Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Cancer/Tumor Markers

A-003

The serum concentration of pro-GRP in diagnosis of lung cancer

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Serum concentration of Pro-GRP in the Diagnosis of Lung Cancer

Background: Lung cancer is classified into two major entities depending on cell type: small cell lung cancer and non-small cell lung cancer (SCLC and NSCLC, respectively). SCLC accounts for up to 15% of all new lung cancer cases and differs biologically from NSCLC by the presence of neuroendocrine differentiation and a higher rate of tumor growth. Clinically, early metastatic spread is very common. SCLC is highly sensitive to initial chemotherapy and radiotherapy. In recent years, a precursor of the neuropeptide gastrin releasing peptide, progastrin-releasing peptide (ProGRP), has been reported as the most promising marker for SCLC. The aim of study was to investigate the diagnostic and prognostic significance of pro-gastrin-releasing peptide (ProGRP) in small cell lungcancer (SCLC) and compare this marker with lung healthy control group.

Methods: The concentrations of Pro-GRP in 100 serum samples were determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 Abbott diagnostic. All of 50 patients were hospitalized at Oncology department in Intensive Care Unit at the University Clinics Center of Sarajevo. Pro-GRP was determined in lung cancer detection with SCLC pathologically confirmed lung cancer serum. We considered Pro-GRP 50 pg/ml as the upper limit of normality. Other group of 50 patients was a lung healthy control group. Collected data were statistically analyzed using programs SPSS version 16.0 and MedCalc.

Results: Serum of patients with lung cancer group, Pro-GRP content (1550.01 ± 287.6 pg/ml) and positive (63%) were higher than benign lesions of the lung healthy control group (33.18 ± 3.92 pg/ml). High concentration of Pro-GRP content have sensitivity of 82.85 % , a specificity of 76.67 % , the largest area under the ROC curve was 0.793. It was found significant difference between lung cancer group and healthy individuals using Mann-Whitney test p= 0.0037.

Conclusion: Our study supports ProGRP as an important marker of SCLC and points the way for further clinical studies to explore its value in monitoring response to therapy and patients' follow-up.

A-004

Comparison of Conventional Chemical Method to Immunochemical Method for Faecal Occult Blood Testing

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Introduction:

Faecal occult blood testing (FOBT) is one effective method to screen for colorectal cancer and to assess for gastrointestinal bleeding in suspected patients. Differences in the sensitivity and the specificity among methods have result in significant effects on further investigations.

Objective:

To check whether there is a significant difference of the results of traditional chemical method with immunochemical method for faecal occult blood testing.

Methods:

We evaluated the analytical performance of two different methods (traditional chemical based method and immunochemical method) using 25 patient samples (3 consecutive samples). Colonoscopy finding of each of the patient was taken as the **"gold standard"**.

Results:

		Patient with positive colonoscopy finding		**Patient with positive colonoscopy finding**			
		Colonoscopy positive	Colonoscopy negative	Colonoscopy positive	Colonoscopy negative		
FOB screen test outcome (traditional Chemical method)	Test outcome positive	True positive (7)	False positive (5)	True positive (12)	False positive (1)	Test outcome positive	**FOB screen test outcome (Immunochemical method)**
	Test outcome negative	False negative (7)	True negative (6)	False negative (2)	True negative (10)	Test outcome negative	
		Sensitivity 58.3%	**Specificity 54.5%**	**Sensitivity 92.3%**	**Specificity 90.9%**		

The analytical sensitivity and specificity were measured and Chemical based FOBT gave 58.3% of sensitivity and 54.5% specificity. Immunochemical FOBT produced 92.3% of sensitivity and 90.9% of specificity.

Conclusions:

These results demonstrate significant differences in the analytical performance among two FOBT methods. The immunochemical FOBT which showed superior sensitivity and specificity is more suitable than the chemical based FOBT when selecting an FOBT method for screening for colorectal cancer or for the assessment of gastrointestinal bleeding in suspected patients.

A-005

Methylation of KLF2 Associates With Its Expression and Non-small Cell Lung Cancer Progression

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Background: Kruppel-like factor 2 (KLF2) is a putative tumor suppressor gene. Our study investigated its role and epigenetic mechanisms in human non-small cell lung cancer (NSCLC) in ex-vivo and in vitro.

Methods: A total of 47 paired NSCLC and normal tissues and six cell lines were analyzed using qRT-PCR for KLF2 expression. KLF2 methylation was assessed using the methylation specific PCR (MSP) or bisulfite sequencing PCR (BSP). Functional KLF2 region 4 (+567 to +906) was confirmed using the dual-luciferase reporter assay, while CCK-8 cell viability and flow cytometric assays were used to assess changes in cell viability, cell cycle distribution, and apoptosis after knockdown or re-expression of KLF2. Western blot was performed to analyze KLF2 expression and p15 and p21 expression in cells.

Results: KLF2 expression was significantly reduced in NSCLC cells and tissues via KLF2 methylation. Reduction of KLF2 expression was associated with KLF2 region 4 hypermethylation in 27 of 47 (57.45%) NSCLC tissues. Furthermore, methylation at KLF2 region 4 was significantly associated with lymph node metastasis and advanced TNM stage. Re-expression of KLF2 suppressed NSCLC cell viability, arrested cells at G0/G1 cell cycle by induction of p15 and p21 expression, and promoted apoptosis, whereas knockdown of KLF2 expression had the opposite effects on cells.

Conclusion: KLF2 possesses tumor suppressor functions in NSCLC and detection of KLF2 methylation should be further evaluated as a tumor or prognostic biomarker for NSCLC.

A-009

Biclonal gammopathy detected not in immunohistochemical stain but in biochemical laboratory test in patient with multiple myeloma accompanying intravertebral plasmacytoma

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Background; Multiple myeloma is a plasma cell neoplasm which is characterized by clonal proliferation of plasma cells. Plasma cells secrete monoclonal immunoglobulins and/or free light chains, and this is referred to as monoclonal gammopathy. Protein

electrophoresis is used for confirming monoclonal gammopathy and diagnosing multiple myeloma. While, biclonal gammopathy is characterized by simultaneous production of two different kinds of monoclonal components. It occurs in 3-6% of all monoclonal gammopathy. In biclonal gammopathy, the most common combination is known to be IgG and IgA (33%), followed by IgG and IgM combination (24%). We experienced a very rare case of biclonal gammopathy with IgA and IgM lambda type confirmed only by biochemical laboratory tests with quantitative difference of secreted monoclonal intact immunoglobulins in patient with multiple myeloma accompanying intravertebral plasmacytoma.

Methods; Bone marrow aspiration and biopsy examinations were done for diagnosing multiple myeloma. Monoclonal components in serum and urine were detected in capillary electrophoresis via capillary 2 (Sebia, France) and they were reconfirmed by a high-resolution gel electrophoresis in a Hydrasys analyzer (Sebia, France) using Hydragel 15 HR gels (Sebia, France). Also, immunohistochemical stains were performed on both bone marrow and intravertebral plasmacytoma specimens.

Results; An 81-year-old man who suffered from lower back pain and both leg weakness for 3 months was admitted to the department of neurosurgery. The patient was transferred to the department of hemato-oncology because plasmacytoma was confirmed by biopsy from vertebral lesion. Immunohistochemical stains in vertebral lesion revealed positive results for IgA and lambda, negative result for IgM. In bone marrow examination, plasma cells are counted upto 66.6%. Samely with vertebral lesion, IgA was positive and IgM was negative, respectively in immunohistochemical stain of bone marrow biopsy. However, serum protein and immunofixation electrophoreses confirmed the presence of two distinctly separated bands in IgA and IgM lambda resulting in biclonal gammopathy. This case showed the difference of the quantity between IgA and IgM serologically, which is that IgM secretion was considerably less than IgA. In urine, abnormal zone of restriction in lambda component was confirmed, too.

Conclusion; IgA positivity was confirmed in immunohistochemical stain in vertebral lesion. Meanwhile, serum electrophoresis showed the positive results of IgA and IgM. According to these results, we considered the possibility of double primary biclonal tumor, that is, IgA type plasmacytoma and IgM type multiple myeloma. To verify this, immunohistochemical stains of both tumor and bone marrow were performed. Eventually, only IgA was positive (and IgM was negative) in both tumor and bone marrow specimens. This suggests that clones from vertebral lesion and bone marrow have the same origin. Because IgM was secreted very small amount, IgM-producing clone cannot be detected by immunohistochemical staining. Instead, the presence of IgM-producing clone can be confirmed only by biochemical laboratory test, capillary electrophoresis. This seems to have occurred because biochemical laboratory tests have better detection limits and higher sensitivity than immunohistochemical staining. After his diagnosis, the patient is now undergoing anticancer treatment.

A-011

Validation of serum-based 4Kscore Test for detection of high grade prostate cancer

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Background:The 4Kscore Test has been clinically demonstrated to improve shared decision making to proceed with a prostate biopsy, by providing a personalized, accurate risk of aggressive prostate cancer (Gleason score ≥7) on biopsy. A prospective study of 1,012 American patients scheduled for prostate biopsy (Parekh et al. 2015) showed excellent discrimination by the 4Kscore Test between men harboring aggressive prostate cancer and those with indolent tumors or no cancer (AUC 0.82). Since 2015, the 4Kscore Test has been recommended by the National Comprehensive Cancer Network Guidelines as a decision-making tool in the prostate cancer diagnosis process. The 4Kscore Test includes the assay of four biomarkers (total prostate specific antigen(tPSA), free PSA(fPSA), intact PSA(iPSA), and human kallikrein 2(hK2)) and was launched as a plasma-based blood test on the observation that some analytes are less stable in serum than in plasma (Woodrum et al. 1996, Christensson et al. 2011). Assay of the 4Kscore in serum allows for immediate reflex testing of a sample originally submitted for serum tPSA assay, with no additional blood draw. Here, we validate the use of 4Kscore in serum by: 1)demonstrating excellent analytical performance of the iPSA and hK2 assays in serum, 2)proving in a prospective clinical study equivalence between 4Kscore generated from serum and plasma samples, and 3)proving sufficient stability of serum for the 4Kscore when handled according to current serum tPSA protocols.

Methods:iPSA and hK2 custom assays were performed on the AutoDELFIA platform utilizing time-resolved fluorescence, and the assays' analytical performances were evaluated according to CLSI protocols at three independent laboratories (OPKO Diagnostics, OPKO Lab, and BioReference Laboratories). The tPSA and fPSA assays were performed with the Roche Elecsys assays on the Cobas instrument. Patient-matched plasma and serum samples from a cohort of 353 men scheduled to undergo prostate biopsy were collected in a prospective clinical study conducted at eight Veterans Administration Hospitals. Samples were measured in a CLIA-certified laboratory and were handled according to the procedures in place for accepting and processing samples submitted for tPSA assay. A subset of samples (N=57) were repeatedly measured over a three-day period.

Results:A complete characterization of analytical performance in three laboratories confirmed robust reproducibility, accuracy, and linearity at clinically relevant ranges of iPSA and hK2 in serum (iPSA LoQ:28.5pg/mL, hK2 LoQ:4.4pg/mL; repeatability and total precision for both assays ≤15%). Mean percent recovery over three days of repeated measurements in serum exhibited no significant change in a mixed model regression analysis (N=57,p>0.05); 4Kscore generated from serum three days after collection was equivalent to first day results (slope=1.014(CI:0.997-1.040,intercept=0.005(CI=.0005-0.012),Passing-Bablok). 4Kscore generated from serum were equivalent to those generated from the same patient's plasma (N=353,slope=1.011(CI:1.003-1.020),intercept=-0.001(CI:-.005-0.001),Passing-Bablok).

Conclusion:Analytical performance of the iPSA and hK2 custom assays in serum meets or exceeds plasma performance. The equivalence of 4Kscore whether generated from serum or plasma and the consistent performance of 4Kscore with routine serum sample handling in a CLIA environment allow the 4Kscore to be used as a reflex option on tPSA results.

A-012

Clinical implications and multiple antitumor effects of miR-651 and miR-708 in renal cell carcinoma

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Background: The mechanisms involved in renal cell carcinoma (RCC) development and progression remain unclear, and new biomarkers are needed in routine practice to improve the diagnostic and/or prognostic accuracy. However, there is no standard serum biomarker to facilitate diagnosis or prognostic stratification in patients with RCC. There is increasing evidence that microRNAs (miRNAs) are involved in cancer development and progression and circulating miRNAs have great potential as biomarkers for diagnosis and prognosis in patients with several types of cancers. Our purpose was to search valuable biomarker for the diagnosis of RCC and to evaluate their functional significance and possible mechanism in RCC.

Methods: An initial microarray survey of 754 miRNAs was firstly performed using the TaqMan Low Density Array followed by a hydrolysis probe-based RT-qPCR validation from serum samples of 33 RCC cases and 33 normal controls to identify significantly dysregulated miRNAs in RCC. Subsequently, the diagnostic values of these miRNAs were evaluated by ROC analysis. The expression levels of miR-651 and miR-708 in tumor tissues (n=17 pairs) were also examined. Furthermore, *in vitro* experiments including CCK8 proliferation, transwell and wound healing assays were conducted to explore the potential functions of miR-651 and miR-708 in RCC. The databases including miRanda, TargetScan and PicTar were used to predict the common target genes of miR-651 and miR-708. Luciferase reporter assays combined with western blotting were employed to validate the target genes. Moreover, xenograft mouse models were used to demonstrate the role of miR-651 and miR-708 in RCC.

Results: The serum levels of four miRNAs were verified to be significantly increased, whereas the levels of four miRNAs were markedly decreased in RCC patients compared with the noncancer controls (at least $P < 0.05$). Of the eight dysregulated miRNAs, miR-651 and miR-708 exhibited the largest AUCs: 0.888 (95% CI 0.833-0.943) and 0.832 (95% CI 0.786-0.878), respectively. Luciferase reporter assays and western blotting showed that both miR-651 and miR-708 directly regulated RAP1B, a Ras-related small GTP-binding oncoprotein implicated in a variety of tumors. In RCC clinical specimens, the RAP1B protein level was inversely correlated with expressions. *In vitro* gain-of-function and loss-of-function studies in human renal carcinoma cell lines A498 and ACHN, demonstrated that miR-651 and miR-708 synergistically suppressed cell proliferation and migration by directly inhibiting RAP1B, and this effect was reversed by co-transfection with RAP1B. Furthermore, *in vivo* xenograft mouse model experiments demonstrated that the tumor weights in the miR-651 & miR-708 group were significantly lower than those in miR-651 or miR-708 group, and the miRNA transfected groups were all lower than the negative control.

Conclusion: miR-651 and miR-708 may potentially serve as novel biomarkers for RCC and may act as tumor suppressors in RCC progression by synergistically

inhibiting the RCC cell proliferation and migration through targeting oncogene RAP1B. Our findings indicate that targeting miR-651 and miR-708 by a genetic approach may provide a novel strategy for the treatment of RCC.

A-013

FIT test prescription patterns and positive conversion rates of serial results of the target population (50-75 yo) in a prescription based colon cancer screening program in the province of Quebec, Canada.

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Backgroud

The fecal immunochemical test (FIT) uses antibodies to detect human hemoglobin protein in stool. This test has been offered in Quebec since 2013 as part of a Provincial Colon Cancer Screening Program and has implemented as a prescription-based colorectal screening strategy. The threshold for a positive test has been set at 175 ng/ml, a higher level as compared to other territories. This study aimed to investigate the prescription pattern and guideline adherence of physicians as well as the positive conversion rates in serial results of subjects with a negative first test.

Methods

In this retrospective study, we analyzed a database of anonymized data from 214,209 subjects with serial FIT testing. Our study population was selected based upon the following criteria: age ranging from 50-74 years and a minimal interval of 90 days between the two tests. To investigate the prescription pattern, intervals between serial tests were calculated for every subject. To evaluate the positive conversion rate in serial testing, subjects were categorized into 8 groups depending on the result of their initial test (A: 0-30, B: 31-49, C: 50-74, D: 75-99, E: 100-124, F: 125-149, G: 150-174, H: 175 ng/ml). The positive conversion rate was calculated for group A to G. Data were analyzed with the statistical package R.

Results.

Our study population included 174,362 subjects (90719 women, 83628 men) with a median age of 62 years. After a 3-year period of the implementation of FIT testing in Quebec, the prescription interval pattern had a bimodal distribution with peaks at 13 and 26 months following the initial test, with a median at 16 months. Furthermore, our data also showed that subjects with an initial FIT result of less than 30 ng/ml had a lower probability of conversion on repeat testing as compared to those of group G (2.6% vs 25.7%, p<0.001) The positive conversion rate increased significantly for each concentration category (A- 2.6%, B-9.1%, C-11.8%, D-15.3%, E-18.4%, F-21.2%, G-25.7)

Conclusion.

In Quebec, the FIT test is currently prescribed with a shorter median interval than that recommended by provincial guidelines. The bimodal distribution of prescription pattern suggests that there may be an ongoing change towards the recommended two-year interval. Furthermore, there is an increased trend in positive conversion rates for each subsequent concentration category. Our results suggest that a multiple threshold screening strategy based on initial results might be considered in FIT screening programs.

A-014

Serum thyroid hormone profile in Breast Cancer of local Libyan Patients.

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Background: Recent studies indicate a possible relationship between thyroid diseases and breast cancer in vivo. In addition, estrogen-like effects of thyroid hormones on breast cancer cell growth are seen in vitro. Therefore, this study evaluated thyroid function in breast cancer patients, women with fibrocystic disease and healthy controls. **Material and methods:** pretreatment group consisted of breast cancer patients who at the initial time of diagnosis (n=28), Post treatment group comprising of patients who had undergone either chemotherapy/ radiotherapy or hormone therapy for their disease (n=37) and fibrocystic group comprising of patients with benign breast tumor (n=21) and 51 healthy control group consisted of members of the public with no prior history of breast cancer or other cancer related disorders (n=51) were included in the study. Thyroid history was reported. Thyroid hormones (fT4, fT3, TSH, T3, T4) and thyroid antibodies (TPO and TG) were determined. Statistical analysis was performed by Kruskal-Wallis test and Mann-Whitney U (p<0.005 significant). **Results:** FT4 which were significantly lower in breast cancer patients (post treatment Ca Breast, pre-treatment CaBreast) compared to control [(p=0.001) (p=0.003)] respectively, No significant difference has been shown between Pre-treatment CaBreast and fibrocystic disease patients and control (P>0.05) There were non-significant differences between Breast disease groups when compared together (P>0.05). The serum levels of FT3 which were elevated significantly in breast diseases patients (post treatment Ca Breast, pre-treatment CaBreast and fibrocystic disease) compared to control [(P=0.018),(0.046),(0.007)] respectively. There were non-significant differences between Breast disease groups when compared together (P>0.05). T3 it was significantly higher in breast cancer post treatment patients compared to controls (p=0.009), No significant difference between respective groups in present study when compared together (p>0.05). Serum levels of TSH, T4, Anti-Tpo and Anti-Tg showed. No difference between breast cancer patients, fibrocystic patients and controls. **Conclusion:** Subclinical hypothyroidism more frequent in breast cancer patients, change level of thyroid hormones suggested deregulation in breast cancer patients, these data must be confirmed in large patients prospective study.

A-017

The differential analysis and functional verification of miRNAs expression profile in breast cancer

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Background: Breast cancer is one of malignant tumor which causes serious damage to the women's health. MiRNA is a fascinating kind of biomolecule due to their vital functions in gene regulation and potential value as biomarkers for serious diseases including cancers. Genome-wide miRNA expression may be useful for predicting risk and/or for the early detection of breast cancer.

Methods: 10 cases diagnosed breast cancer patients and 10 cases of normal controls were selected. By high-throughput miRNA sequencing from 10 pairs breast cancer tissues, adjacent normal tissues, serum and serum from normal persons, and using Target Scan and Clip-seq database, we selected miRNAs specifically expressed in breast cancer. Then we used real-time fluorescence quantitative PCR test the level of miRNA expression from serum of large samples. We picked the miRNA with statistical difference. For the miRNA function, we designed and synthesised the miRNAs inhibitors, and transfected to breast cancer cells MDA-MB-435, MDA-MB-468 to detected cell vitality by CCK8.

Results: After the analysing of miRNA high-throughput sequencing from breast cancer pathology organization, adjacent tissues, serum and serum from normal persons, we picked out 28 cases difference expression of miRNAs in 986 cases.More than 2 times different expression and less than 30 CT value of RT-PCR as the standard, we selected 4 miRNAs: miR-374a-5p, miR-223-3p, miR-423-5p and miR-320a. To RT-PCR results showed the stable and different expression of miRNA in 113 cases of breast cancer patients from 104 cases of healthy control group. The miRNA expression of miR-374a-5p, miR-223-3p, miR-423-5p and miR-320a in the breast cancer group was up-regulated with diffenence respectively($P < 0.05$). Besides, the expression of miRNA had significant correlation with clinical pathological characteristics. MiR-374a-5P had higher expression in lymph node metastasis group ($P = 0.001$); MiR-223-3P expressed differently between the different molecular classification ($P = 0.040$), the higher expression of miR-223-3P associated with ER negative estrogen receptor status ($P = 0.035$); The expression of miR-423-5p, miR-320a are positive correlation with clinical stages($P = 0.001$,0.014) and Ki-67 ($P = 0.001$,0.015). Functionally, we designed and synthesised the miRNAs inhibitors, and transfected to breast cancer cells MDA-MB-435, MDA-MB-468 to detected cell vitality by CCK8. We found that miR-223-3p inhibitor could inhibit miR-223-3p in MDA-MB-468, result in the inhibition of MDA-MB-468 energy. At the same time, miR-423-5p could inhibit in MDA-MB-468 and MDA-MB-435,suggesting that miR-223-3p, miR-423-5p could be inhibitors of tumor suppressor genes and regulate activity of breast cancer cells.

Conclusion: With help of the new generation of high throughput sequencing method of breast cancer, we have picked out specific differentially expressed miRNAs, including miR-374a-5p, miR-223-3p, miR-423-5p, miR-320a. The expression of the miRNAs were stable in serum and tissue. Meanwhile, The miR-223-3p inhibitor and miR-423-5p inhibitor can effectively inhibit breast cancer cell activity, which lay a foundation for subsequent function study. So we hypothesized they can be used as potential biomarkers of breast cancer for early diagnosis, prognosis and therapy target.

A-018

Development of Multiplexed Mass Spectrometry-based Assays for Urine Biomarkers of Aggressive Prostate Cancer

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Background: Currently, biochemical testing for prostate cancer utilizes serum total, free, and p2PSA to screen males over 50 years of age for the presence of disease. Follow-up of a positive serological result is a multi-core biopsy and pathological grading using a Gleason score. Measurement of biomarkers in urine would enable less invasive disease monitoring and prognosis determination. We have generated a workflow for developing mass spectrometric multiplexed selected reaction monitoring (SRM)-based assays for the detection of urine protein biomarkers for aggressive prostate cancer. This strategy exploits prostate cancer proteins previously found both in tumor tissue and in the urine of prostate cancer patients. To generate a multiplexed MS assay, urine PSA will be monitored, along with urine proteins zinc-alpha-2-glycoprotein (AZGP1) and inactive tyrosine protein kinase 7 (PTK7). PTK7 has been reported to have higher expression levels in aggressive prostate tumor tissues while the opposite trend has been found for AZGP1. **Methods:** A purified extract or recombinant form of each target protein was denatured with urea, reduced, alkylated, and digested with chymotrypsin. The resulting peptides were analyzed via LC-MS/MS on a Thermo Velos linear ion trap MS to identify candidate precursor peptides. MaxQuant searches of the human proteome positively identified peptides and confirmed that each peptide was unique to its corresponding protein. Following the identification of candidate peptides, selected ion monitoring and product ion scan experiments were carried out on a Shimadzu 8040 triple quadrupole to select appropriate transitions. Target peptides eluted between 20-35 minutes using a 50 minute acetonitrile/water LC gradient on a Restek C18 column. Urine specimens were concentrated with a 10K filter before processing proteins as above, then analyzed using the previously selected transitions in a multiplexed SRM assay. **Results:** This workflow enabled the identification of peptides and transitions for PSA, PTK7, and AZGP1. Of note, 19 candidate PSA peptides were identified by MS/MS and 11 were further selected based on high peak intensity and amino acid composition (excluding methionine and asparagine-containing peptides). For each peptide, product ion scans were performed to identify specific transitions. Four PSA peptides were chosen for use in SRM measurement of total urine PSA levels: ASGWGSIEPEEF (mass 1307.57, charge +2, 654.79 *m/z*, product ions at 521.2, 788.3, and 1014.4 *m/z*), DLPTQEPALGTTCY (mass 1564.71, charge +2, 783.36 *m/z*, product ions at 882.2, 965.5, 714.3 *m/z*), GSEPCALPERPSLY (mass 1574.73, charge +2, 788.38 *m/z*, product ions at 861.4, 715.3, 974.5 *m/z*), and TGGKSTCSGDSGGPL (mass 1379.59, charge +2, 286.1, 344.1, 692.3 *m/z*). . These transitions were recapitulated in patient samples and the chosen peptides were detected using multiplexed SRM. These peptides are detectable in urine from prostate cancer patients ranging from stage T1c to T4. **Conclusion:** This workflow enables the systematic development of multiplexed mass spectrometric SRM methods for the relative quantification of protein biomarkers from the urine of prostate cancer patients. The overall benefit of this work is the ability to generate assays for the assessment of protein biomarkers in urine, which is of particular interest for the non-invasive identification of aggressive prostate cancers.

A-019

Validation of the Ella™ chromogranin A (CgA) immunoassay

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Background: Chromogranin A (CgA) is a 48 kDa acidic glycoprotein comprised of 439 amino acids belonging to the granin family. It is considered a highly sensitive, nonspecific marker for the presence of neuroendocrine tumors due to its role in the formation of secretory granules within the neuroendocrine system. In addition CgA is used as a marker for neuroendocrine differentiation in prostate cancer and is used to monitor for disease progression. Currently, there is no established FDA-approved assay for the analysis of CgA and there is no gold standard reference method. Existing methods yield results that can vary significantly due to the lack of standardization and utilization of different antibodies between assay methods. Simple Plex (ProteinSimple, San Jose, CA) is an attractive immunoassay platform as its novel microfluidic design with separate channels for antigen/antibody reactions prevents cross-reactivity and allows the process to be automated. The objective of this study was to evaluate the performance of the Ella™ single-plexed CgA immunoassay and compare its results with those of the Quest Diagnostics™ (Madison, NJ) assay.

Methods: Quality control (QC) material, sample diluent, running buffer, Simple Plex cartridges and the Ella instrument were obtained from ProteinSimple. Concentrations were calculated using vendor-determined calibration curves. High and low QC material was used to determine assay precision. The limit of detection was established by running blank samples (n=12) and calculating the mean + 2 SD. Analytical linearity was determined by serially diluting high quality control material and 1 patient sample. Reference ranges were determined by measuring CgA levels in 120 apparently healthy volunteers. Accuracy was determined by splitting 120 patient samples that were sent to a reference laboratory and run on the Ella for comparison. Both serum and plasma was measured as part of this study.

Results: Within-run precision ranged from 2.8 - 5.2%; between-run precision ranged from 2.9 - 6.1%. The limit of detection was 0.017 ng/mL. Overall, the linearity of the assay was acceptable; the slopes ranged from 0.910 - 0.9348, R2 values ranged from 0.9959 - 0.9976. The reference range among healthy volunteers showed a non-parametric distribution. The calculated reference range was 10.82-15.70 ng/mL. Method comparison to the Quest method showed poor correlation (R2 value of 0.3961). Plasma samples showed consistently higher values compared to serum. Age range varied from 22-87 years and the mean age was 66 years. 108 samples were from male patients and 12 patients were from female patients. Samples were obtained from patients with various disease states including: neuroendocrine (10.8%), carcinoid (7.5%), pheochromocytoma/paraganglioma (1.67%), other (5.83%) and prostate adenocarcinoma (74.2%). **Conclusion:** The Simple Plex has acceptable precision, limits of detection and linearity for measurement of chromogranin A. Method comparison with Quest Diagnostics™ showed poor correlation and may be due to the fact that different immunoassays recognize different epitopes and because chromogranin A is highly heterogeneous. We will investigate this further and evaluate the clinical performance of the Simple Plex assay. The Ella instrument is easy to use and may be a good alternative to traditional ELISA assays.

A-020

Genetic variants of the aryl hydrocarbon receptor-interacting protein gene (*AIP*) in patients with clinical features of familiar isolated pituitary adenomas(FIPA): First series in Argentina

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Background: Familiar isolated pituitary adenomas (FIPA) encompasses the familiar occurrence of isolated pituitary adenomas in at least two members of the same family outside the setting of syndromic conditions such as MEN1 and Carney's complex, and comprise about 2-3% of pituitary adenomas. About 20% of FIPA have germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene (*AIP*), usually associated with a worse outcome because these tumors are large, occur at a young age and also demonstrate features of aggressiveness and treatment resistance. It is an autosomal dominantly inherited disease with a penetrance of *AIP* mutation between 15-30%.Objective:The aim of this study was to study the prevalence of germinal mutations of *AIP* gene and polymorphisms(SNPs) in a cohort of patients with FIPA or with diagnosis of pituitary macroadenomas under the age of 36

Methods: We studied 29 potential carriers with a family history of pituitary tumor or clinical features of FIPA and 66 healthy subjects (mean age 34,3 +/- 0,7 years, 50 women and 16 men) was analyzed as control group. In controls, age, gender and medical history were recorded and we also exclude subjects with personal or family history of MEN1 or Carney complex. Genomic DNA was prepared from blood samples.The promoter and exons 1 to 6 and intronic flanking regions were amplified by PCR using specific primers. The DNA fragments were analyzed by direct sequencing. All genetic alterations were confirmed by a second PCR and direct sequencing. All subjects gave informed consent to genetic studies

Results: We found the following SNPs: c.682C> A (Q228K) in 29 of the 29 patients studied, c.920A > G(Q307R) in 26 of 29, IVS 3 + 111 C> T in 17 of 29, c.-810T> G in 1 of 29, c.993+60G> C 3' UTR in 1of 29 and , c.- 941 A> G in 1 of 29 this variant has not been previously described in the literature.We did not find any mutations in the patients. Due to the high frequency of the Q228K and Q307R in the patients group we decided to study them in the control group and we found the Q228K in heterozygous form in 1 of 66 and 65 of 66 in homozygous form and the Q307R all in homozygous form.

Conclusion: There are limited data on AIP SNPs with potential functional consequences. In the literature Q228K and Q307R have been found significantly different between FIPA patients and healthy controls however in our population are equally distributed.Our results suggest that these variants do not have pathological implications. The functional consequences should be evaluated and synergism between the SNPs founded cannot be excluded. For genetic counseling, genetic testing could be proposed to evaluate relatives as a high-risk population for developing pituitary

tumors. The investigation for mutations in the AIP gene in families with pituitary adenomas is necessary, since it is associated with poor outcome and resistance to treatment.

A-021

High detection rate of CRC-related mutants in stool samples of patients with polyps by MassARRAY

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Background: Fecal occult blood test (FOBT) is adapted globally as a colorectal cancer (CRC) screening tool to identify high risk individuals. However, FOBT has some pitfalls, only 20% of the adenomas can be detected and at least half of FOBT(+) individuals undergo unnecessary colonoscopy procedures. In the present study, we aim to establish a stool DNA (sDNA) test by using a Single Allele Base Extension Reaction (SABER) MassARRAY platform to increase the detection accuracy. The sDNA panel contains 30 hot spots on 5 CRC-associated genes.

Methods: 2-fold serially diluted LoVo cell line DNAs were tested for analytic sensitivity. 10 FOBT (+) stools from individuals with negative colonoscopy finding were collected for specificity assessment. 21 CRC and 15 polyps patients whose neoplastic tissues had been pre-screened by Thermal NGS Cancer Panel provided pre-operational stool samples for representativeness evaluation. Albumin housekeeping gene is included in the panel for internal control.

Results: The SABER test can detect as low as 1.4% of the variants. 52.4% of CRC and 40% of adenoma patients have positive signals on any one of five genes in their stool samples with 100% specificity of negative reaction in 10 control samples. By comparison of mutations found in their matched tissue samples, 44.4% (16/36) of patients have concordant mutation pattern in stool samples and 19.4% (7/36) of patients appear unexpected variants which were not present in tissue samples. The sDNA detection rate is affected by lesion size (25% polyps <1cm, 45.5% polyps≧1 cm, 52.4% tumor ~3.8cm) and location (33% right, 50% left, 56% rectum).

Conclusion: SABER MassARRAY is a cost-effective and sensitive method with only 100 ng stool DNA input. Although the neoplasm size and anatomic site affected the detection rate, sDNA test demonstrates better sensitivity in detection of large adenomas and excellent specificity than FOBT. High mutation concordance rate found between stool and original lesions reveal the trueness of sDNA mutations. Further studies are warranted to validate the findings in a larger population and optimize the sDNA test.

A-022

Accurate Sequencing and Enrichment-Based qPCR as a Combinatorial Approach for Liquid Biopsy Detection and Monitoring

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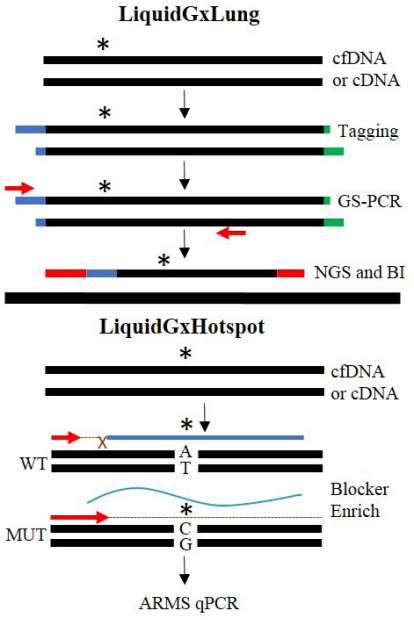
Clinical relevance: Liquid biopsy as a non-invasive method for detection and monitoring of rare variants is a rapidly emerging field. Lung cancer is the leading cause of cancer death and lung biopsies are invasive and prone to result in insufficient material for diagnosis.

Objective: To develop a non-invasive test to identify DNA and RNA variants present at low frequencies in lung cancer patients, we utilize a combinatorial approach of accurate sequencing and enrichment-based qPCR.

Methodology: Our accurate sequencing uses gene-specific PCR of individually tagged cfDNA or cDNA molecules. Our current NGS panel (LiquidGxLung) detects ≈75 variants in 10 genes (AKT1, ALK, BRAF, EGFR, ERBB2, KRAS, MET, PIK3CA, RET, and ROS1). Sensitivities are 94% and 90% for 0.1% SNPs and indels, respectively, while fusions are detected as low as 0.1%. Our CLIA-approved qPCR tests (LiquidGxHotspot) utilize blocker-based enrichment of mutant DNA followed by qPCR detection. These seven separate tests (ALK, BRAF, EGFR, KRAS, MET, RET, ROS1) have a limit of detection of as low as 0.01%. LiquidGxLung and LiquidGxHotspot display ≈100% specificity with TAT's of 4-6 and 1-2 days, respectively.

Validation: To assess the performance in a clinical setting, accurate sequencing was performed on 103 lung cancer patients while qPCR was performed on 71 of these patients. The mutations identified closely mirrored published lung cancer tissue biopsy data, while the two assays exhibited 90% concordance with each other. In 5 out of 7 non-concordant instances, the qPCR enriched a mutation that was below the 0.1% detection threshold of the accurate sequencing.

Conclusions: This NGS panel can be performed for the identification of variants in late-stage lung tumors, with monitoring of treatment response using the more targeted enrichment-based qPCR. This technology is easily adaptable to the diagnosis and monitoring of other cancer types.



A-023

Alpha-Fetoprotein (AFP) in Peritoneal, Pleural and Pericardial Fluids: A Body Fluid Matrix Evaluation

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Background: Alpha-fetoprotein (AFP) is glycoprotein produced during fetal development. It is measured clinically as a tumor marker for malignancies such as hepatocellular carcinoma and germ cell tumors. It is also used for the assessment of fetal neural tube defects during pregnancy. In association with imaging and cytology, AFP may provide supportive evidence for malignancy when measured in body fluids. A College of American Pathologists (CAP) Laboratory Accreditation Program checklist item for body fluid analysis (COM.40620) permits the use of method performance specifications from blood specimens for body fluids if the laboratory can reasonably exclude the possibility of matrix interference. The objective of this study was therefore to conduct a matrix evaluation using the Access AFP assay (Beckman Coulter; Brea, CA) with three body fluids - peritoneal (ascites), pleural, and pericardial.

Methods: The UniCel DxI 800 immunoassay platform (Beckman Coulter) was used for AFP testing. Previously collected clinical body fluid specimens were retrieved from frozen storage (-20°C) and de-identified according to an IRB-approved protocol. For spiked recovery studies, 20 low AFP pools (near zero) for each fluid type were created from patient body fluid specimens. These were spiked with patient serum samples containing elevated AFP concentrations (spiked volume 2.5 to 10%). Spiked body fluid samples were analyzed in duplicate for AFP. Percent recovery was calculated for each specimen. Mixed recovery and linearity studies were conducted covering the analytic measurement range (AMR) of the assay (0.5–3,000 ng/mL). For these studies, admixtures of high and low (near zero) AFP pools for each fluid were created and tested for AFP in duplicate. Percent recovery was calculated and linearity was assessed. For evaluation of precision, high and low AFP pools for each fluid were prepared to provide 5 aliquots each. These aliquots were frozen at -70^{0}C. Aliquots were thawed, mixed, centrifuged, and tested for AFP in four replicates each day over five days of testing. Results were analyzed using Excel 2010 (Microsoft; Redmond, WA) and EP Evaluator 11 (Data Innovations; Burlington, VT).

Results: In spiking and mixed recovery studies, the average percent recovery was within predefined acceptable limits (less than ±15%) for all three body fluids. Linearity was observed over the AMR for all three body fluids (slope, intercept, % total error): peritoneal, 0.986, 0.0, 4.1%; pleural, 1.016, 0.0, 1.6%; pericardial, 0.988,

-0.1, 6.1%. Imprecision was acceptable (%CV, <10%) for all three body fluids at both the high and low AFP concentrations.

Conclusion: Exclusion of matrix interference should be conducted prior to performing body fluid testing on assays whose performance specifications were originally derived using blood. The present studies provide evidence against the presence of any systematic matrix interference for AFP in peritoneal, pleural, and pericardial fluids on the Access AFP assay.

A-024

Serum Concentrations of Open Reading Frame 1 (ORF1) protein Produced by Activation of Long Interspersed Nuclear Element 1 (L1) In Subject Being Screened for Lung Cancer with CT scan

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Background. Retrotransposons are repetitive DNA sequences capable of copying and moving themselves and other sequences to new locations throughout the genome. One of the most abundant and active groups of them is the Long interspersed nuclear element 1 which becomes activated by hypomethylation of its promoter region. The major causes of L1 activation include environmental stressors such as particulate air pollutions, chemical carcinogens and inducers of oxidative stress. We investigated the serum ORF1 protein concentrations in a group of smokers presenting for CT scan for lung cancer at Baptist Health Floyd hospital (New Albany, IN). **Methods:** Subjects (>50 years of age) with the history of smoking for more than 30 pack/year and presenting to the radiology department for routine lung cancer screening by chest CT scan were screened, consented and enrolled in this study. Upon completion of chest CT scan, a non-fasting blood sample was collected for measuring ORF1 protein concentration. Using the CT scan report, 72 subjects were initially grouped into two major categories. Those with negative chest CT result (45) and others who were considered to be positive (27) and were asked to return for further work up as a part of their routine clinical care. In the chest CT negative group, 5 were found to have nodules or cysts in kidneys, liver or thyroid. Ultimately, 40 subjects were entirely negative for any nodules or cysts in the organs or tissues assessed on the CT scan. In order to measure ORF1 protein concentration in serum of each subject, we used an in-house competitive ELISA assay. It involved a custom-made antibody against the select amino acid sequence of ORF1 protein. The same peptide sequence, coupled with biotin was used as an anchor in streptavidin coated 96-well plates. Then, a secondary antibody (GAR-HRP) and colorimetric substrate were used to generate a blue color. Absorbance values at 450 nm were measured and patient sample ORF1 concentrations were calculated based on a logistic 4-parameter standard curve generated from calibrators of known ORF1 concentrations. **Results:** The mean ORF-1 protein concentration in subjects with suspicious nodule(s) based on CT scan who were recommended for further follow up (n=27) was 13.6 ng/ml (SD=13.5). In 45 subjects with negative lung CT scans, the mean ORF1 was 8.08 ng/ml (SD=8.2). The difference between these groups was significant (P=0.039, Mann-Whitney Rank Sum). The mean concentration of ORF1 in subjects with no nodules or cysts found in any of the studied tissues (lung, kidney, liver and thyroid) was 15.4 ng/ml (SD=8.3). The difference between the means of positive lung CT versus a completely negative CT results was also significant (P=0.016, Mann-Whitney Rank Sum). **Conclusion:** Measurement of serum concentration of ORF1 protein generated by L1 activation shows promise as a non-invasive and potentially widely available risk assessment tool for lung cancer screening in smokers.

A-025

Suppression of the non-involved heavy/light chains pair isotype as new biomarker of poor prognosis in Multiple Myeloma

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Background: The outcome for patients with Multiple Myeloma (MM) is highly variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunoassay Hevylite (HLC) allows us a precise measurement of monoclonal and non-monoclonal immunoglobulins of the same isotype. In this study we evaluate i) the impact of the "HLC ratio" defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated "HLC-matched pair suppression" and III) the effect of "systemic immunoparesis" at diagnosis and at +100 days after autologous stem cell transplantation (ASCT).

Material and methods: 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chain pairs (HLC) were assessed by Hevylite assays (The Binding Site). Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method. Statistical analysis was made with Prism 6.0.

Results: The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.71). **At diagnosis**, a i/u HLC ratio>80 was significantly associated with worse OS (48 vs. 61%, p=0,005) and shorter PFS (23% vs. 42%, p=0,006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% vs. 81%, p=0,004) and PFS (21% vs. 50%, p=0,013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32% vs. 81%, p=0,030) but not with shorter PFS (26% vs. 44%, p=0,306).

In the post-ASCT evaluation of the patients (n=30), normalization of HLC ratio was observed in 22 patients (73%). An altered HLC ratio was significantly associated with shorter PFS after ASCT (25% vs. 70%, HR: 3,42, 95%CI 1,12-11,97, p=0,039) and with a trend towards a worse OS (p=0,072). Severe HLC-matched pair suppression was found in 12 patients (40%) and was predictive of worse OS (0% vs 70%, HR: 10,63, 95%CI: 1,11-114,11, p=0,023) and shorter PFS (35% vs. 71%, HR: 8,87, 95%CI: 1,72-45,92, p=0,002). On the other hand, the severe systemic immunoparesis observed in 17 patients (57%) was not associated with OS (p=0,644) and PFS (p=0,750).

Conclusions: Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

A-026

Screening and Diagnosis of Monoclonal Gammopathies: An International Survey of Laboratory Practice

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Objectives: Serum tests used for the screening and diagnosis of monoclonal gammopathies include serum protein electrophoresis (SPE; either agarose gel [AGE] or capillary zone [CZE]), immunofixation (IFE) and immunosubtraction capillary electrophoresis (IS-CE), serum free light chains (sFLCs), quantitative immunoglobulins, and heavy / light chain combinations. Urine protein electrophoresis (UPE) and urine IFE may also be used to identify Bence Jones proteinuria. International Myeloma Working Group (IMWG) guidelines recommend the combination of SPE, serum IFE, and sFLC when screening for new monoclonal gammopathies. The objective of this study was to assess the current state of laboratory practice for monoclonal gammopathy testing.

Methods: In April 2016, a voluntary questionnaire was distributed to 923 laboratories participating in a College of American Pathologists (CAP) protein electrophoresis proficiency testing survey.

Results: 774 laboratories from 38 countries and regions completed the questionnaire (84% response rate). The majority of participants (68.6%) use AGE as their SPE method, while 31.4% use CZE. The most common test combinations used in screening were: SPE w/ reflex to IFE/IS-CE (39.3%); SPE only (19.1%); SPE and IFE or IS-CE (13.9%); and SPE w/ IFE, sFLC, and quantitative immunoglobulins (11.8%). Only 39.8% of laboratories offered panel testing for ordering convenience. While SPE was

used by most laboratories in diagnosing new cases of myeloma, when laboratories had to select only one test used to follow patients with monoclonal gammopathy, 55.7% of laboratories chose SPE, with the next most common selections being IFE (18.9%), sFLC (11.7%), and IS-CE (2.1%). Few laboratories (13.4%) cancel IFE if an M-protein has previously been characterized. A slightly higher percent (21.8%) cancel IFE if the M-protein has the same migration as the original specimen. Less than half of laboratories (40.4%) comment in their reports on whether monoclonal proteins increased, decreased, or did not change from a prior specimen.

Conclusions: Current practices vary widely across laboratories and often do not reflect IMWG guidelines. Efforts on improving utilization management and report content, as well as further recognition and development of lab-directed testing guidelines, may serve to enhance the clinical value of testing while decreasing cost-of-care.

A-027

Novel reference materials for *EML4-ALK* testing

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Background:

In the emerging clinical paradigm of precision medicine, accurate detection of the rearrangements of echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene is important to select the subgroup patients for crizotinib therapy. Despite many attempts have been done to improve the *EML4-ALK* detecting in clinical practice, the inaccuracy of testing is still an important yet to be solved problem within all the methodologies, including fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), reverse transcription real-time PCR (RT-PCR) and next-generation sequencing (NGS). For the clinical laboratories, in order to improve accuracy and reliability of detection, a proper reference material for validation of laboratory-developed tests, verification of commercial detection kits, internal quality control and proficiency testing is of prime important. Therefore, to ensure the accuracy and reproducibility of detection, here we developed a kind of well-characterized candidate reference materials for *EML4-ALK* testing.

Methods:

In this study, clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9 (CRISPR/Cas9) technique was used to edited three types of cell lines containing *EML4-ALK* rearrangements variant 1, 2, and 3a/b. Single guide RNAs (sgRNAs) were designed in silico to target specific *EML4* locus and *ALK* locus. Px330 vector expressing Cas9 and specific sgRNA was transfected into HEK293T cells. The edited individual positive clones were verified by PCR, RT-PCR, and western blotting. Followed by subcutaneous inoculation, the formalin fixed paraffin embedded (FFPE) samples based on CRISPR/Cas9 and xenograft were prepared and tested for suitability as candidate reference materials by FISH, IHC, RT-qPCR and NGS. All the results were compared with the authentic clinical specimens to assess the commutability. In addition, homogeneity and stability assessments were also performed.

Results:

By editing HEK293T cells by CRISPR/Cas9 system, three kinds of cells containing *EML4-ALK* variant 1, 2, and 3a/b have been constructed and named as 1-F8-G9, 9-E11-G5, 14-F9-E8, respectively. The edited cells were all verified on DNA, RNA and protein levels, and can be observed a high frequency of exact fusion events. Via subcutaneously injection, the corresponding xenograft tumors were obtained in their fourth week and embedded as FFPE blocks. All kind of FFPE samples derived from xenograft tumors were found with typical histological structures by using HE staining, such as tumor infiltrating, inflammation, and partial necrosis. In addition, by FISH, IHC, RT-qPCR, and NGS, all the materials were verified to be *EML4-ALK* rearrangements-positive. Among four methodologies for *EML4-ALK* detection, the validation test showed 100% concordance. Meanwhile, compared with clinical ALK positive-NSCLC specimens, the novel FFPE samples showed great commutability. Furthermore, the materials were also completely homogeneous and stable for at least 2 months.

Conclusion:

Without limitations on variant types and production, our novel FFPE samples based on CRISPR/Cas9 editing and xenograft are suitable for all the methodologies as candidate reference materials in the validation, verification, internal quality control and proficiency testing of *EML4-ALK* detection.

A-028

LacdiNAc-PSA improves differential diagnosis and predicting aggressiveness of prostate cancer

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Background: Prostate cancer (PCa) is the second most common cancer among men worldwide. Prostate specific antigen (PSA) is a widely used biomarker for screening and monitoring patients of PCa, although the lack of specificity causes overdiagnosis and overtreatment. It is known that the *N*-glycan structure on PSA changes according to carcinogenesis. LacdiNAc-PSA is an aberrant glycosylation isoform of PSA which has LacdiNAc structure on its *N*-linked glycan terminal. We have demonstrated a pilot study of LacdiNAc-PSA by automated immunoassay system which utilizes surface plasmon field-enhanced fluorescence spectroscopy (SPFS) as its detection principle. The aim of this study is to evaluate the clinical utility of serum LacdiNAc-PSA compared to serum total PSA and the ratio of free PSA to total PSA (%free PSA).

Methods: A total of 407 patients with PCa (n=223) and benign prostatic hyperplasia (BPH, n=184) were investigated. 92 PCa patients who underwent radical prostatectomy (RP) were investigated before prostate biopsies (Pbx) to evaluate the pre-operative prognostic performance. The final diagnosis of PCa or BPH were confirmed using the histopathological findings of Pbx. The grade group (GG) of PCa specimens were evaluated according to the International Society of Urological Pathology guidelines. The serum LacdiNAc-PSA was measured by SPFS-based automated two step sandwich immunoassay system [Kaya, T. *et al.* Anal. Chem. 2015;87:1797-1803.]. Serum total PSA and free PSA was tested on automated immunoassay analyzer Architect i1000 (Abbott Japan). Diagnostic accuracy was assessed for LacdiNAc-PSA, total PSA, and %free PSA.

Results: Among PCa and BPH patients, serum LacdiNAc-PSA levels in the both range of total PSA <20 ng/mL and <10 ng/mL were significantly higher in patients with PCa (median: 0.1700 U/mL and median: 0.1140 U/mL, respectively) than BPH (median: 0.0715 U/mL and median: 0.0670 U/mL, respectively), $p<0.0001$. At the cutoff LacdiNAc-PSA level (0.0495 U/mL) for the prediction of PCa, the specificity was 40.8% at its 90% sensitivity, which was much higher than that of %free PSA (32.5 %) and total PSA (13.4%). The AUC of LacdiNAc-PSA showed quite better performance in each total PSA range (0.807 and 0.755, respectively) than that of total PSA (0.641 and 0.542, respectively) and %free PSA (0.716 and 0.689, respectively). Serum LacdiNAc-PSA levels of PCa patients were much higher at Pbx GG 3 (median: 0.2500 U/mL) than Pbx GG 2 (median: 0.1280 U/mL), $p=0.0118$, while total PSA and %free PSA could not discriminate between Pbx GG 2 and 3. This trend was also found for PCa patients with GG after RP (ope GG) ≥3 (median: 0.1885 U/mL) and ope GG ≤2 (median: 0.0985), $p=0.0068$.

Conclusion: These data suggest that serum LacdiNAc-PSA improves diagnostic accuracy of PCa against BPH, which lead to large reduction of unnecessary biopsies. Predicting performance of LacdiNAc-PSA could be used as a clinical index of patients under active surveillance. Moreover, correlations between LacdiNAc-PSA level and histopathological evaluation indicate the potential use for stratification of patients according to the aggressiveness of PCa.

A-029

Ligand Binding Assay Development for Full Length KRT19 Measurement in Serum

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Background: Keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Keratin 19 is one of the three main keratins besides CK8 and CK18 expressed in simple or stratified epithelium and in various carcinomas including lung cancer, bladder cancer, breast cancer, and other cancer types. KRT19 is cleaved by caspase 3, and the soluble fragments are released and detected in cancer patients' serum. CYFRA21-1 assay which measures the c-terminal fragment of KRT19 has been cleared by FDA for aiding in monitoring disease progression during the course of disease and treatment in lung cancer patients.

Though CYFRA21-1 is the predominant assay used for clinical studies on various cancer types, literature showed that cancer cells actually release full length KRT19 (FL KRT19) actively. The observation based on cancer cell lines was extended to breast cancer patients with existence of FL KRT19 releasing- bone marrow cancer cells linked to metastatic progression. The theory is that CYFRA21-1 is passively

released from tumor cells undergoing apoptosis or necrosis, while active release of full length KRT19 could promote tumor metastasis.

Results: Assay was developed to specifically measure FL KRT19 but not CYFRA21-1, with two antibodies' binding epitopes flanking the KRT19 caspase 3 cleavage site. Electrochemiluminescence technology was used to enhance assay analytical sensitivity. The assay has an analytical range of 6.9 pg/ml to 5 ng/ml. Spike and recovery accuracy study showed average of 71.5%, 75%, and 68% of recovery efficiency for high, middle, and low levels of analyte spiked. The limit of quantification of the assay was 22.6 pg/ml. With FL KRT19 above 22.6 pg/ml, samples demonstrate intra-assay variation of <20.8% and inter-assay variation of <20.0%. Specificity and interferences test included two keratin family members KRT13 and KRT17 and four common interferents: unconjugated bilirubin, hemolysates, lipids (triglyceride-rich lipoproteins) and biotin. The only interference observed was from high concentration of hemolysates (positive bias of 41% at 500 mg/dL of hemoglobin). Sample stability was also studied, FL KRT19 was sensitive to temperature change, showing significant deterioration under 37°C and room temperature, or for prolonged time in 4°C (> 2hrs). Sample incubation condition (time and temperature) is critical for reproducible quantification. In contrast, up to 5 freeze/thaw cycles do not affect the FL KRT19 stability significantly.

A-030

Development and Characterization of Multiplex Immunoassays for Ovarian Cancer Serum Biomarkers: An NCI Early Detection Research Network Study

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Background: Ovarian cancer is the fifth leading cause of cancer death among U.S. women and has the highest mortality rate of all gynecologic cancers. Due to the lack of effective screening tools and therapy, the mortality of ovarian cancer has not declined significantly in the past three decades. Most cases of ovarian cancer (~75%) are diagnosed at an advanced stage of the disease. Whereas patients with early-stage disease will have a 5-year survival of >74%, those with advanced-stage cancer will have overall survival rates of only 19% to 30%. Although serum CA-125 followed by ultrasound for elevated tests has the necessary specificity, there remains concern about its sensitivity for early stage disease. The U-PLEX platform enables flexible multiplexing of immunoassays using Meso Scale Discovery (MSD)'s MULTI-ARRAY technology. Multiplex immunoassay simultaneously measuring multiple analytes in the same patient sample using minimum volume allows us to evaluate serum biomarker panels that can potentially complement CA-125 in the early detection of ovarian cancer.

Objective: To develop and characterize multiplex immunoassays for a panel of serum biomarkers for ovarian cancer as part of the NCI's Early Detection Research Network (EDRN) program.

Method: Existing candidate serum biomarkers identified by multiple EDRN sites were triaged first through *in silico* mRNA analysis and then by a Mass Spectrometry-based approach. U-PLEX assays were developed for 8 selected biomarkers using a MESO QuickPlex SQ 120 system (MSD). Briefly, antibodies were conjugated with biotin and/or SULFO-TAG and antibody pairs targeting the specific isoform of the MS-identified peptides were screened. Immunoassays for the individual candidates were first developed on small spot streptavidin plates followed by development and optimization of multiplex assays on U-PLEX plates. Spike & recovery, linearity, and cross-reactivity studies were performed. Patient samples including 10 healthy control sera and 11 ovarian cancer sera were analyzed.

Results: The U-PLEX assays of 4 candidate biomarkers or 2 candidate biomarkers including two FDA approved markers (CA-125 & HE4) had negligible cross-reactivity among analytes, recovery of 72-124%, intra-assay precision of 1.0-10.3% for 21 patient samples, and wide dynamic ranges for target measurements. The sensitivity of the U-PLEX assay was at 1.95 pg/ml for CA-125 assay. The U-PLEX assay significantly correlated with its respective monoplex assay using 21 patient sera (CA-125: Pearson R/p value = 0.9967/<0.00001). Among 11 ovarian cancer sera with available CA-125 values analyzed on the Tosoh Bioscience AIA-2000, the U-PLEX assay also significantly correlated with their clinical measurements (Pearson R/p value = 0.9986/<0.00001).

Conclusion: The utility of the developed multiplex assays was demonstrated with sufficient analytical performance. The developed multiplex assays can be used to evaluate serum biomarker panels of ovarian cancer and other cancers.

A-031

Improved detection of hidden Beta-2 monoclonal proteins by capillary electrophoresis using the Beta-2 to Beta-1 ratio

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Background: Serum protein electrophoresis (SPE) is used for the diagnosis and monitoring of multiple myeloma and its related disorders by detecting monoclonal paraproteins. Capillary electrophoresis separates serum protein into 6 fractions: Albumin, Alpha-1, Alpha-2, Beta-1 (B1), Beta-2 (B2), and Gamma. While paraproteins are often detected in the gamma and beta fractions as an extra peak on the electropherogram, paraproteins have also been identified hidden in the B2 fraction. Identification of these paraproteins by inspection of the electropherogram alone is difficult. However, an elevated ratio of the B2 fraction over the B1 fraction increases suspicion of a hidden monoclonal protein in the B2 fraction. The manufacturer recommends flagging electropherograms with a B2/B1 ratio of 1.00 or higher for further investigation. However, in the setting of a community population, we questioned the value of a 1.00 threshold which, in our hands, was producing excessive false negative results. This study investigated the threshold necessary to achieve the most efficient positive detection rate of hidden B2 monoclonal proteins using the B2/B1 ratio.

Methods: Patient SPE data was collected retrospectively across 3 months for a total of 18,376 data points. The B2/B1 ratios were compared between cases with B2 monoclonal proteins confirmed by immunofixation electrophoresis, and cases without B2 monoclonal proteins. The data were used to predict the positive detection rate of B2 monoclonal proteins when the threshold for B2/B1 was set between 1.00 and 1.20.

Results: In this data set, 0.9% (n=165) of all cases had a B2 monoclonal protein and their B2/B1 ratios ranged from 0.58 to 19.42. When the B2/B1 ratio was set to a threshold of 1.00, 90.9% of all B2 monoclonal protein cases (n=150) had a ratio greater than the threshold. However, 1,947 cases negative for any monoclonal protein also had a ratio greater than the threshold. This produced a positive detection rate for B2 monoclonal proteins of 7.2%. Setting the threshold to 1.20 flagged 85.5% of all B2 monoclonal protein cases (n=141) as well as 156 negative cases. This produced a positive detection rate of 46.5%. Increasing the threshold to 1.20 considerably improved the likelihood of finding a B2 monoclonal protein by reducing the number of false negative cases flagged for further investigation. However, 24 B2 monoclonal protein cases had a B2/B1 ratio less than 1.20 and would be missed for further investigation. Similarly, 15 B2 monoclonal protein cases had a ratio less than 1.00 (the manufacturer recommended threshold). To detect 100% of B2 monoclonal proteins by capillary electrophoresis only, the positive detection rate would be 1.2% which is not a sustainable approach to screening for monoclonal proteins.

Conclusion: In a community population, setting the B2/B1 ratio to 1.20 for further investigation of suspected hidden B2 monoclonal proteins will help identify B2 monoclonal proteins at a detection rate of 46.5% without compromising patient care. This is a considerable increase compared to a threshold of 1.00 where the detection rate was 7.2%, flagging an excessive amount of false negative cases.

A-032

Performance Evaluation of an Automated Assay for the Measurement of Calcitonin on the Siemens ADVIA Centaur® Immunoassay Systems

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Background: Calcitonin, a 32-amino acid polypeptide secreted by the C cells of the thyroid gland, reduces calcium levels in the blood. Calcitonin is used as an aid in the diagnosis and treatment of diseases involving the thyroid and parathyroid glands, including carcinoma and hyperparathyroidism. The objective of this study was to develop and evaluate the initial analytical performance of a calcitonin assay* on the ADVIA Centaur® XP Immunoassay System (Siemens Healthcare Diagnostics Inc.).

Methods: A sandwich immunoassay is being developed using direct chemiluminescent technology for the quantitative measurement of calcitonin in human serum and plasma. Calcitonin is bound to mouse monoclonal anti-calcitonin antibody-coated particles and is then detected by an acridinium-labeled anti-calcitonin mouse monoclonal

Fab. Following a wash stage and magnetic separation, acidic and basic reagents are added to the reaction mixture and the resulting chemiluminescence is measured. Assay performance was evaluated for precision, linearity, limit of quantitation (LoQ), and method comparison to the cobas e 411 assay (Roche Diagnostics). The method comparison study was performed per CLSI EP-09-A3 using 107 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively.

Results: Observed reportable range of the prototype Calcitonin assay was up to 2000 pg/mL without dilution or up to 200,000 pg/mL with automated or manual dilution. The assay demonstrated linearity up to 2000 pg/mL. The limit of quantitation was observed at 1.74 pg/mL, with a total error of 20%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 2.6-4.4%. Passing-Bablok procedure comparison of the Calcitonin assay to the cobas e 411 assay using 107 patient samples gave a slope and intercept of 0.98 and 0.50 pg/mL, respectively, with a correlation coefficient (r) value of 0.99.

Conclusions: The feasibility of an automated Calcitonin assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of calcitonin.

*Assay under development by Axis Shield Diagnostics Inc for Siemens Healthcare Diagnostics Inc. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

A-033

A biomarker discovery platform for non-serous ovarian cancers using integrative proteomics

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Background: Ovarian cancer (OvCa) is not a single disease but rather, several subtypes including serous, endometrioid (EC), clear cell (CC) and mucinous (MC) carcinoma. In fact, each subtype represents a distinct disease that differs in etiology, progression and treatment response. While timely and accurate diagnoses are critical to patient management, the current markers (CA125 and HE4) are specific for serous OvCa and perform poorly for the non-serous subtypes. As such, biomarkers with the ability to definitively diagnose the non-serous subtypes are sorely needed. **Methods:** Tissues from EC (n=7), CC (n=6), and MC (n=7) were compared to their respective controls - endometriosis and benign endometrium (n=8) for EC and CC; mucinous cystadenoma (n=6) for MC - using 2D-offline liquid chromatography tandem mass spectrometry on the Q Exactive Plus. MaxQuant was used for protein identification and quantification while statistical and bioinformatics analyses were performed using Perseus and various proteomic and transcriptomic databases. To identify high-priority candidates, a scoring algorithm was developed based on five criteria: (1) method of identification; (2) statistical metrics; (3) tissue specificity; (4) transcriptomic annotation; and (5) cellular localization. **Results:** Approximately 10,000 unique proteins were identified in total, with roughly 7000 proteins being identified within each patient cohort. Overall, the expression profiles of EC and CC were associated with endometriosis and benign endometrium while those of MC were associated with mucinous cystadenoma. Using a scoring algorithm (Figure 1), high-priority candidates were identified for each subtype. Several high-priority candidates have shown strong associations to their respective subtypes, such as napsin A for CC and meprin A for MC. **Conclusions:** We have identified high-priority biomarker candidates for non-serous subtypes with the use of a novel scoring algorithm. Several of the candidates have shown strong relevance to their respective subtypes, demonstrating the utility of the biomarker discovery platform for subtype-specific biomarkers.

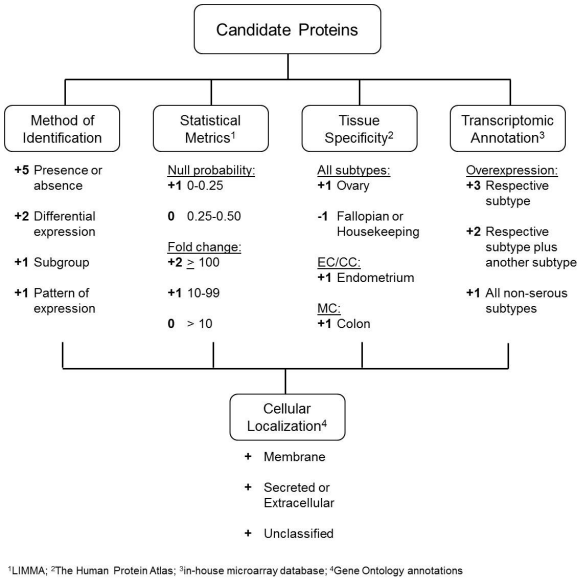

Figure 1 – Scoring algorithm for candidate proteins.

A-034

Diagnostic Performance of Serum Dickkopf-1 in Egyptian Patients with HCV Related Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most common cause of cancer deaths worldwide. In Egypt, HCC was reported to develop in about 5% of patients with chronic liver disease. Early detection of HCC is critical as poor prognosis is correlated with diagnostic delay. Serum α-fetoprotein (AFP) is the tumor marker universally utilized for HCC, however studies revealed its poor sensitivity and specificity for proper surveillance and diagnosis. Therefore, additional and more sensitive diagnostic tools are needed. Dickkopf-1 (DKK-1) was considered a diagnostic and prognostic biomarker in multiple cancers. DKK-1 is a secretory antagonist of canonical Wnt signalling pathway which is hardly expressed in normal tissues except in placental and embryonic. DKK-1 could serve as a tumor suppressor or metastasis promoter. Recently, it was reported to be involved in HCC migration and invasion.

The aim of the present work was to evaluate the serum level of DKK-1 in patients with chronic HCV related liver cirrhosis with HCC and to compare it with its level in chronic HCV related liver cirrhosis without HCC. The diagnostic performance of DKK-1 was also evaluated.

Methods: 80 subjects were included in the present study and were divided into 3 groups: Group I (Control group) included 20 apparently healthy volunteers, Group II included 30 patients with HCV related liver cirrhosis and Group III included 30 patients with HCC on top of HCV related liver cirrhosis. Child-Pugh classification, Abdominal US, Triphasic CT and determination of serum levels of AFP and DKK-1 were done.

Results: The median value of AFP was significantly higher in patients with chronic HCV related liver cirrhosis with HCC compared to the other two groups (p=0.001). DKK-1 showed a significant increase in chronic HCV related liver cirrhosis with HCC when compared to chronic HCV related liver cirrhosis without HCC (p=0.001). There was a significant decrease in DKK-1 in chronic HCV related liver cirrhosis with HCC when compared to the control group (p=0.001). Receiver operating characteristics (ROC) curve showed that AFP surpassed that of DKK-1 in the diagnosis of chronic HCV related liver cirrhosis with HCC when compared to the control group with diagnostic sensitivity 83.33%, specificity 90%, positive predictive value 92.6% and negative predictive value 78.3%. While DKK-1 showed diagnostic sensitivity 60%, specificity 95%, positive predictive value 94.7% and negative predictive value 61.3%.

Also, when comparing the diagnostic performance in chronic HCV related liver cirrhosis without HCC with chronic HCV related liver cirrhosis with HCC, there was superior performance for AFP with diagnostic sensitivity 73.33%, specificity 76.67%, positive predictive value 75.9% and negative predictive value 74.2 %. While DKK-1 showed diagnostic sensitivity 56.67%, specificity 70.0%, positive predictive value 65.4% and negative predictive value 61.8%.

Conclusion: Serum AFP is better than serum DKK-1 in the diagnosis of HCC in patients with chronic HCV related liver cirrhosis due to its higher diagnostic sensitivity and specificity. Further studies are needed to indicate if serum DKK-1 can be used as tumor biomarker and to address its role in diagnosis and prognosis.

A-036

Expression of miRNAs Dysregulated by Human Papilloma Virus 16 E5, E6, E7 Oncoproteins in Cervical Carcinogenesis

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Background: Cervical cancer is the third most common malignancy in women worldwide. Almost all cervical cancers are associated with human papilloma viruses (HPV) but the majority of women with HPV do not develop cervical cancer. This study is aimed to find the microRNAs (miRNAs) as an explanatory variable and early tumor marker in cervical carcinogenesis.

Methods: We analyzed the miRNAs expression profiles in 12 cervical tissues by NanoString nCounter system miRNA Assay (813 miRNAs panel) using the digital multiplexed counting method. Significant miRNAs were selected based on arbitrary |fold change| ≥ 2 and p-value ≤ 0.01 using R software (v3.1.1). To validate selected significant miRNAs, thirty-four cervical tissues performed real-time RT-PCR using the miScript II RT Kit (Qiagen, Hilden, Germany) and the StepOnePlusTM Real Time PCR System (Applied Biosystems, CA, USA) using 2X QuantiTect SYBR Green PCR Master Mix (Qiagen). The data were analyzed using the StepOne software v2.2.2 (Applied Biosystems). The expression levels of each miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with endogenous control small RNA, U6.

Results: Six genes (miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p) were expressed significantly higher and two genes (miR-597-5p, -655-3p) were expressed lower in HPV16-positive cervical cancer group than the HPV16-positive normal group and pooled normal group (HPV-negative and HPV16-positive) in clinical tissues experiments.

Conclusion: Eight dysregulated miRNAs in cervical cancer will be possible early diagnostic biomarkers for women with HPV16 to predict cancer development. Especially, the miR-9-5p and miR-199b-5p might be expected most significant among them considering with previous reports consistent with our results.

A-039

Normal reference range of PIVKA-II in Chinese population: preliminary data from the Asian multiple-centers study

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Background

Clinical application of protein induced by vitamin K absence or antagonist-II (PIVKA-II) has been widely used in Japan for a couple of years while it's still new to other Asian countries and needs more evidence supports in other population. From the preliminary reports, the normal reference ranges vary between Europe (95% confidence interval, CI: 11.12-32.01 mAU/ml) and Japan(95% confidence interval, CI: 17.36-50.90 mAU/ml). It indicated the ethical variation in different regions. Previously, we initiated an Asian study on the application of PIVKA-II in HCC diagnosis and differential diagnosis, including China, Singapore, Vietnam and Thailand. Here we report the preliminary results of the normal reference range from three sites in China.

Methods

A total of 309 cases of healthy subjects were recruited from routine health check subjects with a complete health examination results from three sites (Peking University First Hospital, Renji Hospital and Xi'an Jiaotong University First hospital) in China. Participants (aged 18-85 years) were with normal liver/kidney function and normal results for routine blood tests/urinalysis, and were excluded with liver disease history, kidney disease history or cancer history of any organ system. The study received ethical approval from the sites.

PIVKA-II levels were examined by the ARCHITECT PIVKA-II assay (Abbott, USA).

Results

of the PIVKA-II distribution is concentrated between 15.41 and 40.01mAU/ml. Among the 309 subjects, PIVKA-II mean level is 25.46 mAU/ml. All 95% reference ranges are estimated by nonparametric method. The 95% reference range of PIVKA-II is 14.26-40.44 mAU/ml. While those of individual centers are 13.68-38.77 mAU/ml (Beijing), 15.96-41.71 mAU/ml (Shanghai) and 14.07-41.76 mAU/ml(Xi'an) respectively .

Conclusions

This is the first multiple-centers study that released the normal reference range of PIVKA-II (14.26-40.44 mAU/ml) in Chinese population to support the local application. However, this is only the preliminary data and more information is still under investigation.

Table.1 Reference Range in Chinese population.

	95% Reference Range
All Centers (n=309)	14.26-40.44
Beijing (n=120)	13.68-38.77
Shanghai(n=112)	15.96-41.71
Xi'an(n=77)	14.07-41.76

A-040

A new panel of SNPs to assess thyroid carcinomas risk: a pilot study in a brazilian admixture population

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Thyroid cancer (TC) is the most common endocrine malignancy, and its incidence rate has been appreciably increasing over the last few years. Hence, if the observed trends are maintained, TC will replace colorectal cancer as the fourth leading cancer diagnosis by 2030 in the United States. According to the Brazilian National Cancer Institute, about 6.960 new cases of this disease were expected in Brazil in 2016 [4]. In addition, the state of Rio Grande do Norte has an estimated incidence of 2.270 new cases, second only to the Southeast region of Brazil. In this study, we aimed to analyze the possible association of 45 single-nucleotide polymorphisms (SNPs) with this cancer in a population from Rio Grande do Norte, Brazil. Considering that the population of this region is characterized by a marked ethnic mixture, this study may significantly contribute to elucidation of molecular basis underlying both predisposition to TC and the effect of interbreed populations on SNP-Based Association Studies.

To this end, 90 thyroid carcinoma samples were collected from the biobank at the Laboratory of Pathology of Liga Norte Riograndense Contra o Câncer. Then, patient's samples were genotyped using the MassARRAY platform (Sequenon, Inc) followed by statistical analysis employing the SNPassoc package of R program. In addition, the genotypic frequencies of all 45 SNPs obtained from the International HapMap Project database and based on data from ancestral populations of European and African origin, were used to compose the control study group. In our study, eleven SNPs were excluded from the analysis because they were not in Hardy-Weinberg equilibrium ($p<0.05$). Another eleven SNPs showed significant differences in their frequency when compared the study and control groups: rs3744962, rs258107, rs1461855, rs9993140, rs4075022, rs9943744, rs4075570, rs2356508, rs17485896 and rs2651339. Furthermore, polymorphisms rs374492 C/T, rs258107 C/T and rs4075022 C/T were associated with a relative risk for thyroid carcinoma of 3.78

(p= 0.0000627), 2.91 (p= 0.00008272) and 2.35 (p= 0.002011), respectively. Thus, these three polymorphisms could be potential biomarkers of predisposition to thyroid carcinoma in the population of Rio Grande do Norte. Furthermore, they may be considered suitable molecular marker for early diagnosis of the disease. In addition, SNPs rs1461855 and rs2356508 might be possibly associated with a protective effect against TC development. However, complementary studies with a control group constitute by samples obtain from healthy subjects from Rio Grande do Norte state should be conducted to confirm these results.

A-041

Identification of a Portuguese founder mutation in *BRCA2* gene in two Brazilian women

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Background: *BRCA1* and *BRCA2* are tumor suppression genes associated with a large number of DNA repair pathways. Alterations in these genes can lead to an improper DNA repair, increasing the probability to develop several cancers types, e.g. breast, ovarian, prostate, pancreatic cancers and melanoma. Hereditary breast and ovarian cancer (HBOC) diagnosis is performed mainly by the identification of heterozygous germline pathogenic variants in *BRCA* genes. Different methodologies can be used for variant screening such as next-generation sequencing, Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). MLPA assays are used for the detection of deletions and duplications, and some specific rearrangements. **Objective:** This report describes two independent cases of a *BRCA2* founder mutation identified in Brazilian women by MLPA. **Methodology:** Genomic DNA was extracted from blood samples of the patients using QIAsymphony (QIAGEN). Gene deletions and duplications analysis were carried out using SALSA MLPA probemixes P002 BRCA1 and P045 BRCA2/CHEK2 (MRC-Holland). **Results:** A pathogenic rearrangement in *BRCA2* was identified in both patients: c.156_157insAlu. This mutation has been first described in a Portuguese family as an Alu insertion at codon 52 of *BRCA2* and is considered as a founder mutation of the Portuguese population, mostly originated from central/southern Portugal. This insertion leads to exon 3 skipping and an in-frame deletion of amino acids 23 to 105 of BRCA2 protein. **Discussion:** Alu elements are considered pathogenic because of their capacity to alter the genome. The exon 3 skipping due to an Alu insertion is important since this exon was reported to encode a transcriptional domain that interacts with the EMSY protein which is involved in chromatin remodeling. The c.156_157insAlu BRCA2 mutation is one of the most common variant identified in Portuguese HBOC families, responsible for more than half of all germline deleterious *BRCA2* mutations observed. However, it is still rare in Brazilian population. Investigation of this rearrangement in suspected HBOC families from countries with Portuguese ancestry (e.g., Brazil, Angola and Mozambique) or with a large community of Portuguese immigrants is strongly recommended. **Conclusions:** Screening of founder mutations in other populations allows evaluation of whether or not it is a population-specific mutation. Furthermore, analysis of founder mutations decreases genetic testing cost since it is more cost-effective to test only for some variants prior the screening of whole gene, making it possible to use more specific approaches for molecular testing.

A-042

Impact of NGS four-gene panel in screening genes with potentially therapies in NSCLC

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Background: Targetable genomic mutation detection has innovated personalized medicine in non-small cell lung cancer (NSCLC) mainly through detection of *EGFR* activation mutations, which predicts susceptibility to tyrosine kinase inhibitors (TKIs). Although patients render positive response to TKIs initially, resistance could eventually arise, due to the secondary mutation T790M, therefore decreasing patient's survival and limiting treatment options. NSCLC patients harbor a variety of mutated genes, that could potentially be treated with drugs already approved, or in ongoing clinical trials for other types of tumor. *KRAS* is one of the most frequently mutated genes in NSCLC and could act as a new target for lung cancer treatment. Although no targeted therapy has yet been approved for mutated-*KRAS*, there are multiple potential treatment strategies under investigation. *BRAF* is another gene that offers a rational therapeutic strategy, since its pathway plays a role in the carcinogenesis of NSCLC. **Objectives:** Determine the prevalence of *KRAS*, *BRAF* and *EGFR* among patients with next generation sequence technology and reveal the importance of multi-gene panel screening. **Methodology:** We used a custom multigene panel for Ion Platform to investigate *EGFR*, *KRAS* and *BRAF* mutations in 491 NSCLC Brazilian patients. Genomic DNA libraries were prepared from FFPE derived DNA and sequenced in the Ion PGM. **Results:** *KRAS* was the most frequently mutated gene, found in 27.6% of all samples, thus could be an appealing target for new therapy strategies as described in other studies. 111 (82.2%) samples harbored mutation in codon 12, being G12C mutation the most common one, present in 37.8%. The second most prevalent mutated gene was *EGFR* with 119 (24.3%) mutated samples. The most common mutations were exon 19 deletions (most frequently E746-A750), found in 42% of the samples and L858R, found in 32% of the samples. *BRAF* displayed prevalence rates of 3.7%. V600E mutation was present in 55.5% of the *BRAF* mutated samples, followed by mutations in codon G469 (22%). We identified 17 double and coexisting mutations. *EGFR* T790M was the most common secondary mutation, found in six (12%) samples concomitantly with p.E746_A750del, indicating that these patients might have a reduced response to TKIs therapy. Interesting is the fact that three of these double mutant samples had prior cobas® *EGFR* Test results from 2-3 previous years with absence of T790M mutation, indicating that resistance may have arrived in a 2 to 3 years life span. Although, *EGFR* gene mutations are reported as mutually exclusive with *KRAS* or *BRAF* mutations in lung cancer, we found three samples harboring concomitant *EGFR* and *KRAS* mutations. Previous studies also reported that mutations in *KRAS* and *BRAF* are mutually exclusive, but we found one sample harboring mutations in both genes, indicating that this is a rare event. **Conclusions:** This study shows that development of future therapies, targeting *KRAS* should be prioritized. Although *BRAF*-mutated samples displayed short prevalence, it could be a potential target among wild-type *EGFR* and *KRAS* cancer patients. This study shows the importance of multi-gene panel screening to reduce overall turnaround time generating results in a single test.

A-043

Conventional Cytogenetics Analysis in Multiple Myeloma in a Routine Laboratory

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Background: Multiple myeloma (MM) is characterized by the accumulation and proliferation of malignant plasma cells, secreting monoclonal immunoglobulins. Genetic abnormalities are powerful prognostic factors in MM for risk stratification and therapy strategies. The standard diagnostic tests to detect genetic abnormalities in MM include Conventional Cytogenetic Analysis (CCA) and Interphase Fluorescence *In Situ* Hybridization (FISH). The interpretation of cytogenetic abnormalities is complicated by the number and complexity of the abnormalities and the methods used to detect them.

Objective: In the present study, we investigated the frequency of chromosomal abnormalities (CA) in samples of patients with MM in a private laboratory on CCA.

Methods: We performed CCA in 474 samples of patients with diagnostic hypothesis that included multiple myeloma, monoclonal gammopathy and/or monoclonal peak, between 2015-2016 in a private laboratory. Bone marrow samples were cultured according to cytogenetics protocols. The karyotype analysis was reported according to ISCN 2016.

Results: Among 474 samples of MM patients, 451 (95%) had normal karyotype and 24 (5%) had cytogenetic abnormalities. The abnormalities were: Complex karyotype, t(1;19)(p22;p13), trisomy 8, t(7;13)(p15;q14), t(6;7)(p21;q22), del 9q, del 11q23, trisomy 12, del 20q, del 13q, monosomy Y and X. Of the 474 samples, the percentage of plasma cells could be done in 292 samples by myelogram. 87 samples (30%) presented more than 10% of plasma cells and 86 (almost 30%) presented less than 1% of plasma cells.

Conclusion: Cytogenetic abnormalities in MM affect every aspect of the disease, from evolution of the malignancy to clinical presentation, response to therapy and prognosis. For clinical purposes, cytogenetics in MM can be assessed by metaphase karyotyping through CCA or by FISH. Metaphase cytogenetics requires proliferating cells and is not sensitive for the detection of either primary or secondary cytogenetic abnormalities in MM. Further, any prognostic impact that is seen with a metaphase detected abnormality is probably not due to that abnormality per se but simply a reflection of the fact that patient had a more aggressive disease. In general, metaphase cytogenetics are mainly useful to determine the presence of myelodysplastic syndrome

that may occur during the course of the disease secondary to therapy. In fact, from twenty cytogenetics abnormalities we found seven samples with complex karyotype, two samples with deletion 11q23 and one with 20q deletion. Most of our karyotypes (95%) did not presented any abnormality. In fact, in newly diagnosed MM, the abnormal plasma cells have a low proliferative activity, and the analyzable metaphase spreads from CCA are derived from normal hematopoietic cells, thus resulting in a normal karyotype. In our analysis, 30% of our samples showed more than 10% of plasma cells and 30% had less than 1%. Cytogenetics assessment of MM is essential for clinical practice, and the importance of this evaluation is indicated by recent incorporation of high-risk cytogenetics abnormalities into Revised International Staging System for MM. However, the conventional technique have limitations which can be overcome partly by the incorporation of FISH analysis of interphase nuclei.

A-044

Alteration of Serum CA125 and HE4 levels in Response to Chemotherapy in Patients with Different Histological Subtypes of Ovarian Cancer

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Background: Serum CA125 is used to monitor response to chemotherapy, relapse, and progression in ovarian cancer patients. Recently, HE4 has been used in combination with CA125 to assess the risk of ovarian malignancy. This study aims to evaluate the change of these biomarkers in response to chemotherapy in patients with different histological subtypes of ovarian cancer.

Methods: 11 ovarian cancer patients with subtypes of serous (5), clear cell (2), mucinous (1) carcinoma, and carcinosarcoma (3) were enrolled in this study. All patients received postoperative adjuvant paclitaxel/carboplatin chemotherapy for up to 6 cycles. No recurrence of ovarian cancer was identified during the course of this study. Serum CA125 and HE4 collected at pre-surgery (baseline) and after each cycle of chemotherapy were concurrently measured using immunochemiluminometric assay (Abbott Architect). The reduction% was calculated as change of CA125 and HE4 from basal level. The overall %reduction was the mean of the reduction% of all cycles for each patient.

Results: Abnormally elevated CA125 and/or HE4 baselines were observed in all patients, except that two normal CA125 baselines were found in one patient with clear cell carcinoma and another with carcinosarcoma, and four normal HE4 baselines were seen in one patient with serous carcinoma, two with clear cell carcinoma, and one with mucinous carcinoma. The overall %reduction of CA125 is significantly higher than that of HE4 in all ovarian cancer patients ($P<0.05$), regardless of their subtypes. Over 50% reduction of CA125 after 2nd cycle of chemotherapy was observed in all subtypes and was stable for up to 6th cycle. The similar reduction pattern for HE4 was only found in serous carcinoma and carcinosarcoma. In contrast, patients with either clear cell or mucinous carcinoma and normal HE4 baseline displayed an increase or a bidirectional alteration in HE4 value, which was inconsistent with the unidirectional decrease of CA125 during the chemotherapy, and might be due to biological variations.

Conclusion: Our data demonstrate consistently large reduction (>50%) of CA125 after successful chemotherapy in all subtypes of ovarian cancer, and indicate that serum CA125 might outperform HE4 as biomarker to monitor the response to chemotherapy in ovarian cancer patients, especially for serous and clear cell carcinoma. However, additional studies with a larger sample size are needed to achieve a definite conclusion.

A-045

Association of INDEL polymorphisms with Colorectal Cancer risk and prognostic follow-up in Brazilian population

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Background: Colorectal cancer (CRC) is the third most common cancer type in men and the second in women. Despite the effective strategies for prevention, early detection, and treatment, there are ethnic differences in CRC incidence and survival. These variances occur specifically in African Americans, who have higher CRC incidence and lower survival rates than other ethnic groups. Thus, in this study was to perform the association between 16 INDEL polymorphisms with CRC risk and prognostic follow-up in an admixture population.

Methods: A total of 280 participants were enrolled in the study, which 140 were diagnostic with CRC and 140 were free-cancer subjects. The polymorphisms and ancestry distribution were genotyped by Multiplex-PCR reaction, separated by capillary electrophoresis on the ABI 3130 Genetic Analyzer instrument and analyzed in GeneMapper ID v3.2. Furthermore, considering that ancestry distribution in an admixed population might influence cancer development susceptibility and affect the polymorphism distribution, all polymorphism analysis were adjusted according the individual proportions of European, African, and Amerindian genetic ancestries.

Results: The logistic regression analysis showed that INDEL polymorphism variations in *ACE* (rs4646994), *IL4* (rs79071878), and *TYMS* (rs151264360) genes were associated with CRC risk. Reference to anatomic localization of tumor Del allele of *NFKB1* (rs28362491) and *CASP8* (rs3834129) were associated with more incidents to colon than rectosigmoid. In relation to the INDEL association with TNM stage risk, the Ins allele of *ACE* (rs4646994), *HLAG* (rs371194629) and *TP53* (rs17880560) were associated with higher TNM stage. Furthermore, regarding INDEL association with relapse risk, the Ins allele of *ACE* (rs4646994), *HLAG* (rs371194629), and *UGT1A1* (rs8175347) were associated with relapse risk, as well as the Del allele of *TYMS* (rs151264360). About INDEL association with death risk, the Ins allele of *SGSM3* (rs56228771) and *UGT1A1* (rs8175347) were associated with death risk.

Conclusion: In summary, in this study, we showed that INDEL polymorphisms in ACE, TYMS, IL4, NFKB1, CASP8, TP53, HLAG, UGT1A1, and SGSM3 might be useful as cancer panel to determine CRC risk and prognostic follow-up, even as auxiliary to the better clinical management.

A-046

ARCHITECT NSE: AN ASSAY FOR MEASUREMENT OF HUMAN NEURON-SPECIFIC ENOLASE

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Background: Neuron-specific enolase (NSE), a glycolytic enzyme (2-phospho-D-Glycerate hydrolase), can be found in a variety of non-neuroendocrine cells (European Group on Tumor Markers, Lung Cancer Guidelines, 2012). NSE is a marker in the management of small cell bronchial carcinoma (Larmerz, 1998). An assay for NSE on ARCHITECT *i* System (ARCHITECT NSE) is being developed, and analytical performance is being presented. **Methods:** The ARCHITECT NSE (in development) is a one-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of NSE in human serum. Determination of NSE is used for monitoring response to therapy and detection of recurrent disease, such as SCLC and neuroendocrine tumors. The specimen is incubated with paramagnetic microparticles coated with the monoclonal antibody (mAb) NSE21, and acridinium-labeled mAb NSE17 conjugate on the ARCHITECT® *i* System. After incubation and washing, Pre-trigger and Trigger are added. The resulting relative light units (RLUs) are directly proportional to the amount of NSE in the sample, allowing for the quantitative determination of NSE in serum. **Results:** The calibration range for the assay is 0.0 to 400.0 ng/mL. The limit of blank (LoB) and limit of detection (LoD) were 0.04 and 0.16, ng/mL, respectively. The limit of quantitation (LoQ) at 20% CV was 0.35 ng/mL. Linearity was demonstrated for a range of 0.8 through 650.8 ng/mL. A Precision study of 3 controls and 4 panels spanning the range of the assay demonstrated a total %CV $\leq$ 3% at all levels. In the sample tube type study, a matrix comparison of 30 matched serum and SST samples were evaluated. A Passing-Bablock slope of 1.01 and r of 0.99 was observed when evaluating samples within the measurement range for serum separator tubes compared to the Red Top serum samples. Six (6) endogenous substances were evaluated for interference in the ARCHITECT NSE assay. The average percent difference between test and control samples for all endogenous interferents was < 10%. Percent difference in the presence of twenty (20) potentially interfering drugs was $\leq$ 4.0%. The potential cross-reactant alpha-enolase was evaluated at a concentration of 900 ng/mL and the percent cross-reactivity was $\leq$ 2%. **Conclusion:** The ARCHITECT NSE assay under development demonstrates a sensitive and precise assay for the quantitative determination of NSE in human serum.

A-047

ARCHITECT Tg: AN ASSAY FOR MEASUREMENT OF HUMAN THYROGLOBULIN

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Background: Circulating levels of thyroglobulin (Tg) are critical in management of patients with differentiated thyroid cancer (DTC), and are measured following thyroidectomy or radioactive iodine ablation (NACB, 2002). ARCHITECT Thyroglobulin (ARCHITECT Tg) is an immunoassay that determines circulating levels of thyroglobulin. **Methods:** The ARCHITECT Tg assay (in development) is a delayed one-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of thyroglobulin in human serum or plasma on the ARCHITECT *i* System. The assay is intended to aid in monitoring DTC patients in the absence of anti-Thyroglobulin autoantibodies. The method utilizes paramagnetic microparticles coated with a highly specific monoclonal antibody (mAb) which capture the Tg in the specimen. After incubation, the acridinium-labeled anti-Tg mAb conjugate is added to complete sandwich format. After another incubation and wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of Tg in the specimen and the RLUs detected by the ARCHITECT *i* System optics. **Results:** The ARCHITECT Tg assay has been standardized against the European Community Bureau of Reference (BCR) CRM 457. The calibration range for the assay is 0 to 500 ng/mL. The limit of blank (LoB) and limit of detection (LoD) were estimated to be 0.026 and 0.048 ng/mL, based on guidance from CLSI EP17-A2. The limit of quantitation (LoQ) defined as the lowest concentration observed at ≤ 20%CV was determined to be 0.072 ng/mL. The assay demonstrated linearity from 0.08 to 521.6 ng/mL. The 20-day precision study tested with 8 samples (3 controls and 5 panels) showed a total within-laboratory imprecision of ≤ 0.013 ng/mL for samples with Tg ranging from 0.1 to 0.8 ng/mL, and ≤ 5%CV for samples above 0.8 ng/mL. Method Comparison to the Roche Cobas Tg II assay demonstrated a linear correlation with a slope of 0.99 and a correlation coefficient of 1.00 (n = 208). In a tube type equivalency study, serum tube was compared to six other tube types including serum separator tube, EDTA and heparin plasma tubes. Passing Bablock slopes ranged from 1.00 to 1.05, and r values were all 0.99. Seven endogenous substances and 15 potentially interfering drugs were evaluated for interference in the ARCHITECT Tg assay. The average percent difference between test samples and control ones for all interferents evaluated was ≤ 10%. The potential cross-reactants TSH and TBG were evaluated at concentrations of 1000 mIU/L and 200,000 ng/mL, respectively, with the percent cross-reactivity ≤ 5%. **Conclusion:** The ARCHITECT Tg assay under development demonstrates a sensitive and precise assay for the quantitative determination of Tg in human serum and plasma that meets the American Thyroid Association guidance for management of Thyroid Nodules and differentiated thyroid cancer.

A-048

Clinical Significance of Pancreatic Cyst Fluid CEA Level

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Background: Measurement of pancreatic cyst fluid (PCF) CEA level is an important first line test to determine mucinous cysts and risk for pancreatic cancer. We have reviewed our large PCF experience (n=11,222) to better understand the predictive value of CEA determination.

Methods: PCFs grouped according to CEA (ng/ml) as follows: very low (VL), 0-4.99 (n=1914), low (L), 5.0-191.9 (n=4326), high (H), 192.0-999.9 (n=2524), very high (VH), over 1000.0 (n=2458). Each group was assessed for mucinous cyst formation based on the presence or absence of 1) gross fluid viscosity, 2) cytologic evidence of stainable mucin, and KRAS/GNAS oncogene point mutational change. Each group was assessed for malignancy risk in each group was evaluated using an established four tier risk classifier based on integrated molecular pathology (IMP) characteristics (Endoscopy, 2015:Feb;47(2):136-42. doi: 10.1055/s-0034-1390742. Epub 2014 Oct 14.).

	Benign	stat indolent	Low risk	stat higher risk	aggressive	High risk	cyto mucin	grossly viscous	KRAS/GNAS
CEA<5 (n=1914)	1713	132	1845 (96.4%)	67	2	69 (3.6%)	194/1424 (13.60%)	276/1728 (16.00%)	98 (5.10%)
CEA 5-192 (n=4326)	3309	777	4086 (94.5%)	234	3	237 (5.5%)	718/3140 (22.90%)	1219/4014 (30.40%)	1570 (36.30%)
CEA 192-1000(n=2524)	1642	638	2280 (90.3%)	235	9	244 (9.7%)	510/1745 (29.20%)	857/2373 (36.10%)	1250 (49.50%)
CEA>1000 (n=2458)	1026	750	1776 (72.2%)	627	55	683 (27.8%)	632/1759 (35.90%)	949/2279 (41.60%)	1263 (51.40%)
Low risk combines benign and stat indolent categories, high risk combines higher risk and aggressive categories									
Assume n is same as in column A unless otherwise stated (see columns H and I)									

Results: Cytologic mucin ranged from 13.6%-VL to 35.9%-VH. Grossly viscous fluid ranged from 16.0%-VL to 41.6%-VH. Positive oncogene mutations ranged from 5.1%-VL to 51.4%-VH. Each of the three methods to affirm mucinous cyst formation was negative in majority of CEA subsets. High risk for malignancy was detected in 3.6%-VL, 5.5%-L, 9.7%-H and 27.8%-VH. Low risk, benign disease was seen in the majority of all CEA subsets, including patient with highly elevated levels. **Conclusion:** While progressive levels of PCF CEA are more likely to be associated with mucinous cyst formation, the majority of mucinous cysts prove to be negative. More than half of cases with PCF-VH are negative for mucin by cytology or gross fluid viscosity. Similarly, while malignancy risk increased with CEA elevation, the vast majority of PCF samples were low risk notwithstanding elevated CEA levels. However, the risk for malignancy nearly triples from PCF-H to PCF-VH, whereas incidence of KRAS/GNAS is nearly the same in both categories.

A-049

Personna Onco: a multi-cancer panel for mutational screening in a Brazilian cohort

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Background: Next-Generation Sequencing (NGS) technology revolutionized the medical diagnosis field through concomitant analysis of multiple regions/genes and several individuals in a single experiment. This is currently allowing the transition from traditional to precision medicine, leading to a more accurate cancer diagnosis and enabling the best selection of molecular target drugs for individual treatments. **Objective:** The aim of this study was to survey the spectrum and prevalence of variants in solid tumor samples using Oncomine™ Focus Assay (Thermo Fisher Scientific), a multi-biomarker NGS assay for the detection of relevant alterations (hotspots, SNVs, indels, CNVs and gene fusions) in 52 oncogenes associated with different solid tumors. **Methodology:** Formalin-Fixed Paraffin-Embedded (FFPE) samples were obtained from 362 patients (186 males and 176 females) and clinical-pathological data were obtained from pathologist/physician responsible for each patient. Genomic DNA and total RNA were extracted from all samples. Libraries were prepared and sequenced on Ion Torrent PGM sequencer. Sequencing data was analyzed using Ion Reporter software integrated with Oncomine® Knowledgebase. **Results and Discussion:** A total of 106 patients (29.3%) did not present any variants and only 13 patients (3.6%) showed inconclusive results due to poor DNA/RNA quality and/or quantity. These patients were excluded from further analysis. In general, 68 CNVs, 36 fusion genes and 245 point mutations/indels were observed for the remaining patients (n=243). The most frequent tumor type observed was lung cancer (46.5%) showing *EGFR* (34.5%) and *KRAS* (31%) as the most frequently altered genes. These mutations are. *KRAS* G12 hotspot (22.1%: p.G12C - 8.0%, p.G12D - 7.1%, p.G12V - 4.4%, p.G12A - 1.8%, p.G12F - 0,9%), *EGFR* p.E746_A750del (6.2%) and *EGFR* p.L858R (6.2%). Other observed tumors were colon, breast, bladder, ovary, liver, uterus, brain, skin and bone. Interestingly, this panel allowed the identification of not commonly observed variants in these tumors such as ovary adenocarcinoma *PIK3CA* p.M1043I, moderately differentiated colon adenocarcinoma *FGFR4* amplification, lung adenocarcinoma *BRAF* p.G469A and *ERBB2* amplification. All these alterations were previously associated with drug response for other cancer types. **Conclusions:** This study demonstrated that a multi-cancer panel plays an important role for clinicians and patients allowing the identification of multiple druggable variants with approved therapies, potentially improving patient outcomes and increasing the chances of getting the best treatment. In addition, the generation of large-scale results of variants in tumors contributes to the description of mutations classified as non-pathogenic and that may eventually become driver mutations.

A-050

Driver mutations in *EGFR* gene identified in tumor cell-free DNA: application of liquid biopsy for use tyrosine kinase inhibitors in non-small cell lung cancer patients

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Background: Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers in world wide. The 5-year survival rate in NSCLC patients is less than 5% thus development of strategies to improve the treatment and survival of these patients is necessary. Activating mutations of epidermal growth factor receptor (*EGFR)* occur in approximately 15% in Caucasian and approximately 30% in Brazilian NSCLC. The EGFR tyrosine kinase inhibitors (TKIs) have been widely used for advanced NSCLC patients and liquid biopsies using plasma-derived cell-free DNA (cfDNA) is a non-invasive test allowing a better selection and monitoring of them. **Objective:** The aim of this study was evaluate the frequency of *EGFR* mutations in cfDNA before and after TKIs treatment in a cohort of NSCLC Brazilian patients submitted to liquid biopsy. **Methods:** Mutations were investigated by Real Time qPCR Cobas® EGFR Mutation Test v2. Clinical data were obtained prior blood collection. Samples were collected at Progenetica Laboratory or sent from other laboratories, centrifuged and plasma were immediately frozen. Fisher´s exact test was calculated to compare frequency distribution between two groups (GraphPad Prism version 6.00). **Results and Discussion:** This study enrolled 535 NSCLC Brazilian patients from Southeast+South (74.9%), North+Northeast (8.8%), and Midwest+Federal District (4.7%). Regional information was missing for 11.3% of cases. NSCLC were more frequent among women (56.4%) compared to men (43.6%). Four-hundred cases (74.8%) not showed any *EGFR* mutations, however 135 cases presented 19Del (57.8%), L858R (16.3%), double 19Del; T790M (11.1%), double T790M; L858R (8.15%), G719X (2.9%), L861Q (1.48%), 20Ins, triple L858R; T790M; 20Ins and S768I; G719X (0.74% each one) mutations spanning 18, 19, 20, and 21 *EGFR* exons. Previous results from liquid biopsy for *EGFR* mutation were obtained for 75/535 cases (14%). Among them, 10/75 cases (13.3%) presented non-detected mutations. Deletions at exon 19 (19Del) and mutations at exon 21 (L858R) were frequently observed accounting for 61.5% and 23%, respectively. Since 19Del and L858R are drug-sensitive mutations we compared the frequency of *EGFR* mutations in patients before and after TKIs treatment. Interestingly, patients harboring these mutations before the treatment were negative for the presence of 19Del (37.5%) and L858R (66.7%) after the treatment, respectively ($p<0.0001$). In addition, *EGFR* T790M mutation already associated with drug-resistance was identified in 3/65 (4.6%) of cases in double with 19Del before the TKIs treatment and one patient was negative and two patients remaining positive for both mutations after treatment, respectively. **Conclusion:** These results showed that liquid biopsy is a powerful tool for personalized medicine for early detection, stratification and monitoring of *EGFR* druggable mutations in cfDNA from NSCLC patients. To our knowledge this is the first report showing the application of liquid biopsies for management of NSCLC patients in Brazil. Clinical data collection is undergoing for a better reliability results. cfDNA has been related to aggressiveness and poor prognosis of NSCLC patients and not all cases present tumor cfDNA. High index of non-detected results was observed at this study however new Next-Generation Sequencing and digital PCR protocols are under development to improve the sensitivity of this test.

A-051

A case of the invisible IgM

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Background: Protein electrophoresis is performed to detect and quantify monoclonal proteins (M-protein) used for diagnosis and monitoring of patients with monoclonal gammopathies. In rare cases, the M-protein can be missed due to the properties of the different types of immunoglobulins, specifically IgM. The objective of this project is to present a case in which M-protein (IgM of 6g/L) using gel electrophoresis was not observed in a 71 years old male patient previously diagnosed with monoclonal gammopathy of undetermined significance.

Methods & Results: Serum and urine protein gel electrophoresis (Sebia Hyadrasys; Hydragel) were requested by the physician. Initial serum protein electrophoresis (SPE) result was unremarkable other than a low background in the gamma region. Serum IgG (2.37g/L; RI: 6.94-16.18g/L) and IgA (0.19 g/L; RI: 0.7-4.0g/L) concentrations were low, whereas IgM was increased (11.57g/L; RI: 0.6 - 3.0 g/L) using immunoturbidimetry (Siemens ADVIA). Urine protein electrophoresis (UPE) (Sebia Hyadrasys; Hydragel) demonstrated a large band in the near gamma region, which was confirmed by urine immunofixation electrophoresis (IFE) as a free kappa light chain. In addition, free light chain (FLC) assay demonstrated abnormally elevated free kappa light chain (182.5 mg/L; RI: 3.3 - 19.4 mg/L). Due to inconsistent results between SPE and UPE, other tests (immunoglobulin quantitation, FLC assay and a serum IFE) were ordered by the laboratory. Similar to the initial SPE results, no distinct band was visible in IFE. After treating the samples with beta-mercaptoethanol or Fluidil to break the disulfide bonds, a distinct band of M-protein of IgM-kappa quantifying at 6 and 5 g/L, respectively was noted. **Conclusion:** The characteristic large size and viscous nature of the pentameric IgM poses challenges for the laboratory to accurately detect the presence of IgM M-protein. In the testing laboratory, mechanisms were in place to alert for any potential false results from SPE, including keeping a laboratory record of all myeloma patients, recognizing the common ordering physicians (i.e. hematologist), and determining any inconsistencies between the related test results. The falsely normal SPE result was initially missed because of the fact that a normal globulin level did not trigger further investigation. However, the recognition that a normal total globulin measurement was inconsistent with a suppressed SPE background prompted additional exploration. Due to the potential of false SPE results in IgM M-protein, additional methods such as UPE, FLC assay, capillary electrophoresis, immunoglobulin quantitation, total protein and globulin should be used in conjunction to interpret monoclonal protein.

A-052

Extended Stability of Free and Total PSA and f/t PSA Ratio% in Frozen Male Serum

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Background: The long term stability of free and total PSA and the f/t PSA Ratio% in male serum stored continuously at -80ºC beyond 9 years has yet to be proven. This study, conducted in 2017, retesting never thawed frozen serum aliquots from the same subjects first collected and tested in 2001 demonstrates stability at -80ºC for each analyte and the ratio for at least 15.5 years. This extended stability allows the performance of important clinical and analytical studies using existing specimens collected around the turn of the last century

Methods: Blood was collected (1999 - 2001) from 174 subjects (50 - 75 years of age) prior to DRE/biopsy during prostate cancer screening. Serum was prepared and kept refrigerated until frozen in 0.5 mL aliquots at -80ºC within 36 hours of collection. In 2001, free and total PSA assays were performed on the Siemens Dimension RxL. Aliquots were thawed immediately prior to testing. The f/t PSA ratio% calculated for each specimen. 15.5 years later, the same instrument was used to measure free and total PSA and calculate f/t PSA Ratio% on freshly thawed aliquots of the same 174 specimens.

Results: y = tPSA 2017 and x = tPSA 2001, $y = 0.9685 + 0.9299\ x$, $R^2 = 0.9929$; y = fPSA 2017 and x = fPSA 2001, $y = 0.0388 + 0.9908\ x$, $R^2 = 0.9931$; y = f/t PSA% 2017 and x = f/t PSA% 2001, $y = 0.0075 + 0.9885\ x$, $R^2 = 0.9714$.

Conclusion: Free and total PSA concentrations, as well as f/t PSA Ratio%, in serum are not affected significantly by continuous storage at -80ºC for at least 15.5 years.

Free and Total PSA Stability Over 15.5 Years at -80 Degrees C

Statistic	tPSA 2001 ng/mL	tPSA 2017 ng/mL	Ratio tPSA 2017/2001	fPSA 2001 ng/mL	fPSA 2017 ng/mL	Ratio fPSA 2017/2001	f/t PSA% 2001	f/t PSA% 2017	Ratio f/t PSA% 2017/2001
Mean	16.32	16.15	**1.03**	1.76	1.70	**0.96**	0.13	0.12	**0.94**
Standard Deviation	14.58	13.61	**0.09**	2.66	2.64	**0.10**	0.10	0.10	**0.10**
Minimum	0.42	0.38	**0.79**	0.05	0.04	**0.65**	0.02	0.02	**0.64**
Maximum	75.04	70.07	**1.29**	30.18	29.98	**1.36**	0.76	0.84	**1.36**
Range	74.62	69.69	**0.51**	30.13	29.94	**0.71**	0.74	0.82	**0.72**

A-055

Sensitive and Specific Detection of Variants in Circulating Tumor DNA by Anchored Multiplex PCR and Next-Generation Sequencing

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Introduction: Liquid biopsies are a promising, minimally invasive alternative to tissue biopsies that have potential cost, time and safety benefits, as well as a greater ability to interrogate heterogeneous tumors. However, except in advanced disease states, cell free DNA (cfDNA) is typically of low abundance and only a small portion of cfDNA originates from tumor cells as circulating tumor DNA (ctDNA), which tends to be highly fragmented (100-300bp). Therefore, NGS-based assays to detect variants in ctDNA must be sensitive enough to detect mutations at allele frequencies (AF) <2% from <100ng of highly fragmented DNA.

Methods: We developed the Archer® Reveal ctDNA™ 28 assay based on Anchored Multiplex PCR (AMP™), a target enrichment method for NGS that uses unidirectional gene-specific primers and molecular barcoded (MBC) adapters for amplification. AMP is well suited to amplify small cfDNA fragments, as it only requires one intact primer-binding site within a fragment. Single primers capture target regions from both strands independently, increasing the sensitivity of variant detection from low-input samples. MBC adapters ligated prior to amplification permit post-sequencing error correction, reducing background noise and increasing analytical sensitivity of ultra low-allele frequency variant detection. Finally, variant filtering in the Archer Analysis pipeline further increases the specificity of variant calls.

Results: Using commercially available reference ctDNA standards, we demonstrate that genomic DNA present in plasma does not significantly impact amplification of small, fragmented ctDNA with the AMP-based Reveal ctDNA 28 assay. Based on sequenced reads, AMP enabled interrogation of more than 65% of the input molecules from 50ng starting material. As a result, we show 100% detection sensitivity for 1% AF variants using 10ng DNA input and 71.9% detection sensitivity for 0.1% AF variants using 50ng DNA input. MBC-enabled post-sequencing error correction and variant filtering reduced the number of false positives by 98%, resulting in 91.7% specificity. Finally, mutations detected from liquid biopsy-derived ctDNA showed cancer type-dependent concordance with tissue biopsy findings, and revealed additional oncogenic driver mutations.

Conclusions: The Archer Reveal ctDNA 28 assay is a powerful tool for sensitive and specific NGS-based detection of variants in ctDNA, demonstrating accurate allele frequency quantification of synthetic reference standards. This assay is a promising approach to characterize solid tumors from liquid biopsies, showing cancer type-dependent concordance of tissue and plasma mutation profiles, as well as identification of additional oncogenic driver mutations in ctDNA.

A-056

Development of ctDNA Reference Materials in a Human Plasma-EDTA Matrix with low interfering endogenous DNA

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Background: Liquid biopsy is a novel, non-invasive method for identifying biomarkers present in circulating tumor DNA (ctDNA). Recent ctDNA detection technologies could potentially revolutionize early cancer detection and therapeutic monitoring in clinical laboratory medicine. However, with the advent of a breakthrough technology comes an equally important need for novel reference materials and quality assessment schemes to ensure quality test implementation. Development of ctDNA reference materials is challenging due to the presence of endogenous, uncharacterized DNA normally present in pooled human plasma. In this study, we developed ctDNA reference materials based on the highly characterized NIST Genome in a Bottle GM24385 in low interfering endogenous DNA human plasma-EDTA.

Methods: Plasma was collected from long term donors and screened for the presence of blood borne pathogens and several cancer mutations. Single or double EGFR/KRAS/NRAS cancer hotspot mutations at 10%, 5%, 1% and 0% allelic frequencies in a background of the NIST genomic DNA was fragmented and spiked into the plasma. The allelic frequency was determined using BioRad® droplet digital PCR and fragment size distribution was analyzed on Bioanalyzer and TapeStation; all tests were performed in triplicate, and the mean and standard deviation were calculated.

Results: Plasma screened negative for all infectious agents tested as well as for cancer mutants described above. Allelic frequencies showed no difference between pre- and post-spiking into plasma, and the observed frequencies were at 10% error of the target frequencies. These data suggest that interfering endogenous plasma DNA levels were negligible, which enabled precise control of allelic frequencies and controlled definition of gDNA background. Notebly, fragmented ctDNA demonstrated a length of 162 ± 2.5 bp on Bioanalyzer, but was 205 ± 2.6 bp on TapeStation, suggesting a need for further nucleic acid sizing technology standardization.

Conclusion: A novel method for producing ctDNA controls has been developed that enables preparation of a multitude of target mutations at a wide range of frequencies with a known gDNA background. Simpler QC materials mimicking patient samples will enable simpler ctDNA test method development and analytical validation, which will be critical for laboratories to introduce ctDNA testing into the field.

A-057

Analytical validation of a blood-based colorectal cancer and advanced adenoma risk assessment LDT

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Background: Colorectal cancer (CRC) is the second leading cause of cancer-related death in men in the United States and the third leading cause in women. With a 5-year survival rate of 90% for early-stage detection versus 13% for late-stage detection, early detection of CRC is critical to reducing associated mortality. Despite the importance of early detection, only ~60% of individuals who would benefit from screening are actually tested. The low compliance rate to colonoscopy and fecal-based screening suggests the need for an alternate blood-based test as a means to improve uptake. We describe here the analytical performance validation of a 15-analyte multiplexed assay that measures protein levels in plasma as a CRC and advanced adenoma (AA) risk assessment to symptomatic patients.

Objective: This poster describes the analytical validation of a Lead Development Test (LDT) multiplexed immunoassay as required by CAP/CLIA and performed in accordance with CLSI guidelines.

Methods: A 5-panel electrochemiluminescent (ECL) multiplexed immunoassay measuring 27 CRC-related proteins was developed on the Meso Scale Discovery (MSD) U-PLEX platform. A discovery and validation study using this 5-panel assay and a large patient population (n=4,435) with symptoms of colorectal neoplasia, revealed a 15-analyte CRC and AA classifier with CRC clinical sensitivity and specificity of 80% and 83%, respectively. The analytical performance of the 15-analyte (4-panel) classifier was further validated in accordance with CAP/CLIA requirements to demonstrate analytical performance and clinical accuracy following CLSI guidelines. A minimum of 74 plasma samples spanning the clinical reportable range (CRR) for each analyte were used to demonstrate assay ruggedness, sample stability, intra-day precision and inter-day relative accuracy. The specificity of each analyte was evaluated using plasma samples spiked with common endogenous interferants. Both the reportable and reference range for each analyte was established, prior to demonstrating clinical accuracy of the 4-panel multiplexed assay using a symptomatic patient cohort.

Results: Deming regression and Pearson correlation coefficients were used to assess the precision and accuracy of each sample measurement across assay runs. Analysis of intra-day precision and inter-day accuracy were within acceptable limits (Correlation coefficient 0.82 - 0.92) for all analytes. Assay specificity was acceptable for all analytes in the presence of a range of bilirubin and intralipid levels and only one analyte displayed interference in grossly hemolytic samples. The dynamic range of each assay spanned 2-3 logs with the LOD 1 to 4-fold below the CRR. Ruggedness was found to be acceptable across all instrument systems tested as measured by Deming regression and Pearson correlation. Sample stability was validated for up to six freeze-thaw cycles for 3 panels and up to five freeze-thaw cycles for the 4th panel. A Clinical accuracy study using the analytically validated multiplexed assay demonstrated greater than 80% concordance with clinical calls.

Conclusion: Analytical validation demonstrates a high quality, low-burden LDT for evaluating CRC and AA risk in a symptomatic patient population. The ability to evaluate CRC risk with the ease of a blood draw provides an important tool in helping to improve CRC screening compliance rates.

A-058

Troponin I levels as an early biomarker for cardiotoxicity after chemotherapy with doxorubicin in women with breast cancer

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Background: Cardiovascular diseases in cancer patients are frequent events due to chemotherapy, which has resulted in increased survival of patients but exposes them to cardiotoxicity. The aim of this study was to evaluate the use of Troponin I (TnI) as a marker to identify early cardiotoxicity in women with breast cancer under chemotherapy with anthracycline doxorubicin.

Methods: This study included 76 women (49.8 ± 11.3 years old) with breast cancer that received neoadjuvant chemotherapy with 372 mg/m^2 mean cumulative dose of doxorubicin, divided in 3-4 cycles. The serum TnI levels were evaluated before the chemotherapy and immediately after the treatment. We used immunometric immunoassay kit. The sensitivity was 95% and the specificity was 93%, intra and inter-assay coefficient of variation were 4.2 and 8.3%, respectively.

Results: No subject presented significant decline in left ventricular ejection fraction evaluated trough echocardiogram. However, the median (interquartile-range) levels of TnI before treatment [0.012 (0.002) ng/mL] was significantly lower than TnI levels observed after chemotherapy [0.028 (0.013) ng/mL] ($p<0.0001$). **Conclusion:** The elevation in TnI levels observed in this group suggest increased risk of myocardium injury after chemotherapy with doxorubicin. The use of TnI may be important as a biomarker for early intervention for cardiotoxicity progression in patients with breast cancer receiving anthracycline therapy.

A-059

Genetic variants in the vascular endothelial growth factor pathway as potential markers of ovarian cancer risk, therapeutic response, and clinical outcome

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Background: As a key regulator of angiogenesis, vascular endothelial growth factor (VEGF) plays an important role in physiology of normal ovaries and pathogenesis of ovarian cancer, such as tumorigenesis and metastasis. The objective of this study was to assess the association of genetic variants in the VEGF pathway with ovarian cancer risk, therapeutic response, and survival. **Patients and methods:** A cohort of 339 ovarian cancer patients matched with 349 healthy controls by age, gender, and ethnicity were tested for single nucleotide polymorphisms (SNPs) in VEGF and VEGF receptor (VEGFR) genes using the Illumina iSelect platform. The statistical analyses were performed using Intercooled STATA software. P-value ≤ 0.05 was considered significant. The overall risk of ovarian cancer and likelihood of poor treatment response were estimated as odds ratios (OR) with 95% confidence intervals (95% CI) for each SNP using unconditional multivariate logistic regression. The overall risk of death was estimated as hazard ratios (HR) and 95% CIs for each SNP using the Cox proportional hazards model. **Results:** 16 SNPs from 5 genes in the VEGF-VEGFR axis were identified as significantly associated with an increased risk of ovarian cancer. Among which, VEGFR-3 rs6877011 showed the highest risk of ovarian cancer (OR, 1.66; 95% CI, 1.09-2.53), while VEGFR-1 rs11149523 showed the lowest risk (OR, 0.51; 95% CI, 0.32-0.83). 10 SNPs from 4 genes were identified as significantly associated with platinum-based chemotherapeutic response. Among which, VEGFR-2 rs1531289 was associated with a favorable treatment response (OR, 0.64; 95% CI, 0.42-0.98), and all the other variants showed a significant association with a poor chemotherapeutic response. In particular, VEGFR-1 rs8000288 showed the highest risk of poor chemotherapeutic response (OR, 4.25; 95% CI, 1.10-16.43). 12 SNPs from 4 genes were identified as significantly associated with the overall survival of ovarian cancer patients. Among which, fms like tyrosine kinase 3 (Flt3) rs3003955 was the most significant variant associated with improved prognosis (HR, 0.59; 95% CI, 0.41-0.84), while VEGFR-1 rs17626553 was the most significant one associated with poor prognosis (HR, 4.07; 95% CI, 1.86-8.92). **Conclusions:** Multiple genetic variants in the VEGF pathway significantly associated with ovarian cancer risk, therapeutic response, and survival are identified in our study. These findings may provide a potential molecular approach for ovarian cancer risk assessment, patient management and clinical outcome prediction.

A-061

A new blood test for colorectal cancer in high-risk subjects

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Background

Colorectal cancer (CRC) is a broadly occurring and lethal cancer. CRC outcome dramatically improves with early detection and curative resection; thus CRC screening is recommended for U.S. patients over 50 years old. The gold standard screening test is colonoscopy, with some stool-based tests also having good performance. However, compliance with CRC screening recommendations is low; only about 60% of the over-50 population undergoes testing.

A low-burden CRC test for non-compliant patients, such as a blood-based test, has been widely sought. Signal may be stronger in patients with developed disease, such as those with symptoms of colorectal neoplasia, than in average risk patients. If so, the appearance of symptoms would offer an opportunity to provide low-burden CRC testing with higher performance; the results could be used to direct only the highest risk patients to colonoscopies.

Objective

The objective was to develop a blood-based CRC test with clinically useful performance in patients with CRC symptoms. This was achieved by building a classifier using samples from a truly representative intent-to-test (ITT) symptomatic population, without filtering based on sample type.

Methods

Subjects. 4,435 patient samples were drawn from the *Endoscopy II* sample set. Samples were collected at seven hospitals across Denmark between 2010 and 2012 from subjects with symptoms of colorectal neoplasia: abnormal bowel habits, abdominal pain, rectal bleeding, unexplained weight loss, meteorism, anemia, and/or palpable mass. Colonoscopies revealed the presence or absence of CRC.

Candidate biomarkers. 27 blood plasma proteins were selected as candidate biomarkers based on a previous targeted-mass spectrometry study. Multiplexed immunoassays were used to measure the concentration of these 27 proteins in all 4,435 samples. Age and gender were also considered biomarkers.

Classifier discovery and validation. 3,066 patients (340 CRC and 2,759 non-CRC) were randomly assigned to the classifier discovery set. Machine learning was used to build and test candidate classifiers in this set. A grid approach was used to examine many combinations of data pre-treatment, predictor selection methods, predictor numbers, and classifier algorithms. For each combination, 10-fold cross-validation was used to assess diagnostic performance. Some classifiers were refined by allowing up to a 25% indeterminate score range. The classifier that gave the best performance was selected for validation.

The remaining 1,336 samples (147 CRC and 1,189 non-CRC) were assigned to the validation set. The classifier selected during discovery was applied to these samples for final performance characterization.

Results

The final classifier was a logistic regression using 10 predictors : eight proteins, age, and gender. In validation, the indeterminate rate was 23.2%, sensitivity/specificity was 0.80/0.83, the PPV was 36.5%, and the NPV was 97.1%. This performance compares favorably to that from other CRC blood tests.

Conclusions

The validated classifier presented here can serve as the basis for a blood-based CRC test for symptomatic patients. Results from a test using this classifier can help assess symptomatic patients' CRC risk, increase their colonoscopy compliance, and manage next steps in their care.

A-062

The Potential for Ubiquitin Occupancy Profiles in Cancer Biomarker Screening

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Background: Post-translational modification (PMT) of oncoproteins can provide a detailed profile of abnormal proliferative signaling in cancer. The pattern of disease specific PTMs can serve as a biomarker for screening, diagnosis and treatment assessment. Ubiquitin PTM is primarily responsible for protein degradation by the 26S proteasome and impaired ubiquitin signaling has been implicated in numerous cancers. Ubiquitination also has non-degradation regulatory functions that contribute

to the overall ubiquitin profile of cells and can be utilized in diagnostic screening. This discovery study examines ubiquitin occupancy, the presence or absence of ubiquitin modification, across the proteome of an ovarian carcinoma cell line. In addition to providing a unique ubiquitin occupancy profile this project has led to the discovery of novel ubiquitination sites in the HER2 oncoprotein. The primary objective of this project is to utilize stable isotope labeling by amino acids in cell culture (SILAC) and LC-MS/MS to characterize the ubiquitin occupancy profile of SKOV-2 ovarian carcinoma cells regardless if the PTM mediates protein degradation or is involved in other regulatory functions. The second aim of study focuses on the novel ubiquitination sites identified in HER2 and addresses their specificity to ovarian cancer.

Methods: SKOV3 cells are cultured in RPMI media with 10% FBS (Light) and SILAC RPMI media containing $^{13}C_6$-L-lysine and $^{13}C_6{}^{15}N_4$-L-arginine (Heavy) media supplemented with 10% FBS. Light cells are treated with MG132 proteasome inhibitor as well as DMSO (control). Both Light and Heavy cells are harvested 6 hours post-treatment and lysed in urea buffer. Protein concentration is determined for each sample and the Heavy and Light lysates are combined at a 1:1 ratio for each treatment. The samples then undergo reduction and alkylation, followed by trypsin digestion, and offline basic reversed phase (bRP) fractionation (Global samples). A sub-faction of the peptides undergoes further K-ε-GG ubiquitin remnant motif peptide enrichment, following the initial bRP fractionation step (Ubiquitin-enriched samples). Samples are subjected to LC-MS/MS using an Orbitrap mass spectrometer, and protein identification and quantification are conducted using MaxQuant. Additionally HER2 co-immunoprecipitation is used to identify changes in HER ubiquitination and examine HER binding-proteins under varying treatment conditions.

Results: Proteasome inhibition by MG132, shows overall enhanced protein up-regulation in Global and Ubiquitin-enriched samples compared to DMSO. Ubiquitin occupancy at all protein sites identified in the proteome is assessed and four HER2 ubiquitinated peptides exhibit a significant increase with proteasome inhibition. Close to 45% of all ubiquitinated peptides do not exhibit increased levels with MG132 inhibition, indicating ubiquitin modification at these lysine residues serves a non-degradation function. Ubiquitin occupancy is assessed by comparing ubiquitin-enriched and global peptides and provides a means of differentiation between protein targets modified by ubiquitin for turnover and those ubiquitinated for trafficking or other non-proteasome signaling.

Conclusion: Protein ubiquitination in SKOV3 ovarian carcinoma cells can be classified into degradation and non-degradation signaling functions. The ability to distinguish these proteins by SILAC-based quantitative proteomics allows

assessment of ubiquitin occupancy and generates a cell specific ubiquitin profile that can be utilized in assessing abnormal proliferative signaling.