistently changes the phenotype of normal myofibroblasts inducing a phenotype similar to that of CAMs in vivo and in vitro. MFDAC are hence a model for epigenetic modification of stromal cells with direct cancer growth promoting effects.

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Genetic profiling of Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma enriched from archival formalin-fixed and paraffin-embedded tissues

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Background. Classical Hodgkin lymphoma (cHL) accounts for 1 % of all human neoplasms. It is among the most curable human neoplasms found in adults. However, 10–20 % especially of advanced-stage patients die after relapse or progressive disease. There are unmet needs for understanding of mechanisms that cause cHL relapses and for development of new prognostic/predictive markers as well as effective targeted therapies that would improve management and outcomes of relapsed/progressive cHL. Comprehensive genetic characterization and advance in understanding the molecular pathology of cHL are indispensable to meeting those needs. However, genetic information on cHL is still scarce mainly due to difficulties of extracting malignant Hodgkin and Reed-Sternberg cells (HRSC), whose overall frequencies in the affected tissues range 0.1–5 %. Therefore, enrichment of neoplastic cells is necessary for the majority of genetic investigations.
Methods. To overcome this issue, we are developing a new high-throughput method for marker-based labelling of archival formalin-fixed and paraffin-embedded (FFPE) tissue-derived nuclei. Labelled nuclei are then enriched by fluorescence-assisted flow sorting (FACS). Sixty eight genes that are frequently mutated in lymphomas were tested for alterations by targeted next-generation sequencing (NGS). Global copy number aberration profiling was performed by aCGH. At the initial stage of the project we demonstrate the feasibility of our approach by processing 4 different cHL cases.
Results. Enzymatically extracted FFPE tissue-derived nuclei retain their antigenicity and can be reliably labelled with monoclonal antibodies against nuclear (MUM1, PAX5) and cytoplasmic (CD30) markers. A ~25 % enrichment (percentage of cells of interest) was reached after FACS sorting when staining HRSC for only one marker (MUM1 or CD30). This could be increased up to 80 % when nuclei were stained and sorted according to positivity for both markers. Using sorted non-malignant cells as a germline control we detected known somatic single nucleotide mutations with oncogenic potential in all cases. Also these cases contained copy number aberrations such as gain of chr2, focal deletions in chr4, chr7, chr16 and chr19 affecting genes like JAK3, CDKN2D, MAP2K3 and NOTCH3. Taken together this demonstrates that DNA extracted from the enriched cell populations is suitable for the wide-scale genomic profiling.
Conclusions. In this study we demonstrate the capacity of the novel rare-cell-enrichment technique for genetic cHL studies. The set of methods applied here opens the possibility for the wider use of archived tissue material that is available in the format of FFPE blocks and therefore enables more robust study designs that would be capable of answering clinically relevant questions in the field.

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A case of cancer immunotherapy-associated inflammation

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Background. Immunologic checkpoint inhibitors such as antibodies targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4, ipilimumab) and programmed cell death-1 (PD-1, nivolumab, pembrolizumab) have significantly improved survival of patients with advanced melanoma and other malignancies in the metastatic phase. Despite important clinical benefits, checkpoint inhibition by immunomodulatory antibodies is associated with a unique spectrum of immune-related adverse events that are typically transient, but occasionally severe or even fatal. The most common adverse effects include dermatologic, gastrointestinal, hepatotoxic, endocrine, and other less common inflammatory events. The combination of nivolumab plus ipilimumab is associated with more toxicity than either agent alone.
Results. Here we report the autopsy case of a 35-year-old female patient who was suffering from metastatic melanoma and was sequentially treated, first with ipilimumab, and then with nivolumab. The autopsy showed marked regression of skin and lymph node metastases. Interestingly, the patient showed striking pulmonary histopathologic features suggesting immune-related adverse events. These features resembled sarcoidosis; foci of organizing pneumonia were also present. In addition, the patient presented with histopathologic features of myocarditis.
Conclusions. Approval of pembrolizumab and nivolumab by the FDA for the treatment of melanoma will lead to the use of these antibodies in a broader patient population. Pathologists need to be aware of the unique spectrum of immunological side effects associated with immunomodulatory cancer treatement both in biopsies and autopsies, as they will be increasingly facing the challenges of these new pathologies in daily routine.

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Inorganic fiber and particle analysis in lung tissue

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Background. Pneumoconioses are induced by inhaled inorganic dust. SILAG investigates the relationship between inorganic dust in lung tissue and pneumoconiosis since 1946 and runs a specialized lab to analyse dust in lung tissue or broncho-alveolar lavage (BAL). Two types of analytical methods can be applied and in most cases both are used complementary. One is the screening for ferruginous bodies (FB) by light microscopy (LM). The second one is transmission electron microscopy (TEM) with energy dispersive X-ray spectroscopy (EDX), used for chemical identification of particles and fibers. Finally, the results of the analysis are compared with the histology of representative slides.
Methods. For FB-screening, samples are digested by sodium hypochlorite and the solution filtered on 1.2 µm Millipore filters. These filters are soaked in immersion oil and the complete filters are searched for FB's. For TEM-analyses, pieces of tissue are freeze dried and afterwards ashed at low temperatures. The residue is filtered on 0.2 µm Nuclepore filters which are then coated with carbon. Through the Jaffe transfer method, the dust is transferred to a Cu-grid ready for TEM-analysis. For fiber analysis, 25 grid fields (300 × 75 µm) are inspected and the fibers counted. Fibers are identified by qualitative chemical analysis.
Results. In the case of asbestos related diseases, the asbestos content is determined by quantifying the number of asbestos fibers per gram dry lung tissue. For other pneumoconioses, chemical analysis of more than 100 particles allows a detection of the dominating particle population and consequently integrated in the histopathological findings to identify the nature of the pneumoconiosis.
Conclusions. Due to the low temperature ashing step during sample preparation, only inorganic dust can be analysed. Typically, mineral dust (e.g. quartz, mica, feldspar), mineral fibers (e.g. asbestos), man-made mineral fibers, welding fume and metal particles are detected.

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Clonal evolution and genomic tumor heterogeneity in non-small cell lung cancer deciphered by Multiparameter Ploidy Profiling

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Background. Genomic tumor heterogeneity is a widely recognized phenomenon and thought to be a main reason for relapses and resistance to therapy. There is a shortage of studies characterizing the genomic landscape of known cancer genes in multiple longitudinal biopsies of individual patients. Additionally a workflow to resolve the complexity of mixed tumor and stromal components is missing. To overcome these limitations and to decipher the genomic heterogeneity and clonal evolution, we developed a method to retrieve pure tumor populations and applied it on matched (primary-recurrence/metastasis) tumors of non-squamous, non-small cell lung cancer (NSCLC) patients.
Methods. Multiparameter Ploidy Profiling (MPP) comprises the isolation of nuclei from frozen or formalin and paraffin embedded (FFPE) tissues, followed by multiparamter flow sorting of tumor specimens. Sorted populations were subjected to genomic profiling by high resolution array comparative genomic hybridization (aCGH) and cancer gene targeted next-generation sequencing (NGS). DAPI was used to separate cell populations by ploidy and TTF1 recognizing antibodies allowed to distinguish tumor from normal cells. Array-CGH, combined with ploidy, was used to calculate the copy numbers at genome wide resolution. The Comprehensive Cancer Panel comrising all-exon coverage of 409 cancer genes was applied on all sorted tumor and stromal populations to detect somatic mutations and their variant allelic frequency.
Results. MPP was successfully applied on > 50 frozen or FFPE tissue specimens from > 20 patients. Array-CGH and NGS of TTF1-negative, normal cells were concordant to germline controls. Overall, genomic analysis by aCGH and NGS showed little heterogeneity between primary tumor and metastases. Three clonal evolutionary patterns were found: (1) direct evolution, (2) indirect evolution, (3) branched evolution, with most patient's disease following the direct evolutionary pattern. Matched tumors without shared chromosomal aberrations or mutations were classified as unrelated primary tumors. Besides common activating mutations in EGFR and KRAS we found additional truncal mutations in other cancer genes, some with allelic frequencies of 100 %.
Conclusions. The power of MPP is to increase the precision of aCGH and NGS analysis. The sorting of pure populations of tumor cells permitted to infer the clonal evolution of tumor populations with unprecedented confidence. The low level of genomic heterogeneity of NSCLC detected in this study is in line with recent data from Zhang et al., who showed that the poor precision of low depth sequencing (< 277 ×) contributes to an overestimation of genomic heterogeneity (Zhang et al., Science 2014). Our data are still preliminary. Integrational analysis of ploidy, chromosomal aberrations and mutations in all 409 cancer genes will allow to comprehensively draw the evolutionary picture of each patient's disease.

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Comprehensive phenotypic characterization of PTLD reveals potential reliance on EBV- or NF-κB-signalling instead of B-cell receptor signaling

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Background. Post-transplant lymphoproliferative disorders (PTLD) are a major problem in transplant medicine. So far, the insights into pathogenesis and potentially druggable pathways in PTLDs remain scarce.
Methods. We investigated a cohort of PTLD patients, consisting of both polymorphic ($n = 3$) and monomorphic ($n = 19$) B-cell lymphoproliferations. Several signaling pathways, cell of origin of PTLDs and their relation to viruses were analyzed by immunohistochemistry and in situ hybridization.
Results. Most PTLDs were of activated B-cell origin. NF-κB signaling components were present in the majority of cases, except for those EBV-infected cases lacking CD19 and upstream B-cell signaling constituents. Proteins involved in B-cell receptor signaling like Bruton tyrosine kinase (BTK) were only present in a minority of cases. Two thirds of cases showed an Epstein Barr virus (EBV) infection of the neoplastic cells. Phosphoinositide 3-kinase (PI3K) was expressed in 94 % of cases and the druggable PI3K class 1 catalytic subunit p110 in 76 %, while proteins of other signaling transduction pathways were expressed only in single cases. Unsupervised cluster analysis revealed three distinct subgroups: (i) related to EBV-infection, mainly latency type III, with lacking CD19, upstream B-cell signaling and NF-κB constituents, (ii) related to EBV-infection with expression of alternative NF-κB pathway compounds, FOXP1 or MUM1p and, finally, (iii) unrelated to virus infection with checkpoint (PD1/PDL1) component-expression.

Conclusions. EBV and NF-κB are important drivers in PTLDs in contrast to B-cell receptor signaling. The main signal transduction pathway is related to PI3K. This links PTLDs to other subgroups of EBV-related lymphomas, highlighting also new potential treatment approaches.

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Comprehensive evaluation of PDL1 expression in lymphomas

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Background. Activation of the programmed death 1 (PD1) pathway is important for tumor cells to escape from immune control. PD1 ligands (PD-L1 and L2) are expressed by tumor cells and induce a reversible inhibition of T-cells. The efficacy of therapeutic modulation of the PD1-PD-L pathway has been recently shown in classical Hodgkin lymphoma (cHL), but little is known on the frequency, diagnostic and prognostic importance of PD-L expression in lymphomas.
Methods. A large cohort of B- cell lymphoma entities, both HL ($n=335$) and non-Hodgkin lymphomas ($n=637$), was examined for the expression of PD-L1 by immunohistochemistry (clone E1L3N). The results were correlated with the expression of other phenotypic markers, FISH data of the 9p24 region (PDL1 locus) as well as with the clinical outcome.
Results. PD-L1 could be detected reliably by immunohistochemistry. As expected, its expression was more abundant in cHL (186/277 evaluable cases, 67 %), nodular lymphocytic predominant HL (NLPHL, 7/13, 54 %) and primary mediastinal B-cell lymphomas (PMBCL, 12/33, 35 %); only in PMBCL, PD-L1 expression correlated with PDL1 gains ($\rho=0.573$). PD-L1 was expressed in 85/306 primary diffuse large B-cell lymphomas (DLBCL, 28 %), 2/9 transformed follicular lymphomas (22 %), 14/59 follicular lymphomas (24 %) and 4/16 (25 %) mantle cell lymphomas (MCL), while Burkitt- (BL), lymphoplasmacytic- and T-cell- and histiocyte-rich B-cell lymphomas (TCRBCL) remained negative and only isolated cases of small lymphocytic- and marginal zone lymphomas (MZL) expressed PD-L1. In cHL expression of PD-L1 correlated with increased numbers of granzyme + T-cells ($\rho=0.251$) and CD68 + macrophages ($\rho=0.221$) but with decreased numbers of FOXP3 + T-cells (Treg, $\rho=0.145$). In EBV-negative HL cases there was a slight trend towards decreased relapse-free survival of PD-L1 + cases. In DLBCL expression of PD-L1 correlated with increased numbers of PD1 + T-cells ($\rho=0.167$) and lacking expression of GCET1 ($\rho=0.239$). In FL and MZL expression of PD-L1 correlated with increased numbers of PD1 + T-cells ($\rho=0.256$ and 0.395). MCL expressing PD-L1 were accompanied by a trend towards adverse outcome ($p=0.059$).
Conclusions. In situ detection of PD-L1 expression in B-cell lymphomas can be diagnostically valuable in some gray zones between DLBCL and BL, and NLPHL and TCRBCL; it identifies an immune escape cluster of cHL with increased granzyme + and PD1 + T-cells and macrophages and decreased Tregs. It might be of prognostic importance in MCL.

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Comprehensive genomic analysis of a donor-derived polyomavirus associated, metastatic urothelial cancer after kidney transplantation

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Background. Although polyomavirus (PV) infection has been associated with development of urothelial carcinoma (UC), its role in malignant transformation remains controversial. We report on a patient with usual UC of the bladder (Ta, G3) 8 years after receiving a kidney graft from a 4 years old male donor. After two recurrences within two years (T1, G3), he was diagnosed with a SV-40 positive metastatic micropapillary and muscle-invasive UC 10 years after kidney transplantation (NTx) with involvement of the graft kidney pelvis (pT3), the bladder (pT3a) and a pelvic lymph node metastasis. The aim of this study was to investigate the genealogy and clonal relationship between these cancer locations and the potential involvement of PV.
Methods. FFPE tumor specimens were sorted based on DNA content by usage of a BD Influx FACS. DNA from sorted aneuploid populations was subjected to whole genome high resolution CGH microarrays (aCGH). In addition, short tandem repeat (STR) analyses were done with the PowerPlex® ESI Kit commercial testing kit. Each run was performed on an Applied Biosystems Genetic Analyzer 3500 and analyzed with the GeneMapper ID-X Software. Integration of PV-genome was investigated by RT-PCR. The IonTorrent platform by LifeTech (PGM, IonAmpliseq Cancer Hotspot Panel v2) was used for next-generation-sequencing (NGS).
Results. STR identified all three tumors diagnosed 10 years after NTx to originate from the allograft donor. Array CGH clarified that the earlier non-muscle invasive bladder tumors differed completely from each other and from the subsequent cancer tissues diagnosed 10 years after NTx. The later tumor manifestations shared the same genomic profile, except of an acquired amplification at 6p12.3 and a deletion at Xp22.33—Xp22.11in the bladder tumor. NGS from paraffin embedded tissue was successful in the UC sites 10 years after NTx. No key driver mutation was found. RT-PCR identified the PV to be integrated in the human cancer genome. Further studies are currently ongoing to define the exact integration sites of PV and the potentially influenced human genes.
Conclusions. To the best of our knowledge this is the first proof of a donor-derived, metastatic micropapillary UC appearing 10 years after deceased kidney transplantation in a patient with a PV-reactivation. This is an impressive example of rapid cancer development under immunosuppression in a presumably healthy tissue of a four years old donor at transplantation. Further results on the potential involvement and role of PV will be presented.

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Analysis of hepatocyte nuclear factor 1 beta (HNF1B) in endometrial lesions

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Background. HNF1B seems to be involved in cancerogenesis of various tumors, including endometrioid carcinoma. Protein expression can be found in various tumor and non-tumor lesions of female genital tract and so the specificity of HNF1B expression for clear cell carcinoma is much lower than previously thought. Recent genome wide association studies have shown that certain single nucleotide polymorphisms (SNPs) are associated with higher risk of endometrial cancer. However, the exact mechanisms by which HNF1B participates in the process of cancerogenesis remain obscure. As the data concerning mutations of HNF1B in various tumors are rare, the frequency and type of these mutations are also unknown.
Methods. Protein expression of HNF1B was assessed immunohistochemically in 320 endometrial lesions. Promoter methylation and genetic variants were evaluated in 30 cases of endometrioid carcinoma (18 fresh frozen, 12 FFPE tumours), and 15 cases of clear cell ovarian carcinoma (CCOC), by using bisulphite and direct sequencing.

Results. HNF1B was expressed in 28 % of endometrioid carcinoma (51/180 cases), 26 % of serous carcinoma (7/27 cases), 83 % of clear cell carcinoma (15/18 cases), 93 % of hyperplastic polyps with atypias (13/14 cases), 100 % of hyperplastic polyps without atypias (16/16 cases), 88 % of hyperplasias with atypias (14/16 cases), 91 % of hyperplasias without atypias (10/11 cases), and 80 % or more samples of normal endometrium. The expression was, however, strong with few exceptions only in clear cell carcinomas. Methylation in promoter region was detected in 13.3 % (4/30) endometrioid carcinomas, but neither in corresponding normal tissue, nor in CCOC (0/15). Truncating variant NM_000458.3: c.454C > T(p.Gln152X) was detected in one endometrioid carcinoma.
Conclusions. In conclusion, we have found variable expression of HNF1B protein in all tested endometrial lesions, with high frequency in non-tumor lesions. Moreover, in endometrioid carcinoma, we have found relatively common methylation in promoter region of HNF1B gene in (in 4 out of 30 cases) and truncating mutation revealed by mutation analysis in one case. However, the exact biological significance of identified genetic and epigenetic changes remains unknown. Acknowledgement: This work was supported by Ministry of Health, Czech Republic (IGA MZ CR project NT14001-3/2013).

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Validation of NGS for lung tumors in a clinical setting

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Background. Molecular cancer diagnostics faces a growing demand for personalized approaches and targeted therapies. Recent developments in massive parallel sequencing (next generation sequencing, NGS) offer novel and promising possibilities to screen for somatic mutations in demanding human tumor samples. Biopsies used for molecular analysis are often small and of variable quality. Especially FFPE samples can be challenging due to DNA fragmentation.
Methods. To validate a semiconductor-based sequencing approach in lung cancer tissues, we compared various DNA and RNA extraction protocols. Three DNA quantification workflows were tested (NanoDrop, Qubit 2.0 and quantitative PCR) and assessed for suitability in downstream NGS application. We performed library preparations with colon and lung-specific (Ion AmpliSeq Colon and Lung Cancer Research Panel v2) and/or RNA fusion (Ion AmpliSeq RNA Fusion Lung Cancer Research Panel) panels of 25 lung adenocarcinomas with known EGFR mutations or ALK fusions. The FFPE samples varied in tissue size, tumor cell content and age of the blocks. The libraries were quantified either with Qubit 2.0 or RNase P assay. QIAxcel instrumentation was tested for usage in library qualification. The sequencing data was analyzed with the panel specific parameters of Torrent Suite software v4.6.
Results. The suitability of DNA for NGS application varied, depending on the extraction methodology used. In our hands fluorometric Qubit 2.0 measurement provided the best quantification. NanoDrop delivered important purity aspects of the nucleic acid preparations. All known mutations could be verified by NGS, and no false positives were detected.
Conclusions. The scope of next-generation DNA sequencing (NGS) is transitioning from research to diagnostics, but the conditions for routine clinical application have not been clearly defined. Here, we analysed some key elements, like sample preparation for transition of NGS from research to diagnostics. Implementing NGS-based diagnostics in routine workflows was successful, but comes with novel challenges for standard analysis and should be considered in the context of future healthcare.

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Nanomechanical characterization of tumors and its implications for clinical practice

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Background. A crucial point in making treatment decisions for breast cancer patients is the assessment of tumor aggressiveness. There exist established prognostic markers such tumor type, size and grade, hormone receptor expression, HER2-amplification, Ki-67, absence or presence of nodal metastases, which are routinely assessed by standard pathological examination. However, these parameters are often not sufficient to stratify patients—especially those with early stages of breast cancer—into distinct risk groups and adjuvant therapy is frequently administered to patients who might have been cured by surgery and anti-hormonal treatment alone. The goal to avoid over- and under-treatment in the curative adjuvant setting has led to an intensive search for prognostic and predictive markers in early breast cancer. There are indeed markers emerging, such as genome based assays (e.g. Oncotype DX) that claim to add more and precise value to the basic data set. Nanomechanical alterations on the (sub-) cellular level in cancer are potentially suitable markers of cancer aggressiveness may help to optimize treatment strategies.
Methods. We developed an atomic force microscope (AFM)-based diagnostic apparatus known as ARTIDIS® ("Automated and Reliable Tissue Diagnostics"). This novel method is based on the use of a microfabricated 20 nm-sharp tip that indents several thousand individual locations across the entire non-fixed biopsy within 60–150 min. We examined invasive breast cancer, lymph node metastases and non-neoplastic breast parenchyma from 187 samples.
Results. We demonstrated by using transgenic mouse as a breast cancer model, that the "softest" nanomechanical phenotype (~ 0.4–0.8 kPa) present in the primary tumor closely corresponds to the stiffness of the metastatic lesions obtained from the lungs of the same mouse (Plodinec et al., Nature Nanotech 2012), and validated these findings in human breast cancer samples. These "nanomechanical" indentations can differentiate between benign and cancerous tissue by measuring mechanical properties (stiffness). ARTIDIS data showed that all carcinoma samples display heterogeneous stiffness phenotypes in comparison to the surrounding non-neoplastic and morphologically normal breast tissue. We could link specific nanomechanical profiles to molecular subtypes and reveal nanomechanical phenotypes that are likely to lead to metastases.
Conclusions. Moreover, our results demonstrate the applicability of nanomechanical profiling in a clinical setting. The relative size and the distribution of stiffness values (nanomechanical profiles) can provide an indicator of cancer aggressiveness, and therefore might help to optimize cancer diagnosis, orientate therapy choice, and support patient follow-up.

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Thyroid cytopathology reporting by the Bethesda system: an 18 month retrospective study at the Geneva University Hospitals

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Background. In order to improve and to standardize reporting and cytological criteria, the Bethesda System for Reporting Thyroid Cytolo-

gy (TBSRTC) was introduced in 2008 and has gained worldwide importance since then. Six diagnostic categories associated with a specific risk of malignancy (ROM) and clinical management are recognized in TBSRTC.

Methods. We report our thyroid FNA experience at HUG between January 2013 and July 2014 (18 months) using TBSRTC. Demographic information, cytology diagnoses, and surgical pathology follow-up were recorded. The prevalence and the ROM for each Bethesda diagnostic category were calculated. The ROM is presented as a range; the lower value including all FNA cases with or without surgical follow-up, the higher value including cases with surgical follow-up only. Incidental papillary microcarcinomas were excluded from the analysis.

Results. Our cohort included 400 patients (320 Female; 80 Male) with a mean age of 53.2 years (range: 18–84). The total number of FNAs was 482 including 37 (7.6 %) nondiagnostic (ND), 190 (40.4 %) benign, 115 (23.8 %) atypia/follicular lesion of undetermined significance (AUS/FLUS), 80 (16.5 %) suspicious for follicular neoplasm (SFN, including 12 cases with oncocytic features), 34 (7.1 %) suspicious for malignancy (SUS), and 26 (4.6 %) malignant cases (POS). Rapid on-site evaluation was performed in > 90 % of cases. Surgical follow-up was available in 172 (35.8 %) cases, and demonstrated benign findings in 103 (59.1 %) and malignant tumors in 69 (40.1 %) cases, including 57 (82.6 %) papillary carcinomas (including 33 follicular variant), 6 follicular carcinomas (including 5 oncocytic variant), 2 medullary carcinomas, 2 poorly differentiated carcinomas and 2 metastasis. The ROM for ND, benign, AUS/FLUS, SFN, SUS, and POS were 0 %, 0 %, 8.7–20.8 %, 16.1–33.3 %, 67.6–79.3 %, and 75–100 %, respectively.

Conclusions. During this time frame, although our rate of AUS/FLUS diagnoses is higher, the ROM of TBSRTC diagnostic categories including AUS/FLUS are within the range of TBSRTC and various previous studies. Follicular variant of papillary carcinoma, mostly encapsulated or non-invasive, was common, representing a significant fraction of all thyroid malignancies, mostly in the indeterminate cytologic categories (AUS/FLUS, SFN and SUS). This may have a significant impact on the ROM for these diagnostic categories if this entity is renamed and no longer considered as a malignancy, as suggested by several expert groups.

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HOXA13 and HOTTIP Expression is associated to worst prognosis and impact on Sorafenib response in HCC

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Background. Despite significant advances in HCC diagnosis and management, for advanced stages no therapeutic options exist beside Sorafenib. Recently, using human liver biopsies, we showed that HOXA13 and HOTTIP (its associated long-non coding RNA) expression in HCC correlates with poor survival and metastasis presence. In addition, we observed that their expression increases HCC cells proliferation in vitro. Here we seek to confirm our data on a larger cohort of samples and to investigate whether HOXA13 and HOTTIP modulate Sorafenib response in HCC.

Methods. A liver TMA (tissue microarray) comprises a total of $n = 305$ specimens, $n = 82$ normal liver tissues, $n = 108$ cirrhotic patients and $n = 115$ HCCs, has been stained for HOXA13, CK-7, CK-19, E-Cad. Protein levels have been correlated with patients' clinical data. In vitro experiments to stably modulate HOXA13 expression (gain and loss of function) have been performed using the HCC derived cell lines: HepG2, SNU449. Subsequently, cells have been treated with Sorafenib and cell cycle analysis, proliferation, migration and drug response have been tested. Finally, HOXA13 and HOTTIP, using RNA in situ, expression has been assessed in a cohort of Sorafenib treated patients.

Results. HOXA13 is altered in 41 % of HCC tested samples, thus confirming our previous results obtained using liver biopsies. In addition, high HOXA13 levels are associated with poorer outcome and higher grading (Edmondson and BCLC). Increased HOXA13 expression is linked with stem progenitor markers, CK-7 and CK19. Furthermore, high HOXA13 expression is coupled with diminished levels of E-Cad, providing a molecular basis for its association with metastasis in HCC. Furthermore, in vitro experiments demonstrate that HOXA13 overexpression results in higher resistance to Sorafenib exposure. Conversely, HOXA13 downregulation sensitize HCC cells to Sorafenib. Finally, both HOXA13 and HOTTIP expression predicts worst overall survival and disease progression in HCC Sorafenib treated patients.

Conclusions. Using a lorge chorht of HCC patients, here we show that HOXA13 IHC-based protein levels can reliably predict HCC outcome and correlates with a number of tumour features (e.g. grade). Thus the usage of HOXA13 as a marker for HCC aggressiveness deserves further investigations. In addition, we observed for the first time that HOXA13 and HOTTIP levels impact on Sorafenib response in HCC patients. These data are further supported by our in vitro set of experiments.

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High mobility group A1 overexpression promotes growth of human cholangiocarcinoma

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Background. High mobility group A1 (HMGA1) protein has been described to play an important role in a wide series of human carcinomas. By modulation of several target genes it promotes proliferation and epithelial-mesenchymal transition of tumor cells. However, its role in cholangiocarcinoma (CCA), an aggressive neoplasia of the biliary tract, has not been addressed.

Methods. Therefore, we determined HMGA1 expression by immunohistochemistry in a tissue microarray (TMA) containing 76 CCA tumor samples and confirmed our results in a smaller cohort by qRT-PCR. In addition, we analyzed changes in cell proliferation and target gene expression after modulation of HMGA1 expression in CCA cell lines.

Results. Immunohistochemistry showed that HMGA1 was overexpressed in 50 % of the CCA specimens. In accordance, we detected an overexpression at RNA level in about half of CCA tumor samples in a smaller cohort. Integration with clinicopathological data revealed that there was a trend for a positive correlation of high HMGA1 expression with lymph node and organ metastasis. In vitro experiments showed that overexpression of HMGA1 in CCA cell lines promoted cell proliferation whereas, its suppression reduced growth rate. HMGA1 overexpression further modulated the expression of several genes involved in CCA carcinogenesis.

Conclusions. In conclusion, our findings indicate that HMGA1 could play an important role in CCA and its expression is increased in about half of CCA specimens. Furthermore, it promotes tumor growth and affects several genes important in CCA development.

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Atomic force microscopy: a novel nanotool for cancer diagnostics

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Background. Emerging evidence indicates that mechanical properties of cancer cells play a critical role for invasion and metastasis. Nanomechanical changes on the (sub-) cellular level in cancer are potentially suitable markers of cancer aggressiveness. Because the established equipment for the measurement of nanomechical properties is adapted from testing industrial material (e.g. metals, alloys), most commercially available products are suitable for the assessment of hard materials (e.g. bones, teeth) but not for soft biological samples. Testing of soft biological samples presents a significant challenge for indenter-based setups since fundamental adaptations of the experimental systems are required.
Methods. In order to achieve optimal accuracy and compatibility for the assessment of nanomechanical properies of soft biological materials we have devised an ARTIDIS ("Automated and Reliable Tissue Diagnostics") apparatus to measure the nanomechanical properties of native living tissues. We mainly optimized the method for the assessment of breast tissue, but other tissue types, such as lung, prostate, colon, kidney and liver were tested as well. Numerous improvements and adjustments such as tip geometry, cantilever spring constant, sample holder design, mechanical models used for stiffness analysis, perfusion/heating systems, bright field and fluorescence optical control, optimal approach and levelling of the sample, software solutions etc. were tested and implemented.
Results. We have optimized the assessment of nanomechanical properties of soft biological tissue by ARTIDIS. By obtaining tens of thousands of force measurements over unadulterated human breast tumor biopsies, we found that malignant tumors give rise to characteristic stiffness profiles in comparison to benign tissue.
Conclusions. Measurements of soft biological material are technically feasible in a variety of tissue types utilizing the ARTIDIS technology. Characteristic nanomechanical profiles can be demonstrated in malignant breast tumors. Measured soft peaks in cancer patients may serve as mechanobiological markers for cancer aggressiveness and and therefore might help to optimize cancer diagnosis, orientate therapy choice, and support patient follow-up.

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Sarcomatoid transdifferentiation in a case of metastatic melanoma

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Background. Metastatic melanomas, mimicking many different tumors, are a morphological challenge for pathologists. Experiments with melanoma cell lines have shown the capacity of tumor cells to transdifferentiate into different lineages and to even have the plasticity to contribute to neovascularization. However, comprehensive melanoma tumor tissue transdifferentiation into another lineage is a little-known phenomenon in diagnostic practice and only few cases have been published so far. Here, we describe a highly unusual case of a metastatic melanoma manifested as groin lymph node metastasis in a 61 years old female with two distinct concomitant subcutaneous sarcomatoid lesions on the same leg, whereas no evidence for a conventional primary tumor was found.
Methods. All three tumorous lesions were formalin-fixed paraffin embedded and examined histologically on Hematoxylin & Eosin staining. Further characterization encompassed extensive immunohistochemical staining, in particular for known melanocytic markers as well as investigation of BRAF (Exon 15) and NRAS (Exons 2–4) genes by Sanger sequencing.
Results. The lymph node metastasis showed obvious morphological and immunohistochemical differentiation of melanoma. The two subcutaneous lesions were conventionally consistent with high-grade myxofibrosarcoma and primarily soft tissue mixed tumor, respectively. All three lesions showed BRAF wildtype and harbored a NRAS p.Q61R mutation.
Conclusions. Although there was no morphological or immunohistochemical conclusive evidence of melanoma in the mesenchymal lesions, the concordant genetic profile of BRAF and NRAS indicates sarcomatoid transdifferentiation of melanoma in this case. Particular in patients with previously known melanoma, unusual presentation of metastasis, even without conventional evidence for melanoma, should raise the possibility of transdifferentiation.

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Cerebriforms cells in cerebrum: an unusual finding

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Background. Mycosis Fungoides (MF) is the most common cutaneous T-cell lymphoma, and large cell trasformation (tMF) is an adverse prognostic event. Extra-cutaneous dissemination can occur in the course of the disease, but dissemination to the central nervous system (CNS) is uncommon. Moreover, CNS lymphomas are overall rare and most often of B-cell phenotype. We report a case of CNS large T-cell lymphoma presenting as multiple cerebral lesions in a patient with a history of MF.
Methods. We report a case of a 33-year-old woman, known since the age of 16 for erythematous plaques thought to be atopic dermatitis, who developed, end 2012, multiple nodular skin lesions and peripheral adenopathies. Two skin lesions were biopsied simultaneously, and diagnosed as MF and tMF. A lymph node biopsy showed dermatopathic changes without lymphoma (Stage IIB). She received local treatment (UVB, PUVA and radiation therapy) and interferon therapy, and experienced almost complete remission. In 2015 neurological symptoms lead to evidence multiple cerebral lesions, suspicious for lymphoma, evaluated by stereotaxic biopsies. We compared histopathological and molecular features of these with previous skin specimens. After negative bone marrow staging biopsy, she was recently started on chemotherapy (MATRIX). Short follow-up shows rapidly worsening clinical conditions.
Results. One of the initial skin biopsies showed atypical lymphoid cells with epidermotropism, Pautrier abcesses and CD4+ CD30- phenotype; the other revealed diffuse dermal infiltration by predominantly large cerebriform tumor cells with high proliferative fraction, and CD2 – CD3 – CD4 +/– CD7 – CD30 + ALK- EMA- non-cytotoxic immunophenotype. Altogether, these results led us to diagnose MF and tMF, respectively. The brain was infiltrated by large atypical lymphoid cells with cerebriform nuclei, somewhat anaplastic features and perivascular distribution. By immunohistochemistry, tumor cells were highly proliferative, with a CD2 – CD3 + CD5 – CD7 + CD30 + activated cytotoxic immunophenotype. A diagnosis of CD30 + cytotoxic peripheral T-cell lymphoma was retained. TRG and TRB clonality analyses revealed clonal rearrangements in skin and CNS biopsies, with identical patterns in both skin specimens but only minimally overlapping profiles when compared to the CNS sample.

Conclusions. The reported case illustrates an uncommon finding of a CNS T-cell lymphoma in a patient with previous MF, questioning the clonal relationship between the two diseases and challenging the adequate classification of this CNS lymphoma as either a progression or a de novo lymphoma. Despite differences in immunophenotype and clonality patterns, this CNS lymphoma could possibly represent an aggressive divergent evolution of a primary cutaneous T-cell lymphoma. Additional sequencing is ongoing to try to solve the question.

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Non-small cell lung cancer: the prevalence of oncogenic driver mutations in Switzerland

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Background. Predictive molecular marker analyses are standard of care in order to select non-small cell lung cancer (NSCLC) patients for targeted therapies. The aim of this study was to determine the prevalence of targetable oncogenic driver mutations including EGFR, KRAS, BRAF, HER2, ALK and ROS1 in Switzerland.
Methods. Eight Swiss pathology institutions provided retrospective and anonymized data on their predictive molecular marker results performed on NSCLC from January 2012 to December 2014. Clinico-pathological data were recorded including age, gender, histological NSCLC-subtype and specimen type (biopsy, conventional cytology and cell block, respectively) used for molecular analyses. The prevalence of oncogenic mutations were calculated and compared between the centres.
Results. A total of 4187 NSCLC were included into the study. The median age was 67 years and 55 % were male patients. The tumor specimens for molecular analysis were mostly derived from biopsies (69 %), 26 % were from conventional cytology specimens and only in 5 % from cell blocks. The most prevalent gene mutation was KRAS with 30.6 % (range: 27.3–33.9 %), followed by EGFR, BRAF and HER2 mutations in 12.2 % (range: 10.2–13.1 %), 3.9 % (range: 2.5–5.6 %) and 1.1 % (range: 0.9–4.0 %), respectively, without significant differences between the eight centers. Concomitant EGFR and KRAS mutations were detected in only 3/2027 NSCLC. In contrast the prevalence of ALK (mean 6.5 %, range: 2.8–11.7 %) and ROS1 (mean 2.4 %, range: 1.5–6.2 %) rearrangements varied significantly between centers.
Conclusions. The Prevalence of EGFR, KRAS, BRAF and HER2 mutations are well in line with data from other West European populations. Concomitant EGFR, KRAS, BRAF or HER2 mutations are exceptional. ALK FISH results vary significantly between the eight centres. Concomitant ALK FISH positive results in NSCLC harbouring other oncogenic driver mutation have only been observed in two smaller centres, highlighting the difficulty in ALK-FISH interpretation.

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The CD70/CD27-interaction regulates cell fate decisions in acute myeloid leukemia blasts

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Background. Acute myeloid leukemia (AML), the most common acute leukemia in adults, is a heterogeneous group of highly lethal hematological cancers. AML is defined by the accumulation of immature malignant blasts that are characterized by aberrant proliferation, increased survival and a block in terminal differentiation. Stem cell gene signatures in AML blasts negatively correlate with patient survival; however, current therapies do not specifically target these deregulated molecular pathways. Cytotoxic chemotherapy remains the standard of care, and long-term survival is achieved in less than 20 % of patients. CD27, a co-stimulatory TNF superfamily receptor, is expressed on lymphocytes and hematopoietic stem cells. Activation of CD27 by its only ligand CD70 induces lymphocyte proliferation and modulates hematopoiesis. Naturally, only activated immune cells express CD70; however, aberrant CD70 expression on cancer cells in solid tumors and lymphomas has recently been reported.
Methods. The CD70/CD27-interaction and downstream signaling pathways were studied in blasts from blood and bone marrow of newly diagnosed AML patients and in AML cell lines using blocking monoclonal antibodies, qRT-PCR, FACS, ImageStreamX and specific knockdowns. Experiments were conducted in vitro and in vivo in murine xenotransplants. Soluble CD27 (sCD27) in patient and mouse sera was determined by ELISA.
Results. AML blasts express the TNF superfamily ligand-receptor pair CD70/CD27. CD70/CD27-interactions result in the activation of stem cell signature pathways such as Wnt-, JAK/STAT- and Hedgehog-signaling and promote an undifferentiated state in AML blasts by increasing symmetric self-renewing cell divisions. Blocking the CD70/CD27-signaling pathway induces asymmetric cell division and differentiation, decreases AML cell growth and colony formation in vitro and prolongs survival in murine AML xenografts in vivo. sCD27, a marker that reflects the extent of CD70/CD27-interactions on blasts in vivo, is profoundly increased in the sera of newly diagnosed AML patients and strongly correlates with adverse outcome, independently of age and molecular risk group.
Conclusions. The cornerstone of AML therapy, unspecific cytotoxic chemotherapy that mainly targets aberrant proliferation of blasts, has been used unchanged for the last 30 years. Our results indicate that CD70/CD27-signaling induces stemness in AML blasts, promotes symmetric self-renewal and inhibits differentiation. Targeting the CD70/CD27-interaction might therefore be a promising novel approach in the treatment of AML. In addition, serum sCD27 might be used as a surrogate marker to predict blast stemness and patient outcome.

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Tyrosine kinase inhibitor treatment results in CD70 expression on chronic myelogenous leukemia stem cells and causes drug resistance via CD27-mediated activation of the Wnt pathway

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Background. In chronic myelogenous leukemia (CML), the Wnt pathway is fundamental for maintenance and survival of the disease-initiating leukemia stem cells (LSCs). The oncogenic BCR-ABL1 tyrosine kinase phosphorylates and stabilizes β-catenin and thereby continuously activates Wnt-signaling in LSCs. Previous studies have shown that genetic deletion or pharmacological inhibition of β-catenin eradicates CML LSCs. In contrast, tyrosine kinase inhibitors (TKIs) that represent the current standard of care for CML do not eliminate LSCs and therefore do not cure the disease. Interestingly, TKI treatment completely inhibits BCR-ABL1 in LSCs and is known to reduce Wnt-signaling. Hence, LSCs must employ different mechanisms to sustain Wnt pathway activity in the presence of TKIs. We have previously shown that signaling via CD70/CD27, a TNF superfamily ligand-receptor pair, activates the Wnt pathway in LSCs and leads to disease progression. We now extended these studies and investigated the possibility whether TKI treatment affects the CD70/CD27-signaling axis in CML LSCs.
Methods. The effects of TKI treatment on the CD70/CD27-signaling axis were studied using CML cell lines and primary patient samples in vitro, as well as in murine xenografts and a murine CML model in vivo. BCR-ABL1 was inhibited using different TKIs such as imatinib, nilotinib and ponatinib, and the CD70/CD27-interaction was blocked by monoclonal antibodies.
Results. TKI treatment of CML cell lines and primary CD34+ stem/progenitor cells reduced the expression of microRNA-29, leading to demethylation of the CD70 promoter and increased expression of SP1, a transcription factor with known binding sites in the CD70 promoter. This induced the expression of CD70 and resulted in CD70/CD27-signaling and consecutive Wnt pathway activation. Blocking the CD70/CD27-interaction in combination with TKI treatment potently inhibited the Wnt pathway, leading to growth arrest of CML cell lines, eradication of human CD34+ stem/progenitor cells in xenografts and elimination of LSCs in the murine CML model.
Conclusions. TKIs have revolutionized CML therapy and turned an invariably fatal disease into a chronic condition. However, because only more differentiated cells are targeted and LSCs remain, the drugs have to be taken life-long with serious side effects and high costs. In addition, there is a significant risk of disease evolution despite therapy. Therefore, novel treatments are needed that directly target the disease-inducing LSCs, and blocking the CD70/CD27-signaling pathway may be an attractive therapeutic opportunity in combination with TKIs.

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Eosinophilic/T-cell chorionic vasculitis: a report of two cases

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Background. Eosinophilic/T cell chorionic Vasculitis (E/TCV) is a rare form of inflammatory lesion of the wall of the placental fetal vessels. It is characterized by an infiltration within the chorionic vessel wall, composed of CD3+ T cells, eosinophils and histiocytes. 2 rare cases of E/TCV are presented here. The first involved a 44 year old patient, on a normotrophic, diamniotic, dichorionic twin placenta at 32 5/7th weeks of gestation. The second concerned a normotrophic singleton bipartita placenta at 41 4/7th weeks of gestation, in a patient aged 34.
Methods. Gross analysis with standard sampling of both placentas was performed, followed by histological examination and immunohistochemistry, with CD3 and CD20 antibodies.
Results. Sampling of the chorionic plate at the umbilical cord insertion showed a localized vascular infiltrate, composed predominantly of lymphocytes and eosinophils. In the twin placenta, inflammation involved only one chorionic plate vessel, whereas in the singleton placenta, many vessels were infiltrated. The latter also presented a stage I chorioamnionitis. Chronic villitis (VUE) and fetal thrombotic vasculopathy, although frequently described in association with E/CTV, were not observed in these 2 cases. All 3 babies are well.
Conclusions. We report 2 cases of E/CTV, an unusual form of chorionic vasculitis. This recently described entity is believed to represent a fetal inflammatory response to intrauterine infection, although not usually related to adverse fetal outcome.

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High intratumoral levels of FoxP3+ lymphocytes are associated with better prognosis in primary resected esophageal adenocarcinomas

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Background. Tumor infiltrating T-lymphocytes (TILs) have been shown to play an important prognostic role in many carcinomas. The identification of prognostic relevant morphological or molecular factors is a major area of interest in the diagnostic process and for the treatment of highly aggressive esophageal adenocarcinoma. Studies about the impact of TILs in this tumor have not shown completely congruent results yet. We present a comprehensive study about the clinical and pathological impact of TIL in esophageal adenocarcinomas.
Methods. A next generation tissue microarray (ngTMA) of 117 primary resected esophageal adenocarcinomas was analyzed for CD3+, CD8+ and FoxP3+ TIL using immunohistochemistry. The TMA contained three cores of the tumor center and the tumor periphery per each case. Slides were scanned with a high-resolution scanner (ScanScope CS; Aperio) and an image analysis software (Aperio Image Scope) was used to determine the TIL counts. The results were correlated with clinicopathological parameters.
Results. CD3+, CD8+ and FoxP3+ TIL counts showed a significant correlation among each other ($p < 0.001$ each, range: 0.27–0.77). TIL counts were categorized as high and low levels, according to the median. Tumors with high FoxP3+ intratumoral lymphocyte counts were more frequently of lower pT category ($p < 0.001$) and without lymph node metastasis ($p = 0.04$). High levels of FoxP3+ lymphocytes in the tumor center and the periphery were also associated with better prognosis ($p < 0.001$ and $p = 0.041$, respectively) in univariate analysis. A sim-

ilar prognostic impact was seen for high levels of CD3 + and CD8 + TIL in the tumor center, but not in the periphery ($p = 0.047$ and $p = 0.011$, respectively). In multivariate analysis high central FoxP3 + TIL levels were an independent prognostic factor (HR = 0.4; $p = 0.023$) which was similar to a combination score of CD3 + /CD8 + /FoxP3 + TIL (HR = 0.54; $p = 0.027$) or CD8 + /Foxp3 + TIL (HR = 0.052; $p = 0.020$) and superior to pT- and pN category ($p > 0.05$ each).
Conclusions. This study demonstrates a significant beneficial prognostic impact of high TIL counts in the tumor center of esophageal adenocarcinomas, in particular with regards to the subpopulation of FoxP3 + and CD8 + T-regulatory cells. The determination of intratumoral lymphocytic counts and application of TIL scores can improve prognostic accuracy of pathologic reports of these tumors and may be a potential tool for better risk stratification of esophageal adenocarcinoma patients.

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Clinical utility of a molecular test in adjuvant treatment decision making in women with endocrine-sensitive early stage breast cancer: a retrospective, single center one year experience

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Background. Molecular tests for breast cancer (BC) risk assessment are reimbursed by health insurances in Switzerland since the beginning of year 2015. The main current role of these tests is to help oncologists to decide about the usefulness of adjuvant chemotherapy in patients with early stage endocrine-sensitive and human epidermal growth factor receptor 2 (HER2)-negative BC. These gene expression signatures aim at predicting the risk of recurrence in this subgroup. One of them (OncotypeDx/OT) also predicts distant metastases rate with or without the addition of cytotoxic chemotherapy to endocrine therapy. The clinical utility of these tests -in addition to existing so-called "clinico-pathological" prognostic and predictive criteria (e.g. stage, grade, biomarkers status)—is still debated. We report a single center one year experience of the use of one molecular test (OT) in clinical decision making.
Methods. We extracted from the CHUV Breast Cancer Center data base the total number of BC cases with estrogen-receptor positive (ER +), HER2-negative early breast cancer (node negative (pN0) disease or micrometastases in up to 3 lymph nodes) operated between September 2014 and August 2015. For the cases from this group in which a molecular test had been decided by the tumor board, we collected the clinicopathologic parameters, the initial tumor board decision, and the final adjuvant systemic therapy decision.
Results. A molecular test (OT) was done in 12.2 % of patients with ER + HER2 negative early BC. The median age was 57.4 years and the median invasive tumor size was 1.7 cm. These patients were classified by ODX testing (Recurrence Score) into low-, intermediate-, and high risk groups, respectively in 27.2 %, 63.6 % and 9 % of cases. Treatment recommendations changed in 18.2 %, predominantly from chemotherapy-endocrine therapy to endocrine treatment alone. Of 8 patients originally recommended chemotherapy, 25 % were recommended endocrine treatment alone after receiving the Recurrence Score result.
Conclusions. Though reimbursed by health insurances since January 2015, molecular tests are used moderately in our institution as per the decision of the multidisciplinary tumor board. It's mainly used to obtain a complementary confirmation supporting the decision of no chemotherapy. The OncotypeDx Recurrence Score results were in the intermediate group in 66 % of the 9 tested cases but contributed to avoid chemotherapy in 2 patients during the last 12 months.

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Polyvinylpyrrolidone storage disease in a drug addicted patient: a lesson from Norway

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Background. A 23 year old man with an 8 year history of i.v. heroin and methadone consumption was referred for renal biopsy because of renal insufficiency. He had no proteinuria or hematuria, was HCV positive, and denied the use of herbal medicines or anabolic steroids.
Methods. Case report. The kidney biopsy was evaluated by light microscopy, immunofluorescence and electron microscopy.
Results. Light microscopy showed peculiar bluish vacuoles in macrophages within glomeruli and the tubulo-interstitium. The material stained with congo red, but was negative in congo red fluorescence and showed no birefringence in polarized light. By electron microscopy, these deposits were empty vacuoles or contained lipid like material and could partly be located to lysosomes. Clinical follow-up, the recognition of more such cases within Norway, and a thorough literature search resulted in the identification of polyvinylpyrrolidone (PVP) as the stored substance. PVP is widely used in oral medications, including oral drugs in the opioid maintenance treatment (OMT) program, to improve dissolution rate and bioavailability. High molecular PVP is not harmful when taken orally. However, some opioid addicted patients inject their OMT medications illicitly. Then high molecular PVP may accumulate in the body resulting in PVP storage disease. This can affect multiple organ systems (e.g. bone marrow, synovia, GI tract, liver).
Conclusions. Paying attention to details matters. Always think of drugs in case of unexplained biopsy findings. As a result of these observations, methadone containing high molecular PVP was withdrawn from the European market.

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Impact on survival of MYC genetic alterations but not MYD88L265P mutation in primary testicular DLBCL

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Background. One of the main prognostic markers in diffuse large B-cell lymphoma (DLBCL) is MYC rearrangement, which is correlated with a high protein expression in most of the cases. The co-expression of MYC and BCL2 is also associated with an unfavorable response to R-CHOP. MYD88 mutation in L265P, which is predominantly described in activated B-cell like (ABC) DLBCL, is correlated with a worse prognosis. Primary testicular DLBCL is characterized by ABC profile, recurrent

MYD88L265P mutation, frequent BCL2 expression, but low incidence of MYC genetic alterations and protein expression. The impact of these features on survival is still unknown.

Methods. We studied the incidence and the prognostic significance of MYC and BCL2 gene and protein and of MYD88L265P mutation, in 33 primary testicular DLBCL patients (24 uniformly treated with R-CHOP immunochemotherapy and intrathecal methotrexate in the IELSG-10 clinical trial). MYC and BCL2 genetic alterations were analyzed by FISH. BCL2 and MYC expression were carried out by IHC. Detection of MYD88L265P was performed by Sanger sequencing.

Results. MYC genetic alteration was observed in 10/33 (30 %): 3 rearrangements and 7 gains. Six of 31 (19 %) were considered positive for MYC expression (cut off > 40 %). All cases with MYC rearrangement, 2/6 (33 %) with gains and 1/20 (5 %) without genetic alterations of MYC showed overexpression of the protein ($p = 0.001$). No rearrangement of BCL2 were detected in any case, but gains were observed in 14/26 (54 %). BCL2 expression was found in 19/30 (63 %). Cases with MYC rearrangement did not showed BCL2 gains, however 5/6 (83 %) of MYC gained also presented gains of BCL2. Among the 6 positive cases for MYC expression, 4 (67 %) co-expressed BCL2. MYD88L265P was detected in 18/33 (55 %), which was more frequently observed in patients older than 70 ($p = 0.009$). The only variable predicting an unfavorable outcome was the presence of MYC genetic alterations which correlated with a worse overall and progression free survival ($p = 0.037$ and $p = 0.017$, respectively). Nor the presence of MYD88L265P neither the expression of MYC protein (with or without the co-expression of BCL2) had an impact on the survival.

Conclusions. This study confirms that MYD88L265P is a common event in DLBCL of the testis, but in contrast to DLBCL of other locations, this mutation has no impact on survival. Only MYC genetic alterations, but not their protein expression, were associated with an adverse outcome. MYC genetic alterations may be a useful marker to identify patients with poor response to R-CHOP.

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Unravelling the driver mutations in endometrial cancer

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Background. Endometrial carcinomas are the most frequently occurring malignancies of the female genital tract. Although many cases are surgically curable, around 30 % of tumours represent an aggressive and untreatable disease. This project aims to identify and understand the molecular causes of these aggressive tumour subtypes disregarding their histological diversity.

Methods. In order to identify the combinations and the chronological order of molecular alterations that accumulate during endometrial cancer progression we utilised our recently developed mouse model of aggressive endometrial cancer. Using the Ampliseq platform (Ion torrent) we designed a mouse custom panel for targeted next generation sequencing. With this panel we sequenced 99 genes suspected to play a role in endometrial cancer development in 64 mouse FFPE samples (endometrial precursor lesions, endometrial carcinomas and non-neoplastic tissue).

Results. Next-generation sequencing provided an overview of the genomic alterations, comparing invasive endometrial cancer samples of different stages with matching precursor lesions and non-neoplastic tissue. We found mutations in 16 genes that are statistically enriched in the precursor and/or tumour samples.

Conclusions. 16 genes seem to be crucial for the development of endometrial cancer in our mouse model. After confirming the candidate mutations by Sanger sequencing, we will design a small custom panel for human FFPE tissue to sequence endometrial cancer samples from a large patient cohort.

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Circulating tumor cells (CTC) as real-time liquid biopsy in patients with lung cancer

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Background. Circulating tumor cells (CTCs) are rare malignant cells that originate from the primary tumor or from metastatic sites and can be detected in the peripheral blood. Analysis of CTCs, also referred to as real-time "liquid biopsy", can be used as a tool in personalized cancer therapy. Furthermore, CTCs are a valuable source to explore tumor biology. In recent years, many different approaches to identify and isolate CTCs have been developed. Previous studies have shown a wide range in the prevalence of CTCs in lung cancer, partly due to different techniques used. The aim of this study was to determine the utility of a size-based filtration technique in a mixed population of patients with lung cancer.

Methods. Peripheral blood from 31 patients with different histological subtypes at different stages was filtered using the ScreenCell (ScreenCell, France) device. The filters were stained with May-Grünwald Giemsa and screened to identify CTCs. Morphology of CTCs was compared to histological biopsies of the primary tumors. Additionally, immunohistochemistry (IHC) was performed to confirm and characterize putative CTCs. IHC has previously been optimized on cultured cell lines spiked into blood from healthy donors.

Results. CTCs, either as single cells but more often as CTC clusters, were identified in 5 of 10 patients with metastatic disease. Morphological criteria for malignancy as defined on spiked cells from cultured cell lines could not be applied to CTCs from patients' samples. CTCs were small, showed subtle cytological atypia and a low nuclear/cytoplasmic (n/c) ratio. There was no morphological correlation between the histological tumor subtype and correspondent CTCs. The level of expression of cytokeratin was lower in CTCs than in cell lines. Additionally, large cells with high n/c ratio and irregular nuclear membrane were present in all samples. These were negative for cytokeratin and partly positive for ERG, corresponding to megakaryocytes and their precursors.

Conclusions. Identification of CTCs based on morphology alone is not reliable due to minimal cytological atypia and missing morphological features of a histological subtype. A subset of large cells with cytological atypia may represent megakaryocytes and their precursors and therefore caution is advised when using size-based methods for CTC isolation without additional confirmation of their origin. Further molecular characterization of CTCs may contribute to understanding mechanisms involved in tumor progression and metastasis.

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Long-term sequelae of penile paraffin injections for aesthetic reasons: report of three cases

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Background. Penile paraffinomas are a rare entity caused by subcutaneous injections of paraffin or similar substances (vaseline, cod liver oil) for penile girth enlargement. Paraffin injections for cosmetic/aesthetic purposes were in vogue at the turn of the last century, but were soon abandoned due to devastating long-term sequelae. Despite well documented complications, laypersons and backstreet practitioners, especially in eastern european countries and Asia, recommend and perform paraffin injections for penis augmentation. Frequent early complications include infections, pain, swelling, painful or uncomfortable erection, paraphimosis and voiding difficulties. Delayed complications such as ulceration, fistulation, deformity and erectile dysfunction seem to be related to the development of foreign body granulomas which are part of a typical histopathology.

Methods. Three patients between 26–32 years old who were treated in the Department of Urology of the University Hospital of Cologne for complications following the subcutaneous injection of paraffin. Resected subcutaneous penile tissue has been routinely formalin fixed and paraffin embedded. Histological H&E slides from selected areas were analyzed using a standard routine microscope. Clinical history including onset of disorders, pain, erectile dysfunction and deformation were recorded by the treating clinical colleague.

Results. The onset of complications occurred with a striking variability of 2 months to 15 years after paraffin injection. All patients showed typical histologic features of sclerosing lipogranulomas with multiple optical empty spaces of varying sizes in dermis and subcutis, embedded in dense, sclerosing collagenous tissue. The vacuoles were lined by syncytial giant cells, between them a variable chronic, often granulomatous, inflammation with multinucleated foreign body giant cells and foam cell macrophages. Because of the clinical correlation the lesions have been classified as penile paraffinomas.

Conclusions. The clinical diagnosis of penile paraffinoma can be challenging if the patient is reluctant to admit the injections. Furthermore, as in our cases, complications can occur with a latency period varying from months to decades, which might obscure the diagnosis as the connection to paraffin injections might not be apparent. The tissue alterations caused by inoculated paraffin are characteristic, thus histopathological evaluation can be helpful. In any case the complete excision of all involved tissue followed by penoplasty is the treatment of choice.