**Category:** Biomarker **Color:** Green **Poster Board #:** 18

# $^{18}$F-Fluciclovine Detection of Primary Prostate Cancer at the Sextant Level and Correlation of Maximum Standardized Uptake Value (SUVmax) with Gleason Grade.

Jed Kendall, Olayinka A. Abiodun-Ojo, Akinyemi Akintayo, Mehrdad Alemozaffar, Dattatraya Patil, Faisal Saeed, Adeboye Osunkoya, Martin Sanda, David Schuster

Emory University, Atlanta, Georgia

**OBJECTIVE**: To evaluate the reliability of $^{18}$F-fluciclovine PET uptake parameters in predicting tumor presence and Gleason grade at the sextant level in patients with primary prostate cancer.

**METHODS**: Twenty-nine surgically eligible patients with primary prostate cancer were diagnosed using standard of care prostate-specific antigen (PSA) and 12-core transrectal prostate biopsy. Prior to prostatectomy, the patients were evaluated with $^{18}$F-fluciclovine PET/CT and each prostate lesion identified was noted. Furthermore, the maximum standardized uptake value (SUVmax) from each prostate sextant was recorded. Following each prostatectomy, the entire prostate was submitted for histologic assessment. Every tumor-containing prostate sextant was assigned a post-surgical Gleason grade. $^{18}$F-Fluciclovine PET findings were compared with the post-surgical pathology results of each prostate. Analysis of variance (ANOVA) was used to determine correlation between $^{18}$F-fluciclovine SUVmax of prostate lesions and post-surgical Gleason grade per sextant level. Sextants with less than 10% tumor volume per histologic analysis were excluded from analysis.

**RESULTS**: $^{18}$F-Fluciclovine was able to predict the presence of prostate tumor per post-surgical pathological sextant analysis with 85.4% sensitivity, 80.6% specificity, 95.3% PPV, and 54.3% NPV. Prostate sextants with higher Gleason grades demonstrated a higher mean SUVmax. Furthermore, ANOVA analysis demonstrated statistical significance ($p < 0.001$) between mean SUVmax and Gleason grade. However, the SUVmax per Gleason grade demonstrated a high degree of variability.

**CONCLUSIONS**: $^{18}$F-Fluciclovine is able to detect the presence of primary prostate cancer with accurate localization to the sextant level making $^{18}$F-fluciclovine PET/CT a valuable pre-surgical planning tool. Furthermore, $^{18}$F-fluciclovine SUVmax correlates significantly with Gleason grade group of primary tumors.

**Source of Funding**: NIH U01-CA113913-11, Blue-Earth Diagnostics.

**Table 1**. Diagnostic performance of fluciclovine primary prostate cancer detection at the sextant level.

| | Fluciclovine |
|---|---|
| True positive (n) | 123 |
| False positive (n) | 6 |
| True negative (n) | 25 |
| False negative (n) | 21 |
| Sensitivity (%) | 85.4 |
| Specificity (%) | 80.6 |
| Positive Predictive Value (%) | 95.3 |
| Negative Predictive Value (%) | 54.3 |

**Table 2**. Correlation between fluciclovine uptake in prostate lesions and sextant Gleason grade group.

| Post-surgical Gleason grade group | Sextants (n) | SUVmax (mean ± SD) |
|---|---|---|
| 1 | 8 | 4.61 ± 0.85 |
| 2 | 26 | 5.65 ± 1.05 |
| 3 | 25 | 6.67 ± 1.78 |
| 4 | 11 | 7.56 ± 1.71 |
| 5 | 39 | 7.85 ± 1.94 |

**Category:** Technology **Color:** Red **Poster Board #:** 4

# Enlarged stromal macrophages found in blood of cancer patient blood are associated with more aggressive disease and poorer prognosis in localized stage I/II solid tumors

Authors: Daniel L. Adams[1], Steven H. Lin[2], Raymond C. Bergan[3,4], Rebecca L. Aft[5], Sreeraj G. Pillai[5], Mark A. Watson[5], Cha-Mei Tang[6], Jeffrey R. Marks[7]

[1] Creatv MicroTech, Inc., Monmouth Junction, NJ,[2]MD Anderson Cancer Center, Houston, TX, [3]OHSU Knight Cancer Institute Portland OR, [4] University of Nebraska Medical Center, Omaha, NE, [5]Washington University School of Medicine, St. Louis, MO, [6]Creatv MicroTech, Inc., Potomac, MD, [7]Duke University Medical Center, Durham, NC

**Background:** Using peripheral blood allows for the recovery of various cancer associated circulating stromal cells, including a previously described phagocytic cell defined as a Cancer Associated Macrophage-Like cell (CAML). CAMLs are phagocytic myeloid cells that derive from an immunological response to tumor presence and emanate from primary tumors. These phagocytic events by CAMLs can be easily quantified by CAML engorgement which has been described by numerous groups as an indicator of poorer clinical outcomes in a variety of cancer types. Using a filtration platform we analyzed the peripheral blood of untreated newly diagnosed breast, prostate and lung cancer patients (n=107) for CAMLs to determined their sensitivity and clinical prediction in localized disease.

**Methods:** A group of single blind multi-institutional prospective study using anonymized peripheral blood was undertaken with written informed consent and with approval of local IRBs. Blood was taken from 107 cancer patients after confirmation of localized solid malignancy [stage I (n=43) or stage II (n=64)] all with pathologically confirmed breast (n=25), lung (n=40), prostate (n=42)]. CAMLs were isolated from whole peripheral blood by the CellSieve™ microfiltration technique and defined as enlarged, multinuclear cells with cytokeratin and/or CD45/CD14. Presence of enlarged CAMLs was then used to evaluate relapse-free survival (RFS) and overall survival (OS) hazard ratios (HRs) by censored univariate analysis at 5 years.

**Results:** CAMLs were found in 83% of all cancer patients regardless of stage, averaging 2.5 CAMLs/7.5mL blood. Specifically CAMLs were found in 72% of Stage I and 91% Stage II disease. In total, 30 patients relapsed and 24 died within the 5 year study. After 5 years, patients with ≥50um CAMLs were found to have had significantly worse clinical outcomes compared with patients with no, or <50um CAMLs, with RFS (HR=9.6 CI95% 3.7-24.7, p<0.00001) and worse OS (HR=6.8 CI95% 2.52-18.8, p=0.00054).

**Conclusions:** We present the results of a combination of prospective studies in three of the most common types of localized cancer. These data suggest that engorged CAMLs, a Circulating Stromal Cell subtype, found in the blood of cancer patients at diagnosis is highly prognostic for presence of highly aggressive tumors likely to relapse within 5 years of diagnosis.

**Category:** Technology **Color:** Red **Poster Board #:** 5

# Sequential Monitoring of PD-L1 on Circulating Stromal Cells Predicts Outcomes in Localized Unresectable Stage II NSCLC Treated with Immunotherapy after Definitive Chemoradiation

Authors: Daniel L. Adams[1], Alexander Augustyn[2], Jianzhong He[2], Yawei Qiao[2], Ting Xu[2], Zhongxing Liao[2], Kirby P Gardner[1,4], Jillian A. Moran[1,3], Cha-Mei Tang[5], Steven H. Lin[2]

[1] Creatv MicroTech, Inc., Monmouth Junction, NJ 08852

[2] MD Anderson Cancer Center, Houston, TX 77030

[3] Rutgers, the State University of New Jersey, NJ 08901

[4] Rutgers University, Graduate School of Biomedical Sciences, Piscataway, NJ 08854

[5] Creatv MicroTech, Inc., Rockville, MD 20850

## Abstract

**Purpose:** Previously we have reported on a type of tumor derived circulating stromal cell found in the blood of cancer patients (pts), described as phagocytic giant macrophages, which appear to represent the inflammatory state of the tumor microenvironment and have been defined as a circulating cancer associated macrophage-like cells (CAMLs). In non-small cell lung carcinoma (NSCLC), CAMLs appear to express PD-L1 that whose expression can dynamically change and be monitored throughout chemoradiation therapy (CRT). However, it is not known if PD-L1 on CAMLs has a relationship to immunotherapy (IMT) efficacy after CRT in local NSCLC. We studied a pilot group of Stage II unresectable NSCLC pts treated with (n=20) or without (n=20) IMT agents after CRT to evaluate CAML PD-L1 expression as it relates to progression free survival (PFS) & overall survival (OS).

**Material/Methods:** A multi-year single blind prospective pilot study was undertaken to test the relationship of PD-L1 expression in CAMLs, for NSCLC pts before and after induction of CRT. Patients were treated either with standard of care CRT with consolidation Durvalumab (n=14) or under a clinical trial treated with concurrent CRT with consolidation Atezolizumab (n=6). A control group patients treated with of CRT alone, absent of IMT (n=20), were evaluated during the same period (n=13 before 2018). In all, we recruited 40 pts with pathologically confirmed unresectable stage II NSCLC prior to CRT. Anonymized blood samples (15mL) were taken ~1 month after completion of CRT with written informed consent and with local IRB approval. Blood was filtered by CellSieve filtration & CAMLs quantified for PD-L1 expression using a binary score (0/1=low & 2/3=high), to evaluate PFS & OS hazard ratios (HRs) by censored univariate analysis at 24 months.

**Results:** CAMLs were found in 83% of all samples, on average 3.8 CAMLs/15mL. Tumor PD-L1 expression analysis was not possible as only n=11 tumor biopsies were available. In blood samples, after completion of CRT, high CAML PD-L1 in patients not treated with IMT was not prognostic for PFS (HR=1.6 CI95% 0.6-6.8 p=0.4760) nor OS (HR=2.2 CI95% 0.4-10.9 p=0.5814). However, in patients treated with CRT and IMT, high CAML PD-L1 patients trended non-significantly for improved PFS (HR=5.9 CI95% 1.2-29.7 p=0.0790) and OS (HR=15.5 CI95% 1.5-164.2 p=0.0931)

**Conclusions:** While a preliminary pilot study, these data suggests that in unresectable stage II NSCLC, high PD-L1 expression on CAMLs after CRT completion may identify patients that are more responsive to IMTs. Larger and more refined studies in the local NSCLC patient population are needed to validate these findings.

**Category:** Biomarker **Color:** Green **Poster Board #:** 9

**Nashville Lung Cancer Screening Trial among the Underserved**

Melinda C. Aldrich, PhD, Vanderbilt University Medical Center, Sanja Antic, MD, Vanderbilt University Medical Center, Subodh Nag, PhD, Meharry Medical Center, Eric L. Grogan, MD, Vanderbilt University Medical Center, Alex Miler, Vanderbilt University, Pierre P. Massion, MD, Vanderbilt University Medical Center

## Abstract

Low-dose computed tomography (LDCT) screening identifies lung cancer at an earlier stage of disease with better curative treatment options and survival. Yet lung cancer screening is greatly underutilized, with the most recent estimates showing only 16% of eligible individuals are screened nationally. Furthermore, current lung cancer screening studies are derived from mostly white and well-educated populations, despite evidence that smoking behaviors vary by race and socioeconomic status. We initiated a pilot screening study to address the lack of molecular and genetic strategies for the early detection of lung cancer among diverse populations.

We initiated recruitment of high-risk individuals from community clinics and patients seeking care at Meharry Medical College. Participants 55-80 years of age and current or former cigarette smokers with a smoking history of 30 pack-years or greater, and, if a former smoker, quit no more than 15 years ago. Participants undergo a clinic visit for sample collection, LDCT, and research bronchoscopy at baseline enrollment and prospectively over a 5-year period.

A total of 67 participants at high-risk for lung cancer have been enrolled to date. Participants are 60% male and 20% Black. We recruited 33% of our cohort from a historically black college, Meharry Medical College. The study population recruited from Meharry Medical College had a mean age of 61 years at enrollment and is 41% Black, 59% female, and mostly comprised of individuals who smoke (91%). Participants recruited from other referral sources are 11% Black, 31% female, and 76% current smokers. Ongoing clinical, imaging and biospecimen data are collected following standard procedures and stored in a biorepository for use by investigators.

We established an ongoing prospective screening study in partnership with community clinics and a historically black college to collected biospecimens from high-risk individuals often underrepresented in lung screening cohorts. Recruitment and follow-up are ongoing. The biorepository will allow the development of candidate biomarkers for lung cancer.

**Category:** Bioinformatics **Color:** Blue **Poster Board #:** 1

**Quantitative Characterization of Tumor Microenvironment**

Alphan Altinok, Ph.D., Jet Propulsion Laboratory
Krysten E. Vance, University of Nebraska Medical Center
Heather Jensen-Smith, Ph.D., University of Nebraska Medical Center
Michael A. Hollingsworth, Ph.D., University of Nebraska Medical Center
Luca Cinquini, Ph.D., Jet Propulsion Laboratory
Ashish Mahabal, Ph.D., Caltech
Daniel Crichton, Jet Propulsion Laboratory

## Abstract

The Tumor Microenvironment (TME) consists of all cellular and molecular components in and around a tumor. The TME has been shown to impact cancer cell proliferation, invasion, metastasis, chemoresistance, and to influence immune responses to cancer, so understanding the structural and functional characteristics of the TME is critical to developing new diagnostics and therapies for cancer patients. The TME is complex, consisting of vasculature, fibroblasts, macrophages, mast cells, basophils, eosinophils, B cells, T cells, and NK cells, along with multiple subtypes of these. Quantitative and spatial characterization of the TME from immunohistochemical analyses of tumor sections requires several post-imaging analysis steps.

Through the use of a multiplex-immunofluorescent technique, we iteratively stain a single slide with up to 34 different antibodies, characterizing the presence, position, and functional states of the antigens that comprise distinct cell types within the TME. Our analysis workflow consists of image registration, nuclei identification, tumor detection, and quantitation of different antibody reporters within the tumor and non-tumor (stroma) regions of tissue scans. Multiple stages of the analysis workflow leverage deep learning methods.

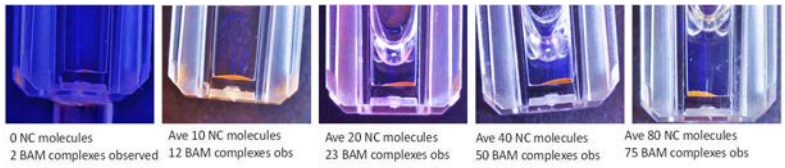
Revealing the structural and functional changes in the TME could significantly benefit from the research tools being built within the EDRN infrastructure. We plan to deploy the analysis pipeline on the JPL LabCAS system developed for the EDRN consortia for efficient scaling on the Amazon's cloud environment and for leveraging the ability to capture, manage, and support the analysis of cancer biomarker data, both raw and processed.

**Category:** Technology **Color:** Red **Poster Board #:** 2

**Rising to the Point of Care Separations and Diagnostics Challenge with Buoyancy And Magnetism (BAM)**

Prof. Jeffrey N. Anker, Ph.D., Clemson University; Chuanlei Wang, B.S., Clemson University; Meena Ranasinghe, B.S., Clemson University; Dr. Brandon McNaughton, Ph.D., Akadeum Life Sciences; Undergraduate Clemson students: Hanna Campbell, Kiersten Barnett, William Pons,

We are developing a rapid screening test that can detect the presence and concentration of SARS-CoV-2 in a patient's saliva using buoyant and magnetic microbeads. Both buoyant and magnetic microbeads are functionalized with antibodies that specifically target and bind to SARS-CoV-2 nucleocapsid protein present in a patient's saliva. When both types of microbeads bind to the same nucleocapsid protein, they form a buoyant and magnet (BAM) complex. These BAM complexes can be pulled to the bottom of a centrifuge tube or cuvette using a magnet and separated from buoyant but non-magnetic beads. Removing the magnet causes the BAM complexes to rise towards the surface, while the non-buoyant magnetic particles stay where they are or sink slowly. At high concentrations, the BAM complexes float to the meniscus, where they are visualized by eye as a brownish line from magnetic beads. At lower concentrations, individual BAM complexes can be observed with a simple digital camera as they rise due to the intense optical scattering of the buoyant beads and counted using motion tracking software. With a 40-minute protocol using 5 uL of artificial saliva spiked with fg/mL levels of nucleocapsid protein, we observe on average 1 BAM complex per molecule with a background of 0-2 BAM complexes with no nucleocapsid protein added (Figure 1). Monte Carlos molecular and bead diffusion simulations indicate efficient capture is due to high particle concentrations and efficient mixing via buoyancy. We also observe spontaneous dissociation of some of the non-specifically bound buoyant-magnetic beads due to the opposing forces between magnetic and buoyant beads. One of our goals is to optimize the technique for higher speed and specificity, even at the expense of sensitivity. Finally, we also applied similar approaches to specific capture and detection of O157 pathogenic E. coli with minimal non-specific capture S. aureus. Overall, these approaches offer a simple, inexpensive, almost equipment-free and scalable approach to sensitive and specific point of care diagnostics.



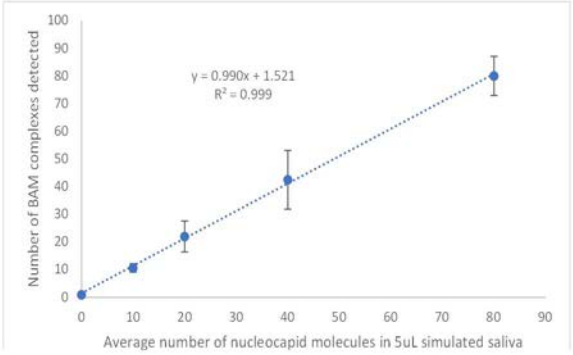




**Figure 1.** Detecting single SARS-COV-2 nucleocapsid molecules in 5 µL of simulated saliva. Photos show cuvettes with brown magnetic particle and superimposed tracks of rising BAM complexes colored blue. One BAM complex is observed per nucleocapsid molecule (plus a background of 0-2 BAM complexes).

**Category:** Biomarker **Color:** Green **Poster Board #:** 7

**Development of Immune Cell-Based Prognostic Signature for Pancreatic Cancer**

Pranita Atri, M.Tech., University of Nebraska Medical Center, Sophia Kisling, B.S., University of Nebraska Medical Center, Sunandini Sharma, M.S., University of Nebraska Medical Center, Lynette Smith, PhD., University of Nebraska Medical Center Sukhwinder Kaur, PhD., University of Nebraska Medical Center, Surinder K. Batra, PhD., University of Nebraska Medical Center.

## Abstract

**Background:** Pancreatic cancer (PC) remains one of the top causes of cancer-related deaths partly due to a lack of understanding of the disease's prognostic and biological heterogeneity. In this regard, the study of the immune system and its many regulatory roles is extremely important. While some studies have elucidated this role as a whole, the specific differences or "immune-signatures" that instill prognostic differences remain poorly understood. Although tools like flow cytometry have proven helpful, their dependence on cell integrity combined with the poor quality of collected samples poses a major challenge. To this end, computational tools like CIBERSORT pose an advantage; hence, the current study presents an in-silico prognostication method for PC.

**Methods:** Gene expression and survival data were curated from two independent patient cohorts (TCGA and GSE28735). Further, this curated patient cohort was then divided into two groups considering the median survival statistic: long-term survivors (LTS, survival >11.3 months) and short-term survivors (STS, survival <11.3 months). CIBERSORT analysis was then carried out comparing the relative abundance of 22 major immune cell types within these two groups. Furthermore, to establish an immune signature, the 547 genes LM22 signature from CIBERSORT was divided into genes with favorable outcomes (providing prognostic benefit to LTS) and genes with unfavorable outcomes. Additionally, a functional assessment of the identified prognostic markers and the associated genes was carried using gene ontology (GO) and gene set enrichment analysis (GSEA).

**Results and Conclusions:** CIBERSORT analysis led to the identification of specific differences in regulatory T-cells, gamma delta cells, natural killer cells, and M2 macrophages in the two STS and LTS prognostic groups. Using the LM22 gene signature, we could successfully identify separate 10 markers "gene-signatures" for each of these groups. Interestingly, unbiased functional assessment of these 10 markers and the associated gene network led to the identification of differential enrichment of immune-based pathways like a response to chemokines and lymphocyte-mediated immunity. Overall, this computational analysis has helped us identify specific immune markers with prognostic significance. Furthermore, this established gene signature has a direct application in clinical decision-making and response to immunotherapies. Current efforts are underway to validate these identified markers independently.

**Category:** Technology **Color:** Red **Poster Board #:** 7

**Optimizing Cancer Early Detection with Circulating Tumor DNA**

Stefano Avanzini, PhD, Stanford University, Alexander Gorelick, PhD, Stanford University, Giulia Simoni, PhD, Stanford University, Johannes G. Reiter, PhD, Stanford University

## Abstract

Early cancer detection aims to find tumors before they progress to an incurable stage. To determine the potential of circulating tumor DNA (ctDNA) for minimally-invasive cancer detection, we developed a stochastic mathematical model of tumor evolution and ctDNA shedding to predict the smallest size at which a tumor becomes detectable with high specificity. From data of 176 stage I-III lung cancer patients, we inferred that on average 0.014% of a tumor cell's fragmented DNA is shed into the bloodstream per cell death. We show how the observed variance in ctDNA shedding rates leads to large differences in tumor detection size. We will consider two clinically different scenarios: cancer screening and cancer relapse detection. For informed monthly relapse testing with a specificity of 99.5%, the model predicts a median detection size of 0.83 cm. For cancer screening with a test specificity of 99% and a sequencing panel that covers 2000 base-pairs, we find a median lung cancer detection size of 2.3 cm. We investigate how copy-number changes and methylation patterns could further increase the sensitivity for ctDNA-based cancer screening. Our new mechanistic framework can help to optimize ctDNA assays and accelerate clinical trials by identifying the most promising cancer early detection strategies.

**Category:** Technology **Color:** Red **Poster Board #:** 9

**Cell Acquiring Fallopian Tube Endoscope**

Richard Cordova[1], Kelli Kiekens[1], Shigehiro Yamada, Ph.D.[2], Ie-Ming Shih, M.D. [2], Jennifer Barton, Ph.D. [1]
[1]University of Arizona, [2]The Johns Hopkins University School of Medicine

**Abstract**

Endoscopic detection of serous tubal intraepithelial carcinoma (STIC) may enable minimally invasive ovarian cancer screening, raising 5-year survival rates to 95% or greater. We present the design, construction, and testing of a flexible and steerable 0.79-millimeter-diameter endoscope capable of both visualizing the fallopian tubes and collecting cells for subsequent analysis. With pseudo white light, multispectral reflectance, and multispectral fluorescence imaging, the Cell Acquiring Fallopian Endoscope (CAFE) could be used to navigate through the tubal lumen and locate sites of potentially pathological tissue indicated by an alteration in endogenous fluorescence, increased microvasculature, or the presence of a targeted fluorophore. A directed scrape biopsy of cells from regions of interest in the fallopian tube epithelium could then be obtained, and the cells collected by the device could be processed to yield biopsy material usable for downstream analyses and diagnosis of pathologies such as STIC. The CAFE utilizes laser sources at wavelengths of 405 nm, 488 nm, 520 nm, and 642 nm, which are multiplexed through a multimode fiber to provide illumination for images captured through a 3000-element coherent fiber bundle. Measured pseudo-white-light imaging resolution was 88 µm at a working distance of 5 mm, and the full field of view was approximately 45° in air. Reflectance and fluorescence images of *ex vivo* human and porcine reproductive tracts were captured. A Nitinol wire with a diameter of 178 µm was shaped for optimal cell collection through the endoscope. Hemocytometry counts of biopsy material taken from *ex vivo* porcine tissue demonstrated that on the order of 10^5 cells per scrape biopsy could be collected, more than enough for karyometric, cytological, and proteomics analyses. A photograph of the cafe shown inserted through a standard hysteroscope and salpingography catheter is shown in Figure 1. Our benchtop findings suggest the potential utility of the CAFE device for *in vivo* imaging and cell collection in future clinical trials.

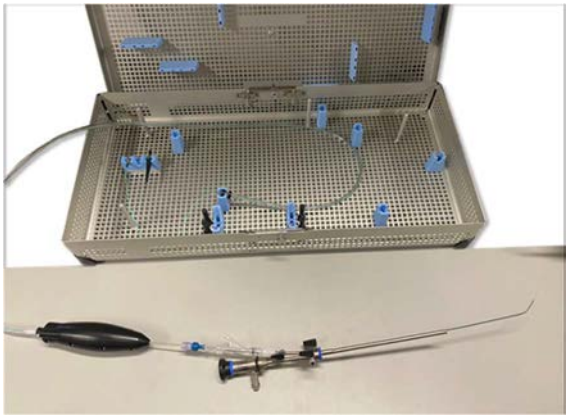


Figure 1. Cell Acquiring Fallopian Endoscope inserted through hysteroscope and positioning catheter.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 18

# CXCR4 Promotes Colon Cancer in the Azoxymethane and APC Mutant Mouse Models

Urszula Dougherty[1], Reba Mustafi[1], Zifeng Deng[1], Diana West Szymanski[1], Arantxa Sanchez[1], Loren Joseph[2], John Hart[1], Joel Pekow[1], Yan Chun Li[1] and Marc Bissonnette[1]
[1]University of Chicago, Chicago IL and [2]Beth Israel Hospital, Harvard Medical School Boston MA

## Abstract

**Introduction**: ADAM17 is responsible for activating EGFR by shedding membrane-bound EGFR ligands. CXCL12-CXCR4 signals also transactivate EGFR by stimulating ADAM17. We previously showed that ADAM17 controls tumor development. *In this study, we hypothesized that CXCR4 blockade or deletion might inhibit colon cancer development.* The aim of this study was to test the ability of a novel CXCR4 antagonist to inhibit azoxymethane (AOM)-induced colon cancer and test the effect of colonocyte *Cxcr4* deletion in mice with conditional mutant *Apc*. **Methods**: HT29 cells were pretreated with CXCR4 inhibitor AMD3100 followed by stimulation with CXCL12. EGFR signals were assayed by Western blotting. *In vivo*, A/J mice (n=40) were given AOM (7.5 mg i.p./kg body weight) weekly for 6 wks and randomized to MSX-122 (2.5 mg/kg body wt), another CXCR4 inhibitor or vehicle intraperitoneally thricely weekly. In separate experiments, we created compound mutant mice *$Apc^{fl/fl}$;$Cxcr4^{+/+}$-Cdx-2-P-Cre-ERT* and *$Apc^{fl/fl}$;$Cxcr4^{fl/fl}$-Cdx-2-Cre-P-ERT* on a C57Bl6 background. Mice were administered Cre-activating tamoxifen (50 mg i.p./kg body wt) and sacrificed 3 wks later. **Results**: CXCR4 inhibitor, AMD3100 inhibited EGFR transactivation by CXCL12 in HT29 colon cancer cells as assessed by reductions in pErbB2, pAKT and pERK. *In vivo*, MSX-122 reduced Ki67 in tumors by 31±6% ($p<0.005$, n=17). MSX-122 also significantly reduced colon tumor burden (2.2 vs. 1.4, $p<0.02$, KW) and appeared to reduce cancer incidence (40% vs. 15%, $p=0.06$). In the mutant *Apc* model, colonocyte *Cxcr4* deletion reduced tumor burden by 31±19% compared to *Cxcr4* wild type mice ($p<0.005$, n = 16 mice per genotype). **Conclusions**: The CXCR4 inhibitor, AMD3100 inhibited EGFR transactivation by CXCL12 in colon cancer cells. MSX-122, another more bioavailable CXCR4 inhibitor, suppressed colon tumor development in the AOM model. Colonocyte *Cxcr4* deletion, moreover, significantly reduced tumor burden in the *Apc* mutant mouse model. *We speculate, therefore, that MSX-122 might be chemopreventative in individuals at risk for colon cancer.*

**Category:** Biomarker **Color:** Purple **Poster Board #:** 8

**Improving Early-Stage Lung Cancer Diagnostic Using Radiomics in the DECAMP 1 cohort**

Kate Bloch, PhD, Boston University/CRUK MI University of Manchester UK; Xingyi Shi, MSC, Boston University; Ehab Billatos, MD, Boston University; Raul Estepar, PhD, Brigham and Women's Hospital; David Elashoff, PhD, University of California, Los Angeles; Avrum Spira, MD, MSC, Boston University; George Washko, MMSc, MD, Brigham and Women's Hospital; Steve Dubinett, MD, University of California, Los Angeles; Denise Aberle, MD, University of California, Los Angeles; Marc Lenburg PhD, Boston University

## Abstract

**Background**

Low dose computed tomography (CT) screening and early detection of high-risk individuals can improve the prognosis and survival rates of lung cancer patients. However, with the increasing adoption of CT, the number of pulmonary nodules being detected has substantially increased. Therefore, there is a need to develop a non-invasive biomarker that could identify the small number of malignant nodules from the large number of benign nodules. Various published studies from different fields in imaging have showed the potential of radiomics to enhance clinical decision-making. In this study we investigated the utility of quantitative radiomic features in predicting malignancy of indeterminate pulmonary nodules detected by CT scans.

**Data**

Data used in this project is from the Detection of Early Lung Cancer Among Military Personnel (DECAMP1) consortium, a prospective and multicentre study that constitutes ever smokers with indeterminate pulmonary nodules (IPNs) ranging from 7-30 mm. Of the 332 pulmonary nodules studied, 125 were adjudicated as benign lung diseases, and 207 were malignant primary lung cancer. A total of 451 radiomic features were extracted from CT images.

**Methods**

Various feature selection methods and machine learning algorithms, including Random Forest, Gradient Boosting Machine, and Support Vector Machine (SVM), with tenfold cross-validation were applied. Accuracy, sensitivity, specificity, and Area Under the ROC Curve (AUC) were utilized to estimate the model performance.

**Results**

Our initial work showed that SVM modeling on 11 selected radiomics features achieves the highest accuracy of 73%, with corresponding AUC of 0.76, sensitivity of 0.88, and specificity of 0.51. The most contributing radiomic features for predicting pulmonary malignancy were Surface Volume Ratio, Minimum Intensity and Spherical Disproportion.

**Conclusion and Further Research**

This study showed the potential of radiomics to distinguish benign from malignant pulmonary nodules in the setting of IPNs. However, further optimization and validation of the radiomics model is required to incorporate radiomics in the diagnostic routine practise. For the future research we are planning to add nasal gene expression data.

**Category:** Methods **Color:** Orange **Poster Board #:** 3

# GPQuant: An Algorithm for Identification and Quantification of Glycopeptides with Different Glycoforms and Its Application to a Large-scale Quantitative Analysis of Urinary Glycopeptides from Aggressive Prostate Cancer

Shao-Yung Chen, Yingwei Hu, T. Mamie Lih, Mingming Dong, and Hui Zhang

Johns Hopkins School of Medicine

## Abstract

Protein glycosylation accounts for one of the most complex protein modifications, with aberrant glycosylation reported to be associated with different diseases. While multiple attempts have been made to characterize the glycosylation event, there is still a need for an algorithm for accurately quantifying glycosylated peptides, and further search for unidentified glycosylated peptide under a LC-MS/MS experiment. There are several reasons for intact glycopeptides (IGPs) being harder to detect, including IGPs are harder to ionize, the intensity of IGPs are usually suppressed by unmodified peptides, which leads lack of MS2 scans under a Data-Dependent Analysis (DDA) setting.

Here, we report an algorithm, GPQuant, which recovers unidentified IGPs by using retention time (RT) of the glycopeptide with different glycan as a reference, comparing the theoretical isotopic distributions based on the molecular composition, and peak integrity using our in-house evaluation system. After recovering the unidentified glycopeptides, GPQuant then proceeds to accurately quantify all the IGPs, identified or previously unidentified. With GPQuant, peptides with different glycoforms are comprehensively described, therefore, a relationship for different glycoforms on the same glycosylation site can be more accurately described. By implementing a previous discovery cohort comprised of 74 aggressive (Gleason score ≥8) and 68 non-aggressive (Gleason score = 6) prostate cancer urine specimens, which were acquired via a data dependent acquisition (DDA) approach. We're able to quantify the glycopeptides along with the glycoform attached, improve the overall yield of intact glycopeptides detected in the LC-MS/MS runs, and further recovers certain glycopeptides that were previously undetected, therefore the overall distribution of glycan occupancy can be characterized.

Together, GPQuant introduces a novel approach in glycoproteomics. By using unidentified IGP recovery, GPQuant improves the yield of IGPs, while also accurately describe the relative glycan distribution of a glycoprotein under a complex biological sample.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 14

**Biomarker-driven Efficiencies In Clinical Trials**

Samantha Chesney, Clarivate Analytics

**Abstract**

Over the past three decades, the life sciences industry has observed considerable progress in the development and application of precision medicine, particularly in oncology. Greater understanding of the specific and varying genetic bases for cancers has driven the tailored approach and provided greater opportunities for clinical trials to take advantage of patient genetic variability for recruitment and segmentation purposes. However, this can also introduce challenges in clinical trial planning. Here, we examine the frequency of biomarker utilization in clinical trials and it's impact on trial efficiency.

At the very basic definition, precision medicine provides the information about what drug to prescribe, at what dose and when, for which patient. The traditional "one-size-fits-all" approach to the development and prescription of treatments has failed many patients. Data have highlighted the heterogeneity that affects how individuals respond to medications, because of differences in absorption, metabolism, excretion and drug therapy target. With a shift toward a more personalized model, we can gain efficiencies throughout the drug development lifecycle (by designing trial protocols that include only those patients most likely to respond) to the clinic (where better care can be provided to the patients in need of treatments).

Analysis of aggregated data from multiple sources has provided broad insights into the current precision medicine and associated clinical trial landscape, enabling data-driven, strategic planning for future clinical trials. Oncology has been the leading therapeutic area in terms of biomarker development and use, setting the stage for biomarker adoption in other therapeutic areas. This includes new and adaptive designs focused on precision medicine.

The percentage of oncology clinical trials using biomarkers has steadily increased over the last ten years, from 32% to 35%. However, there has been a slight decrease since 2017(43% to 35%). This could be partially explained by the increase in studies of immune-oncology drugs at that time as these trials do not require a biomarker because of their universal application. The two types of biomarkers that are predominantly being used in oncology trials are disease markers, which have increased in usage over 300% over the last 10 years, and therapeutic effect markers, which have increased 30% over the last 10 years. Although the use of biomarkers for patient segmentation in oncology trials is increasing and still significantly greater than in other therapeutic areas, the number of trials using biomarkers for this purpose (e.g., PDL expression, EGFR) still only represents a small proportion of trials (6% of trials starting in 2019), and Phase II trials are still the majority. This represents a potentially missed opportunity to shorten clinical trial timelines based on patient segmentation.

This analysis indicated that although great progress has been made in precision medicine, providing patients with more effective treatments, biomarker use is still being underutilized in clinical trials. Opportunity remains to incorporate biomarkers, and particularly biomarker-based stratification, into clinical trials — to shorten trial durations and increase the chances of successful outcomes.

**Category:** Imaging **Color:** Orange **Poster Board #:** 3

# Deep Transfer Learning for Malignancy Evaluation of Intraductal Papillary Mucinous Neoplasms from Endoscopic Ultrasound

Tasadduk H. Chowdhury, PhD[1], Sachi Khemka[2], Aatur D. Singhi, MD, PhD[3], Eugene J. Koay, MD, PhD[1], Randall E. Brand, MD[3]

[1]University of Texas MD Anderson Cancer Center, [2]Rice University, [3]University of Pittsburgh

## Abstract

**Background:** Mucinous cysts, either intraductal papillary mucinous neoplasms (IPMNs) or mucinous cystic neoplasms (MCNs) of the pancreas, are a recognized precursor lesion for the development of pancreatic ductal adenocarcinoma (PDAC) for 15 to 20% of cases. Since millions of individuals have mucinous cysts, the vast majority of which are IPMNs, and only thousands will develop a PDAC annually, a surveillance strategy is required to identify those cysts that have advanced neoplasia. Endoscopic ultrasound (EUS) with fine needle aspiration is commonly utilized to characterize IPMNs by looking for high-risk sonographic features, such as a mural nodule, or as a tool to obtain cyst fluid for biomarker analysis. In recent years, the application of deep learning methods has been effective for many tasks in radiology, including classification. Transfer learning, a deep learning method that adjusts a pre-trained model to new data, is commonly used when the size of the training data is small. The aim of this pilot study was to develop a transfer learning-based convolutional neural network (CNN) for predicting advanced neoplasia from EUS images of IPMNs. **Methods:** In this pilot study, we retrospectively studied a cohort of 32 patients (17 male, 15 female, median age 66.5 [range 43-82]), diagnosed with IPMNs who subsequently underwent surgical resection. The histological grade of the IPMN was assessed from surgical pathology using standard histological criteria. Out of the 32 patients, 16 patients were graded as high-grade dysplasia (HGD) and the remaining 16 patients were graded as low-grade dysplasia (LGD). A total of 88 EUS images were used (40 HGD, 48 LGD) for training, validation, and testing of the model. Each EUS image was first cropped around the cyst and then resized to 299x299 pixels. The processed images were randomly divided into 3 separate sets: 70% for training, 15% for validation, and 15% for independent testing. The Xception, an image classification CNN pre-trained on the ImageNet database, was used as the baseline model for transfer learning. The top layer of the baseline network was replaced by a sequence of new layers: a global average pooling layer, a fully connected layer with 256 nodes, a 50% dropout layer, and a sigmoid activation layer for binary classification. The weights of the baseline model's layers were not updated during training. The transfer learning CNN was trained on EUS data in 100 epochs with a batch size of 5 for both training and validation data. The Adam optimizer was used with an initial learning rate of 0.0001 and the binary cross-entropy loss function. **Results:** The CNN differentiated HGD and LGD lesions from the validation set with an area under the receiver operating characteristic curve (ROC-AUC) of 0.79 at an accuracy of 69%, sensitivity of 67% and specificity of 71%. To be specific, the model misclassified 2 HGD cases (out of 6) and 2 LGD cases (out of 7) in the validation set. In the independent test set, the model discriminated HGD from LGD with a ROC-AUC of 0.96 at an accuracy of 86%, sensitivity of 83% and specificity of 87%. Here, the model misclassified 1 HGD (out of 6) and 1 LGD (out of 8). **Conclusion:** Our pilot study demonstrated the malignancy risk in IPMNs can be evaluated by a transfer learning-based CNN from EUS images. Based on these promising results, validation of our findings is planned with an independent cohort to determine if this may aid clinical management of patients with IPMNs.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 12

# Low Frequency TP53 Mutations in Airway Epithelial Cells as a Biomarker for Lung Cancer Risk

**Authors:** Daniel J. Craig, PhD, The University of Toledo College of Medicine and Life Sciences, Erin L. Crawford, MS, The University of Toledo College of Medicine and Life Sciences, Pierre P. Massion, MD, Vanderbilt University School of Medicine, Nashville, TN, Thomas Morrison, PhD, Accugenomics, Inc., Wilmington, N.C., James C. Willey, MD, The University of Toledo College of Medicine and Life Sciences

## Abstract

**Background:** Lung cancer is the leading cause of cancer-related death in men and women in the United States. United States Preventative Services Tasks Force (USPSTF) guidelines recommend low dose CT (LDCT) screening for individuals with high demographic risk based on age and smoking history. However, a large fraction of lung cancers occurs in individuals who do not meet LDCT screening threshold criteria. Thus, there is a need for biomarkers that supplement demographic factors so that it may be possible to more accurately detect those at highest lung cancer risk. In a biomarker discovery study TP53 mutations with variant allele fraction (VAF) of 0.05-1.0% were significantly more prevalent ($p<0.005$) in grossly normal airway epithelial cell (AEC) specimens from lung cancer cases compared to non-cancer controls matched for smoking and age (Craig et al, BMC Cancer, 2019). Based on these preliminary data, we conducted a case-control study to test this biomarker.

**Methods:** Based on preliminary data, analysis of AEC specimens from 30 cancer and 30 non-cancer subjects would provide sufficient power to test the hypothesis that a cut-off of 0.02 mutations/bp would have 55% sensitivity and 95% specificity and positive likelihood ratio of 11. AEC specimens were collected by bronchoscopic brush biopsy at Vanderbilt University Medical Center from 30 subjects diagnosed with lung cancer (cases) and 30 subjects matched for age, smoking history, and age (controls). AEC genomic (g)DNA from each AEC specimen was shipped to UToledo. Synthetic DNA internal standards (IS) were prepared at UToledo for multiple lung cancer driver mutations within the TP53 gene. A competitive multiplex PCR amplicon NGS library was prepared for each AEC sample gDNA with >50,000 **gDNA** copies and equivalent **IS** genome copies included in each library preparation. Technical error at each genome position within each IS controlled for corresponding position-specific technical error in the corresponding sample gDNA. By controlling for technical error, this approach enables reliable detection of mutations with VAF as low as $5 \times 10^{-4}$ (0.05%).

**Results:** For all subjects measured (N=60; 30 CA, 30 NC) a TP53 mutation prevalence biomarker cut-off (CA = >5 TP53 mutations/subject) was associated with sensitivity 60%; specificity 93%; ositive likelihood ratio 8.7. For low/intermediate risk subjects (based on Tammamagi index) (N= 26; 6 CA, 20 NC) the biomarker cut-off had sensitivity 83%; specificity 100%, and Positive likelihood ratio unmeasurable due to Specificity 100%.

**Conclusions:** The analytical goal was reached for the most important group, those with low/intermediate risk. Based on this result the next priority is to further refine the biomarker cut-off in a comparable case-control cohort (N=60) and assess the biomarker in nasal brush specimens as a suitable non-invasively obtained surrogate clinical sample. Toard this goal, we have identified a second case-control cohort for validation of the biomarker in AEC, and a set of matched AEC, nasal brushing, and bronchial biopsy specimens from >20 subjects.

**Category:** Bioinformatics **Color:** Blue **Poster Board #:** 2

# Developing Knowledge Environments for Consortium-based Clinical Cancer Biomarker Studies

Daniel Crichton, NASA Jet Propulsion Laboratory, Heather Kincaid, NASA Jet Propulsion Laboratory, Alphan Altinok, PhD, NASA Jet Propulsion Laboratory, Kristen Anton, University of North Carolina, Chapel Hill, Luca Cinquini, PhD, NASA Jet Propulsion Laboratory, Maureen Ryan Colbert, University of North Carolina, Chapel Hill, David Liu, NASA Jet Propulsion Laboratory, Sean Kelly, NASA Jet Propulsion Laboratory, Ashish Mahabal, PhD, California Institute of Technology, Asitang Mishra, NASA Jet Propulsion Laboratory, Christos Patriotis, PhD, National Cancer Institute, Sudhir Srivastava, PhD, MPH, National Cancer Institute

## Abstract

As data generated by disciplines including imaging, genomics, and proteomics increases, the need to systematically capture, manage and analyze data through an advanced knowledge system is becoming vital. The use of automated data processing, intelligent algorithms for data reduction, inference and analysis, and consistent data pipelines from laboratory data management—and across distributed, collaborative research teams—presents new challenges. The data ecosystem must ensure that systematic capabilities are in place to support the reproducibility obstacles inherent in the collaborative biomedical research environment. Through its collaboration with NASA/JPL, the EDRN and MCL programs are well positioned to take on many of the challenges that will be presented in cancer biomarker research as the need for data-intensive science increases. Integrating informatics into science research continues to be critical as these programs move forward and enhance their pioneering efforts to provide a next generation infrastructure for discovery and validation of cancer biomarkers. JPL is responsible for the research and development of novel data science architectures to support the capture, management, and analysis of scientific data sets acquired across NIH-supported biomarker research consortia. The architecture facilitates team science in cancer biomarker research, bringing together leading scientists throughout the U.S. The Integrated Knowledge Data Environment supports the integration of science and informatics across the entire data lifecycle in order to scale investigation and increase collaboration and reproducibility. Importantly, the environment unifies consortium data into a rich data commons that supports collaborative analysis and the ability to apply data-driven techniques and tools such as machine learning.

**Category:** Biospecimens **Color:** Blue **Poster Board #:** 6

**EDRN Standard Specimen Reference Sets**

Jackie Dahlgren (DMCC), Deanna Stelling (DMCC), Karl Krueger (PhD, NCI), Christos Patriotis (PhD, NCI), Paul Wagner (PhD, NCI), Jacob Kagan (PhD, MSc, NCI), Lynn Sorbara (PhD, NCI), Jo Ann Rinaudo (PhD, NCI), Matthew Young (PhD, NCI), Richard Mazurchuk (PhD, NCI), Natalie Abrams (PhD, NCI), Guillermo Marquez (PhD, NCI), Sharmistha-Ghosh Janjigian (PhD, NCI), Ying Huang (PhD, DMCC), Yingye Zheng (PhD, DMCC), Yingqi Zhao, (PhD, DMCC), James Dai (PhD, DMCC), Tracey Marsh (PhD, DMCC), Eardi Lila, (PhD, DMCC), Ziding Feng (PhD, DMCC), Sudhir Srivastava (PhD, MPH, NCI)

## Abstract

In our effort to standardize performance evaluation of biomarkers, the NCI Early Detection Research Network (EDRN) has created and continues to create specimen reference sets to facilitate testing and implementation of biomarkers useful for the detection of solid malignancies for the purpose of down-staging incident cancers in the population. Much of the biomarker work to date has been performed on convenience samples from cases and controls. Since these samples have been collected in a variety of ways, comparisons have been difficult. Further, cases and controls may not have been selected and matched using appropriate rigor to reduce bias. Finally, since there has not been a common resource with sufficient amounts of sample, comparison or integration of multiple markers has not been feasible. With the creation of shared and common sets of specimens from well characterized and matched cases and controls from specific disease spectra, the EDRN will overcome many of the logistic and design issues in preliminary and advanced biomarker validation. These resources are accessible to any investigator within or outside of the EDRN based upon a common and transparent set of criteria used to evaluate applications. It is anticipated that results from these studies will be made publicly available. To date EDRN has established or is in the process of establishing standard specimen reference sets for the following organs: Breast, Cancers in Women, Colon, Liver, Lung, Pancreas and Prostate.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 3

**Auto-Antibodies Targeting Recurrent Serous Ovarian Cancer Associated Neoantigens as Candidate Markers for Ovarian Cancer Early Detection**

Lindsay Bergan, Sarah Hawley, Robert Pearhill, Martin McIntosh and Charles Drescher
Translational Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA

## Abstract

Ovarian cancer is a lethal disease primarily due to advanced stage at clinical diagnosis. Serous Ovarian Cancer (SOC) is the most common subtype accounting for roughly 60% of ovarian cancer deaths. Currently there are no effective screening strategies for ovarian cancer. The United Kingdom Cooperative Trial of Ovarian Cancer Screening demonstrated a non-significant 15% mortality reduction using rising serum CA125 levels to select women for transvaginal ultrasongraphy as a second line test. A goal of our EDRN CVC is to identify and validate auto-antibody markers that can complement CA125 as first line ovarian cancer screening test. We focus on antibodies targeting a novel set of recurrent SOC associated neoantigens.

We developed a bioinformatic pipeline to identify candidate antigens correspond to the translation products of Cancer Selected Transcripts (CSTs). Our methods allow us to reliably predict CSTs that maintain high coding potential by integrating large-scale public data sources with innovative RNA-seq assays that profile the translational activity of identified variants. Data resources from the TCGA, GTEX and EST databases are combined to identify all splicing events and translation start sites that appear to be unique to SOC. The coding potential of a specific variant (e.g., a species that harbors a cancer specific exon-skip) is determined from profiles of ribosome-protected mRNA fragments spanning the variant. Ribosome-protected CSTs prevalent in 20% or more of SOCs cases were selected for manual curation and experimental confirmation using PCR or Sanger Sequencing. Serum IgG responses to the translation products of selected CSTs, were evaluated by screening SOC case and healthy control sera using bead based assays generated by coupling IVTT expressed proteins or tiled 15mer synthetic peptides to the bead surface. We confirmed low frequency antibody responses targeting several SOC associated neoantigens including CSTs arising from PON1 and Chr 7.442, an annotated lincRNA we identified as expressed in SOC. These auto-antibodies discriminate SOC cases from controls at high specificity and appear to complement antibodies targeting TP53, a known SOC associated antigen. We also confirm antibody responses to MAGEC1, a known cancer-testis antigen, in SOC patients.

**Category:** Biomarker **Color:** Green **Poster Board #:** 2

**Lead-Time Trajectory of CA19-9 as an Anchor Marker for Pancreatic Cancer Early Detection**

Johannes F. Fahrmann[1], C. Max Schmidt[2], Xiangying Mao[1]*, Ehsan Irajizad[1]*, Maureen Loftus[3], Jinming Zhang[3], Nikul Patel[1], Jody Vykoukal[1], Jennifer B. Dennison[1], James P. Long[4], Kim-Anh Do[4], Jianjun Zhang[2], John A. Chabot[8], Michael D. Kluger[8], Fay Kastrinos[9,10], Lauren Brais[3], Ana Babic[3], Kunal Jajoo[5], Linda S. Lee[5], Thomas E. Clancy[6], Kimmie Ng[3], Andrea Bullock[7], Jeanine Genkinger [8], Michele T. Yip-Schneider[2], Anirban Maitra[9], Brian M. Wolpin[3], Samir Hanash[1]

[1] Department of Clinical Cancer Prevention, [4] Department of Biostatistics, [9] Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA
[2] Department of Surgery, Indiana University School of Medicine, Indianapolis, IN
[3] Dana-Farber Brigham and Women's Cancer Center, Division of Gastrointestinal Oncology, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[5] Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[6] Dana-Farber Brigham and Women's Cancer Center, Division of Surgical Oncology, Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA USA
[7] Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[8] Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, Department of Epidemiology, Columbia Mailman School of Public Health, New York, New York, USA.
[9] Division of Digestive and Liver Diseases, Columbia University Irving Medical Cancer and the Vagelos College of Physicians and Surgeons, New York, New York
[10] Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, Department of Surgery, New York, NY

## Abstract

**Background & Aims:** There is substantial interest in liquid biopsy approaches for cancer early detection, among subjects at risk, using multi-marker panels. CA19-9 is an established circulating biomarker for pancreatic cancer. However, its relevance for pancreatic cancer early detection or for monitoring subjects at risk has not been established.

**Methods:** CA19-9 levels were assessed in blinded sera from 175 subjects collected up to 5 years prior to diagnosis of pancreatic cancer and from 875 matched controls from the PLCO Cancer Screening Trial. For comparison of performance, CA19-9 was assayed in blinded independent sets of samples collected at diagnosis from 129 subjects with resectable pancreatic cancer and 275 controls (100 healthy subjects; 50 with chronic pancreatitis; and 125 with non-cancerous pancreatic cysts). The complementary value of two additional protein markers, TIMP1 and LRG1, was determined.

**Results:** In the PLCO cohort, levels of CA19-9 increased exponentially starting at two years prior to diagnosis with sensitivities reaching 60% at 99% specificity within 0-6 months prior to

diagnosis for all cases and 50% at 99% specificity for cases diagnosed with early-stage disease. Performance was comparable for distinguishing newly diagnosed cases with resectable pancreatic cancer from healthy controls (64% sensitivity at 99% specificity). Comparison of resectable pancreatic cancer cases to subjects with chronic pancreatitis yielded 46% sensitivity at 99% specificity and for subjects with non-cancerous cysts 30% sensitivity at 99% specificity. For pre-diagnostic cases below cut-off value for CA19-9, the combination with LRG1 and TIMP1 yielded an increment of 13.2% in sensitivity at 99% specificity ($p=0.031$) in identifying cases diagnosed within 1 year of blood collection.

**Conclusion:** CA19-9 can serve as an anchor marker for pancreatic cancer early detection applications. Inclusion of additional markers as demonstrated for LRG1 and TIMP1 and potentially in combination with other marker types, would have value for identifying cases that do not meet CA19-9 cutoff thresholds.

**Category:** Imaging **Color:** Orange **Poster Board #:** 1

**Digital Breast Tomosynthesis and Breast Cancer Risk**

Moffitt Cancer Center: Erin Fowler, MPH; John Heine, PhD; Jared Weinfurtner, MD; Shelley Tworoger, PhD; Emma Hume, MPH

**Abstract**

Mammograms contain information that informs on the future risk of breast cancer (i.e. breast density). The best way to summarize this content has been studied for many years. Breast density is often measured as the mammographically dense area within a two-dimensional (2D) mammogram normalized by the total breast area. Broad classes of automated measures related to image texture have also been investigated. The search for the optimal measure has been confounded by advancing technologies in mammography: film, to digitized film, to full field digital mammography (FFDM), and currently to digital breast tomosynthesis (DBT). Within a given technology, there are also design differences producing image variations. In this project we evaluated images from DBT units.

This work is part of an ongoing EDRN parent grant project within the CVC at Moffitt Cancer Center, Tampa FL. The goal is to evaluate image measurements as possible risk factors within and across imaging technologies. The DBT acquisition produces breast volume images (i.e. stacked 2D slices). These units also provide 2D synthetic mammograms constructed from the volume slices (i.e. termed C-Views for our units). Reconstructed volume and C-View images result from heavy processing. In addition, these images have varying pixel spacing based on paddle size: 85-90μm for women imaged with the small compression paddle and 103-110μm for women imaged with the large compression paddle in our dataset.

We investigated C-View images from a matched case-control study (n = 426 pairs) with anisotropic diffusion filtering (ADF). Matching variables include age, mammography unit, and hormone therapy profile. ADF is a multiscale iterative filtering process that produces a series of blurred images ($I_j$) starting with the raw image ($I_0$). In theory, the filter kernel adjusts locally to achieve both maximal smoothing in areas where $I_0$ varies slowly spatially and minimal smoothing in areas that change rapidly. We considered the variance ($V_j$) of the difference images: $d_j = I_j - I_{j-1}$, for j = 1-200, where $d_j$ contains the spatial information captured between iterations. We investigated two related measures: (1) $V_{100}$; and (2) the entire diffusion process by analyzing $\log(V_j)$ vs. $\log(j)$ plots for each image, where the absolute value of the slope (m) was used as the measure. Linear correlation analysis (R) was used to assess these plots and compare both measures. Before filtering, we used an approximation to adjust for pixel spacing differences. All small paddle images were placed at the respective average (i.e. 87 μm) and processed with a Butterworth low-pass filter constructed to permit downsampling to match the average pixel spacing for the large paddle images (i.e. 106 μm). Conditional logistic regression was used to analyze both measures ($V_{100}$ and m) controlling for BMI, exposure time product (mAs), ethnicity, and compression paddle (binary). Odds ratios (ORs) were used as the measure of breast cancer association in per standard deviation increment for each measure with 95% confidence intervals. Both measures produced significant associations: OR = 1.43 (1.18, 1.72) for $V_{100}$ and OR = 0.73 (0.61, 0.87) for m. Log-log plots were nearly linear (average R = 0.99), indicating the process is well defined as an inverse power law: $V_j \propto j^{-m}$. Both measures are elements of the same process and highly correlated (R = 0.86).

These OR findings agree with breast density measured from 2D mammograms. Although mammograms can be considered as complex random fields, the diffusion process is described by simple power law. The overall findings will require replication with varying populations and imaging technologies.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 17

# Elevated EVL Methylation Level in Normal Colon Mucosa is a Potential Risk Biomarker for Metachronous Polyps

Authors:
Ming Yu[1], Kelly T Carter[1], Kelsey Baker[1], Mary W. Redman[1], Ting Wang[1], Kathy Vickers[2], Lynda Ann Dzubinski[3], Robert E Schoen[3], William M. Grady[1,4]

Affiliations:
[1]Clinical Research Division, [2]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA
[3]Department of Medicine and Epidemiology, University of Pittsburgh Medical Center, Pittsburgh, PA
[4]Department of Medicine, University of Washington School of Medicine, Seattle, WA

## Abstract

**Background:** Individuals with adenomatous polyps are advised to undergo repeated colonoscopy surveillance to identify and remove metachronous adenomas in order to prevent incident colorectal cancer (CRC). However, many subjects with adenomas do not develop recurrent adenomas, which leads to a need for more accurate methods to evaluate those people who would most benefit from colonoscopy-based polyp surveillance. We evaluated the association of altered *EVL* methylation in normal mucosa as a potential biomarker for the risk of developing metachronous adenomas.
**Methods:** We conducted a retrospective cohort study that included subjects who were polyp-free at all colonoscopic exams done within 8-16 years of the observation window (Group 1; N=11); and subjects who had polyps detected within 12 years of the index colonoscopy (Group 2, N=71). An ultra-sensitive methylation-specific droplet digital PCR assay was used to accurately determine *EVL* methylation in normal colon mucosa samples taken from these subjects at the time of their index colonoscopy. We correlated the *EVL* methylation status with the primary outcome of metachronous polyp occurrence during follow-up using the standard Inverse Probability Weighted Logistic Regression model.
**Results:** Elevated EVL methylation in the normal colon mucosa at the index colonoscopy indicated an increased risk of having metachronous polyps detected within 12 years, compared to subjects with no metachronous polyps detected (*Odds Ratio (95% CI) = 4.35 (1.14, 16.60); p-value = 0.0317)*.
**Conclusion:** Our results suggest that the EVL methylation level in the normal colon mucosa can be used as a risk biomarker for metachronous adenomas.
**Impact:** These findings support the potential utility of EVL methylation for improving the accuracy for assigning the need for surveillance colonoscopy.

**Category:** Biomarker **Color:** Green **Poster Board #:** 4

# The sTRA Plasma Biomarker: Blinded Validation of Improved Accuracy over CA19-9 for Pancreatic Cancer Diagnosis in the EDRN Team Projects

Ben Staal[1], David Ayala-Talavera[1], ChongFeng Gao[1], Daniel Barnett[1], Ying Liu[1], Zonglin He[2], Mark Hurd[3], Ying Huang[2], Aatur Singhi[4], Anirban Maitra[3], Randall E. Brand[4], and Brian B. Haab[1]

[1]*Van Andel Institute, Grand Rapids, MI 49503, USA*
[2]*Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA*
[3]*Sheikh Ahmed Center for Pancreatic Cancer Research, MD Anderson Cancer Center, Houston, TX 77030, USA*
[4]*University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA*

## Abstract

The EDRN Pancreatic Cancer Working Group recently completed two multi-laboratory studies for the blinded validation of the best biomarkers from EDRN laboratories for the detection or diagnosis of pancreatic ductal adenocarcinoma (PDAC). A set of plasma specimens provided by EDRN Clinical Validation Centers was blinded and distributed to each site, and the investigators at each site returned case/control calls to the EDRN biostatistician. The studies resulted in the validation of a biomarker panel that achieved significant improvement over CA19-9, the current best biomarker for PDAC. The biomarker panel is a combination between CA19-9 and another marker called sTRA, which detects a glycan that is produced by a different subtype of cancer cells than make CA19-9. Using preset thresholds and classification rules, the panel achieved statistical improvement ($p < 0.001$) in the first study: 95% specificity and 54% sensitivity (75% accuracy), compared to 97%/30% (65% accuracy) for CA19-9. The adjusted thresholds for the panel gave 96% specificity and 65% sensitivity, better than the optimized CA19-9 performance of 96% specificity and 46% sensitivity. The panel achieved similar performance improvement over CA19-9 in the second blinded study. The collaborative group is now moving this marker panel forward to the next round of blinded validation. The new panel may enable surveillance for PDAC among people with elevated risk or improved differential diagnosis among patients with suspected pancreatic cancer.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 6

**Multi-Biomarker Panel Assessment of Serological Assaysin Early Detection of Ovarian Cancer***

Chae Young Han[1], Wei-Lei Yang[1], Jing Guo[1], Yiling Liu[2], Hailing Yang[1], Joseph Celestino[3], Karen H. Lu[3], Zhen Lu[1], Karen S. Anderson[4], Charles W. Drescher[5], Steven J. Skates[2], Robert C. Bast Jr.[1]

[1]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, Texas; [2]Massachusetts General Hospital, Boston, Massachusetts; [3]Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas; [4]Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, Arizona; [5]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington;.

**Background:** Computer simulation suggests that early detection of ovarian cancer could reduce mortality by 10-30%. At present only 20-25% of ovarian cancers are diagnosed in early stage (I-II). Two-stage screening strategies appear most promising where rising serum markers prompt ultrasound and abnormal ultrasound prompts surgery. CA125 has been used in most trials but is elevated in serum from 70% and in tissue in 80% of early stage ovarian cancers. An EDRN collaborative group has evaluated a panel of autoantibodies, antigen-autoantibody complexes and antigens as potential biomarkers to augment the sensitivity of CA125 for early detection.

**Methods:** Multiple serological immunoassays were performed using an EDRN discovery sera set of ovarian cancer cases with 64 early stage (I-II), 186 late stage (III-IV), 250 pelvic masses, and 502 healthy controls. Anti-TP53 autoantibody assays were performed with RAPID (ASU), Magpix/xMAP, Meso Scale Discovery, SeroMAP, and Roche Elecsys Cobas to compare the sensitivity of each assays. Other autoantibody candidates (CTAG1A, CTAG2, CA4, ICAM3, KSR1, NUDT11, NXF3, POMC, PVRB, STXL1, TRIM-39, UHMK1) were assayed. In addition, HE4 antigen-autoantibody complexes and Osteopontin (OPN) antigen were measured with a Luminex assay.

**Results:** The sensitivity of anti-TP53 autoantibody immunoassays ranged from 13 - 23% across the assays for all stages at 98% specificity. The RAPID assay (ASU) exhibited the greatest sensitivity of 13% for early stage cases and 22% for total cases, whereas the Roche Cobas assay (MDACC) detected 11% of early stage cases and 23% of total cases. The anti-TP53 autoantibody assays detected 1 of 18 (6%) early stage cases missed by CA125. Among other autoantibodies tested, CTAG1A (ASU) showed the greatest sensitivity of 8% for early stage cases and 16% for total cases, detecting 1 of 18 (6%) early stage cases missed by CA125. HE4 Ag-Ab complexes detected 19% of early stage cases, including 6 of 18 (33%) missed by CA125. OPN detected 39% of early stage cases including 7 of 18 (39%) missed by CA125. While CA125 detected 72% of early stage cases at 98% specificity, a combination of CA125, HE4 Ag-Ab complexes and OPN detected 89% of early stage cases at a specificity of 94%.

**Conclusions**: The RAPID and Roche anti-TP53 autoantibody assays detected 11-13% of early stage cases at 98% specificity and detected 6% of cases missed by CA125. Addition of HE4 antigen-autoantibody complexes and OPN to CA125 detected 89% of early stage cases at a specificity of 94%.

* This research was conducted as an EDRN collaborative project by investigators at MD Anderson Cancer Center (MDACC), Arizona State University (ASU), Fred Hutchinson Cancer Center (FHCRC), and Massachusetts General Hospital (MGH). EDRN discovery sets were assembled from MDACC and FHCRC.

**Category:** Technology **Color:** Red **Poster Board #:** 3

# Development of Reference Materials for Cancer Biomarker Measurements in Liquid Biopsy and Beyond

Hua-Jun He[1], Ph.D., Elzafir Elsheikh[1], Ph.D., Jamie Almeida[1], M.S., Zhiyong He[1], Ph.D., Nathanael Olson[1], Ph.D., Lindsay Harris[1], B.S., Lili Wang[1], Ph.D., Kenneth D. Cole[1], Ph.D., Lynn Sorbara[2], Ph.D., Matthew R. Young[2], Ph.D., and Sudhir Srivastava[2], Ph.D. [1]The National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899. [2] The Early Detection Research Network, National Cancer Institute, Bethesda, Maryland

## Abstract

Various components of tumor cells released into the body fluids can be analyzed in liquid biopsy, some of which include circulating cell-free tumor DNA (ctDNA), circulating tumor cells (CTCs), circulating cell-free RNA (cfRNA), tumor-educated platelets (TEPs), and extracellular vesicles (EVs). The molecular profiles gathered from ctDNA such as gene alteration and DNA methylation can be further complemented with the analysis of CTCs, RNA, proteins, and EVs. The measurement of these biomarkers has the potential to non-invasively screen and diagnose cancer at an early stage, monitor tumor progression, and evaluate therapy responses. However, the reliable detection of cancer biomarkers in liquid biopsy is difficult because these biomarkers are fragmented and present at low concentrations compared to wild type form of the biomarkers in the blood derived from normal cells.

NIST, in collaboration with the Early Detection Research Network and National Cancer Institute, is developing reference materials for measurements in liquid biopsy. We work with the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium to address the absence of well-validated quality control materials (QCM) for ctDNA testing. Recently, we have completed a phase I study by using QCM with 14 clinically relevant mutations representing single nucleotide variants (SNVs), insertion/deletions (indels), translocations, and copy number variants (CNVs) were sourced from 3 QCM commercial manufacturers. The results of this validation support the utility of the QCM in testing analytical assay performance of ctDNA assays. We have developed research test materials at NIST, using synthetic DNA spiked into fragmented human background DNA either by physically shearing or enzymatic digestion. The research test materials have been characterized, and the cancer biomarker variants have been quantified by validated digital PCR (dPCR) assays. This approach is rapid and very flexible to enable the rapid design cancer panel of mutations and customize copy number and size of the resulting DNA fragments. Secondly, we are developing methylated DNA test material sets by mixing different ratio of *in vitro* methylated human genomic DNA with native state genomic DNA. These materials were characterized by Sanger sequencing, digital PCR, targeted NGS, EPIC array and reduced representation bisulfite sequencing (RRBS) assays. Thirdly, we are developing EVs reference control materials with reasonably uniform size distribution and a set of techniques for characterizing and quantifying EVs with sufficient measurement confidence. Furthermore, we are working on a project to develop NIST 21$^{st}$ Century cell lines, fully consented and characterized cancer tumor/normal cell lines as reference materials (focus on early stage and pre-cancer markers). The development of these reference materials would improve the reliability and confidence of the measurement for cell-free DNA biomarkers that show great promise for cancer detection.

**Category:** Biomarker **Color:** Green **Poster Board #:** 9

**Urine Based PSA Assay- A Surrogate Marker For Invasive Prostate Cancer**

Naseruddin Höti, PhD, Tung-Shing Lih, PhD, and Hui Zhang, PhD, Johns Hopkins School of Medicine.

**Abstract**

Serum PSA together with digital rectal examination and imaging of the prostate gland remained the gold standard in urological practices for managing and intervention of prostate cancer. Therefore, a great challenge remains for any novel prostate cancer biomarker that can be adopted into these settings and their eligibility for any medical reimbursements. Based on these adopted practices and at the same time the poor performance of serum PSA towards prostate cancer has led us to evaluate whether urinary PSA levels might have any clinical utility in prostate cancer diagnosis. Utilizing the established diagnostic platform for serum PSA ELISA assay, we evaluated a total of n=439 urine specimens that were obtained from prostate cancer patients. In our initially cohort a total of hundred and forty-six (n=146) urine specimens for PSA were obtained from aggressive (GS ≥8) and the non-aggressive (GS=6) prostate cancer patients, followed by a validation set of n=293 samples. Our data demonstrated that patients with aggressive disease (GS ≥8) expressed lower levels of urinary PSA compared to the non-aggressive patients (GS=6). Interestingly, when we evaluated the serum PSA in the same individuals, we found that unlike urinary PSA that showed lower PSA levels for aggressive disease, the serum PSA levels were higher in patient with aggressive prostate disease (GS ≥8). The discordance among serum and urine PSA levels was further validated by immuno-histochemistry (IHC) assay in biopsied tumors and several metastatic lesions (n=65). Our data demonstrated that invasiveness might be negatively regulating the PSA production in prostate cancer tissues and unlike serum PSA, urinary PSA might serve a better surrogate in capitulating tissue milieus in detecting prostate cancer. We further explored the utility of urine PSA as cancer biomarker alone or in combination with serum PSA to predict the disease status. Comparing the AUCs for the urine and serum PSA we found that urinary PSA have a higher predictive power (AUC= 0.741) in detecting aggressive disease. Furthermore, the addition of urinary PSA with serum PSA enhanced the sensitivity and specificity of serum PSA (AUC= 0.767) in predicting aggressive prostate diseases. These studies strongly support the role of urine PSA in combination with serum to increase the sensitivity of detecting aggressive prostate cancer.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 7

# Detection of Circulating EGFR Mutations by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Feng Jiang PhD and Sanford A Stass MD, Department of Pathology, University of Maryland School of Medicine

## Abstract

The detection of EGFR mutations in circulating cell-free DNA can enable diagnosis of lung cancer and personalized therapy for the disease. The current techniques for detecting circulating EGFR mutations are expensive and time-consuming with moderate sensitivity. Emerging CRISPR is revolutionizing medical diagnostics and showing a great promise for nucleic acid detection. This study aims to develop CRISPR-Cas12a as a simple test to sensitively detect circulating EGFR mutations in plasma. Serially diluted samples of DNA containing heterozygous EGFR mutations (L858R and T790M) in wild-type genomic DNA are concurrently tested for the mutations by a CRISPR-Cas12a system and droplet digital PCR (ddPCR). The CRISPR-Cas12a system can detect both L858R and T790M with a limit of detection of 0.005% in less than three hours. ddPCR detects the mutations with a limit of detection of 0.05% for more than five hours. CRISPR is ten times more sensitive than ddPCR. Plasma samples of 28 lung cancer patients and 20 cancer-free individuals are tested for the EGFR mutations by both CRISPR-Cas12a system and ddPCR. The CRISPR-Cas12a system could detect L858R in plasma of two lung cancer patients whose tissue biopsies are positive for L858R, and one plasma sample of three lung cancer patients whose tissue biopsies are positive for T790M. ddPCR detects L858R in the same two plasm samples, however, does not detect T790M in any of the plasma samples. This proof of principle study demonstrates that the CRISPR-Cas12a system could rapidly and sensitively detect circulating EGFR mutations, and thus has potential diagnostic or therapeutic implications.

**Category:** Technology **Color:** Red **Poster Board #:** 8

**Multiplexed Profiling of Single Extracellular Vesicles**

Ala Jo, Ph.D[1], Ralph Weissleder, MD[1, 2, 3], Hakho Lee, Ph.D[1, 2]

1. Center for Systems Biology, Massachusetts General Hospital, Boston, MA
2. Department of Radiology, Massachusetts General Hospital, Harvard Medical School, MA
3. Department of Systems Biology, Harvard Medical School, Boston, MA

## Abstract

Cancer-derived extracellular vesicles (EVs) are an emerging new class of cancer biomarker: most types of cancer shed EVs that carry molecular information about the parent tumor, providing a new avenue to probe and serially monitor tumor molecular status. Technical challenges, however, remain when using EVs for early cancer detection: tumor-derived EVs comprise a small fraction of total vesicles in circulation. Conventional assays may fail to differentiate true tumor signal from biological background, missing unique molecular signature pertaining to tumor heterogeneity and phenotypic changes. Here we describe a new platform technology for single EV analysis with multiplexing (SEAM) [1, 2]. We adopted a cycling imaging method that allowed for multiplexed protein profiling of individual EVs. When applied to profile glioblastoma-derived EVs, the SEAM technique i) revealed heterogeneity in marker expression and ii) identified EV subpopulations even among isogenic cell types. This proof-of-concept study highlights SEAM's potential as a transformative analytical tool to study nearly all types of vesicles at a single particle level – this bottom-up approach could uncover new biological processes that are currently masked by bulk measurements.

References

[1] ACS Nano 2018, 12, 494–503
[2] Neuro-Oncology 2019, 21(5), 606-615

**Category:** Imaging **Color:** Orange **Poster Board #:** 6

**Radiomics and Its Application in the Stratification of Pancreatic Cystic Lesion(S)**

Sukhwinder Kaur, Ph.D. [1], Vipin Dalal M.B.B.S. [1], Wang Shuo, Ph.D. [2], Chi Lin, Ph.D. [2], Maneesh Jain[1] Ph.D., Dandan Zheng[2], Ph.D., Chi Zheng Ph.D.[3], Randall E. Brand, MD. [4], Surinder K. Batra Ph. D. [1]

[1]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE

[2]Department of Radiation Oncology, Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE,

[3]School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE

[4]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

**ABSTRACT**: Pancreatic cystic lesions (PCLs) are a clinically identifiable and histologically diverse groups of precursor lesions that present a variable risk of malignant transformation into pancreatic ductal adenocarcinoma (PDAC). An estimated 3-13% of patients undergoing CT or MRI are incidentally discovered to harbor PCLs, making them a prime target population for the screening and surveillance modalities. However, complexities arise as cystic lesion presents a variable risk for malignant progression: while some PCLs including Intraductal papillary mucinous neoplasms (IPMN) carry up to a threefold increase risk of developing PDAC, others present with a marginal risk or low probability of developing into PDAC. With limited knowledge of time and frequency of malignant progression, there is an urgent need to identify features associated with the malignant progression of PCLs. Radiomics and deep learning framework, with their ability to carry out high-throughput extraction of comprehensible data and identify hidden pattern from standard-of-care images, can provide potential markers/features for early identification of invasive features of PCLS. Based on this, we hypothesize that Radiomics based quantitative image analysis can identify unique imaging biomarkers to predict the risk of a malignant cyst. In order to test this hypothesis, in our pilot study, 41 patients diagnosed with PCLs were analyzed for the identification of unique radiomics features. Briefly, contrast-enhanced CT images prior to surgery were retrospectively collected from 24 patients with benign cysts and 17 patients with the high-grade malignant cysts. PCLs were segmented (contoured) in BrainLab iPlan Treatment Planning System (BrainLab AG, Germany) by two physicians. The exported DICOM images and RT Structures were processed by an in-house developed pipeline, and 924 radiomics features were extracted using PyRadiomics (an Open-source tool developed by Computational Imaging & Bioinformatics Lab at Harvard University). To identify unique features associated with malignant cyst development, extracted features were analyzed by one-way ANOVA analyses followed by hierarchical clustering of key Radiomic features. Pearson coefficient analyses of stable radiomics features were carried out to identify highly correlated features. Finally, a unique classifier from the unique set of features from Pearson coefficient analyses was analyzed using 5-fold cross-validation using XGBoost, Random Forest, AdaBoost, and Extra Tree classifier. Our pilot study identified wavelet-LLL-firstorder_totalEnergy, wavelet-LLL-firstorder_90Percentile, wavelet-HHL-kurtosis, and wavelet-HLH-firstorder_skewness as key characteristic features across benign and malignant cyst. Studies are underway to evaluate these models across the independent datasets and develop deep learning models for the distinction of the malignant cyst from benign. Overall, Radiomics and deep learning-based cost-effective approach would guide clinical decision-making leading to better utilization of healthcare resources.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 1

**A unique approach combining avatar mice and mass spectrometry to identify blood biomarkers for early detection of breast cancer**

Jacob J. Kennedy*, Richard G. Ivey*, John Landua†, Aura Burian*, Rachel Lundeen*, Pei Wang§, Shrabanti Chowdhury§, Steven Skates‖, Jeffrey R. Whiteaker,* Michael T. Lewis†, and Amanda G. Paulovich*
*Fred Hutchinson Cancer Research Center; †Baylor College of Medicine; §Icahn School of Medicine at Mount Sinai; ‖Massachusetts General Hospital

**ABSTRACT**

Tens of millions of women undergo population-based screening for breast cancer by mammography. Despite the life-saving advantage of early detection, screening guidelines for mammography are controversial and continue to evolve. At issue are the specificity of mammography for distinguishing benign vs. cancerous lesions, the sensitivity of mammography in some patient populations (e.g. women with dense breasts), and concerns regarding over-diagnosis (e.g. some ductal carcinoma in situ). A blood-based biomarker that could be used in conjunction with mammography to improve sensitivity and specificity could thus have enormous clinical impact by detecting treatable cancers missed by mammography (thereby reducing mortality), and by allowing patients to avoid unnecessary invasive procedures for benign or non-life-threatening disease.

Our ultimate clinical aim is to develop a blood test for early detection of breast cancer that can be used to complement mammography (±ultrasound). Our novel approach builds on our observation that human breast cancer-derived protein tumor markers can be identified in the blood of "avatar mice" that harbor human breast cancer xenografts. (Since transplanted breast cancer xenografts are the only human tissue present in host mice, uniquely human proteins identified in the mouse plasma must be derived from the transplanted tumor.) We hypothesize that a panel of these plasma-borne tumor biomarkers can be used as a clinical diagnostic for early detection of breast cancer in women. We propose a novel strategy to conduct the initial discovery of candidate biomarkers in the plasma of "avatar mice," followed by triage and validation of candidate breast cancer biomarkers in humans.

Using this approach, we identified 1,314 candidate breast cancer protein biomarkers. 90% of these proteins have been detected in human breast cancer tissue, and a subset has not previously been detected in normal breast epithelial cells nor in the plasma of non-cancer patients. 5,178 peptides to the candidate proteins were quantified by targeted proteomic analysis on a Thermo Eclipse Tribrid Orbitrap mass spectrometer using a SureQuant method. Briefly, this method quantifies heavy-labeled peptide standards and the corresponding endogenous ("light") peptide by first observing and identifying the spiked-in heavy peptide which then triggers a high-resolution MS2 scan of the light peptide. Both the heavy and light versions of the peptide are quantified using a method called parallel reaction monitoring (PRM). PRM allows conclusive peptide sequence identification and relative quantification of the endogenous peptide, which is reported as the peak area ratio (PAR) of the light over heavy peptide peak areas.

The candidate proteins were further vetted in plasma of human breast cancer patients. 138 samples were received from the EDRN biorepository, depleted of high- and mid-abundance proteins and pooled into 7 samples (4 breast cancer subtype pools and 3 confounding controls). The pools underwent proteolytic digestion, spiked with all 5,178 heavy peptide standards, fractionated by basic reverse phase (bRP) liquid chromatography into 24 fractions, and evaluated using the SureQuant method. Nearly all heavy peptides were targeted for quantification in the 7 plasma pools (98.8% - 99.6%) with most resulting in a good heavy signal (82.0% - 88.6%). We measured endogenous signals (light signals that were > 2x above the blank runs) for 28.8% - 34.6% of the target peptides, corresponding to a majority of the 1,314 candidate proteins (52.8% - 60.9%). The analytical reproducibility of endogenous measurements was determined by comparing PAR in neighboring bRP fractions. The median relative difference was 11.0%, and 75% were below 20%.

Preliminary candidate biomarker validation has focused on the triple negative breast cancer (TNBC) plasma pool. The goal was to prioritize proteins elevated in TNBC plasma compared to 3 control pools in the 734 candidate proteins with endogenous signal measured in these 4 pools. A rank order analysis identified 67 proteins higher in the TNBC plasma pool with a p-value < 0.01, and 102 proteins with a p-values < 0.1. Future work will extend this analysis to the other breast cancer plasma pools.

**Category:** Bioinformatics **Color:** Blue **Poster Board #:** 3

**LabCAS: An Integrated Data-Intensive Computing Environment for Archiving, Processing and Sharing Data**

Heather Kincaid, NASA Jet Propulsion Laboratory, Alphan Altinok, PhD, NASA Jet Propulsion Laboratory, Chris Amos, PhD, Baylor College of Medicine, Kristen Anton, University of North Carolina, Chapel Hill, Alexander D Borowsky, MD, University of California, Davis, Luca Cinquini, PhD, NASA Jet Propulsion Laboratory, Maureen Ryan Colbert, University of North Carolina, Chapel Hill, Daniel Crichton, NASA Jet Propulsion Laboratory, John Heine, PhD, H.Lee Moffitt Cancer Center and Research Institute, Olivier Harismendy, PhD, University of California, San Diego, Sean Kelly, NASA Jet Propulsion Laboratory, David Liu, NASA Jet Propulsion Laboratory, Joseph Lo, PhD, Duke University Medical Center, Ashish Mahabal, PhD, California Institute of Technology, Anirban Maitra, MBBS, MD Anderson Cancer Research Center, Jeffrey Marks, PhD, Duke University Medical Center, Asitang Mishra, NASA Jet Propulsion Laboratory, Christos Patriotis, PhD, National Cancer Institute, Yinhao Ren, Duke University Medical Center, Sudhir Srivastava, PhD, MPH, National Cancer Institute, Radka Stoyanova, PhD, University of Miami

## Abstract

LabCAS ("Laboratory Catalog and Archive Services") is a web-enabled environment that provides a comprehensive suite of services for managing scientific data sets captured in biomedical research through its full lifecycle, supporting both pre-publication restricted access and post-publication open access. The LabCAS architecture is composed of a front-end web portal, where users can login to browse, inspect, visualize and download data; and a back-end software stack that exposes a rich set of data and metadata APIs for programmatic client access. The LabCAS front-end allows for external tool integration to provide visualization and other analytical tools to be plugged-in. Currently in LabCAS, image files such as DICOM, svs, scn, and tiff can be viewed in an integrated viewer (the Digital Slide Archive or DSA) to provide seamless visualization and interactivity.

Additionally, LabCAS can be used to execute data intensive processing pipelines as structured workflows with scalable computation on the cloud. It also provides customizable user input, automatic publication of output with controlled access and allows for repeatable and reproducible results.

This poster will present a general overview of LabCAS, the open-source image viewer and examples of existing machine learning algorithms plugged into a processing pipeline as a structured workflow.

**Category:** Biomarker **Color:** Green **Poster Board #:** 5

**Prognostic Markers of Pancreatic Cancer: HOXA10 and its Driven Signature**

Sophia G. Kisling, BS, University of Nebraska Medical Center, Pranita Atri, MTech, University of Nebraska Medical Center, Sunandini Sharma, MS, University of Nebraska Medical Center, Surinder K. Batra, PhD, University of Nebraska Medical Center, Sukhwinder Kaur, PhD, University of Nebraska Medical Center

**Abstract**

Pancreatic cancer (PC) is a highly lethal malignancy with a five-year survival rate of less than 10%. This is partly due to the late stage at which the disease is diagnosed and the high level of therapy resistance seen in many cases. The high heterogeneity and presence of molecular subtypes in PC are a significant factor in this resistance, and there are currently no effective treatment modalities to address these challenges. Moreover, although more subtypes are being identified and studied every year, little efforts have been made to identify the underlying pathway(s) associated with pancreatic cancer's poor prognosis. Identifying a prognosis-based gene signature may be the key to addressing these gaps and improving patient outcomes. Therefore, we hypothesize that identifying a prognostic signature associated with short-term survivors will elucidate novel pathways and lead to the development of novel markers and therapies for optimal targeting of PC. Analyses of transcriptomic profiles of long- and short-term PC survivors followed by gene set enrichment analysis (GSEA) led to identifying a short-surviving prognostic subtype with a high-degree of significance (p-value=0.018). Interestingly, a plethora of genes (130 genes) in this novel subtype were found to be regulated by a master regulator, HOXA10, and 69 genes were found to be significantly differentially expressed in short-term survivors compared to long-term survivors. These genes are novel compared to traditional biomarkers of pancreatic cancer prognosis, including carbohydrate antigen 19-9, due to their associations with immune suppression, enhanced tumorigenesis, and chemoresistance, which was demonstrated by pathway analysis. Overall, our in silico and human PC tissue analyses revealed distinct groups of long- and short-term survivors of PC and enhanced expression of HOXA10 and its gene signature in short-term survivors of PC compared to long-term survivors. This indicates that there may be a potential link between HOXA10 expression and the advancement of PC, and validating and targeting HOXA10 could potentially improve the management of pancreatic tumors. Further studies are underway to validate this short-term survival signature (69 genes) at the tissue and serum level in PDAC patient samples and determine the mechanistic significance of this signature. In the future, we hope to develop a HOXA10-based biomarker panel that can differentiate long- and short-term survivors for specific therapeutic targeting.

**Category:** Biomarker **Color:** Green **Poster Board #:** 11

**Identification of Aggressive Prostate Cancers: In-depth Proteomics of Tissues and Urines**

Amanda Khoo, University of Toronto, Zhuyu Qui, University of California, Los Angeles, Joseph Otto, Eastern Virginia Medical School, Vanessa Correll, Eastern Virginia Medical School, Andrew Macklin, Princess Margaret Cancer Centre, Katharina Fritsch, University of Toronto, Vladimir Ignatchenko, Princess Margaret Cancer Centre, Meinusha Govindarajan, University of Toronto, Lydia Liu, University of Toronto, Julie Livingstone, University of California, Los Angeles, Lifting Yang, Eastern Virginia Medical School, Brian Main, Eastern Virginia Medical School, Danny Vesprini, Sunnybrook Health Sciences Centre, Stanley Liu, Sunnybrook Health Sciences Centre, Julius O. Nyalwidhe, Eastern Virginia Medical School, O. John Semmes, Eastern Virginia Medical School, Paul Boutros, University of California, Los Angeles, Thomas Kislinger, University of Toronto

For the 135,000 North American men diagnosed with low- or intermediate-risk prostate cancer (PC) each year, clinical outcomes are heterogeneous: 50-80% will be disease-free 10 years following curative-intent therapy while 10-20% will experience recurrence within 18 months, portending lethality. Conversely, 25% diagnosed with low-risk PC elect to enter Active Surveillance (AS), where their disease is monitored by repeat prostate-specific antigen (PSA) tests and ultrasound guided biopsy to rule out the presence of aggressive PC. Current clinical prognostic factors (Gleason Score, PSA, and T stage) do not accurately predict disease aggression and clinical outcome for individual men resulting in under-treatment of occult aggressive disease and over-treatment of indolent disease. Our prostate cancer proteomics program has analyzed 1676 samples from 656 individual patients. All proteomics data is performed by label-free shotgun proteomics using 2-hour or 4-hour nano-flow UPLC gradient coupled to either a Q-Exactive HF or an Orbitrap Fusion mass spectrometer. Obtained data is searched by MaxQuant and resulting output files are parsed into a relational database for downstream analysis. Samples were process either by MStern for total proteome analyses or via ultracentrifugation to obtain extracellular vesicles. We have applied proteomics analyses of direct expressed prostatic secretions (dEPS), post-DRE-urines and frozen tissues to identify novel proteomics and proteogenomics signatures of aggressive disease. Combining comprehensive proteomics profiling of stratified dEPS fluids with targeted proteomics and computational biology we discovered robust proteomic signatures for extracapsular prostate cancer (Kim et al. Nat Commun. 2016). We are extending on these discoveries by developing novel approaches for in-depth proteomics profiling of prostate fluids stratified into low, intermediate and high-risk prostate cancer. Our goals are to develop liquid biopsy signatures to follow patients on active surveillance. Our data contains both quantitative information for soluble proteins, extracellular vesicle cargo and tissue protein expression. All data has been parsed into a relational database that currently contains quantitative data for over 9,000 proteins. Integration of these data with the rich clinical annotation will enable objective data mining and selection of candidate biomarkers for validation by targeted proteomics via Parallel Reaction Monitoring Mass Spectrometry (PRM-MS).

**Category:** Biospecimens **Color:** Blue **Poster Board #:** 7

**Roles and Collaborations of DOD-USU-CPDR under NCI-EDRN**

Indu Kohaar[1,2], Shyh-Han Tan[1,2], Jacob Kagan[3], Sudhir Srivastava[3], Albert Dobi[1,2], Gyorgy Petrovics[1,2], Gregory Chesnut[1,4]

[1]Center for Prostate Disease Research, Murtha Cancer Center Research Program, Department of Surgery, Uniformed Services University of the Health, Bethesda, Maryland, 20817, USA;
[2]Henry M. Jackson Foundation for the Advancement of Military Medicine Bethesda, Maryland, 20817 USA;
[3]Cancer Biomarkers Research Group, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, 20817 USA;
[4]Urology Service, Walter Reed National Military Medical Center, Bethesda, Maryland, 20814, USA

## Abstract

The goal of this abstract is to highlight the roles of CPDR under the Inter-Agency Agreement (IAA) between the National Cancer Institute-Early Detection Research Network (NCI-EDRN) and the Uniformed Services University-Center for Prostate Disease Research (USU-CPDR) that started on August 5, 2017. CPDR contributes to the overall mission of the EDRN to identify, develop, and validate biomarkers to improve the detection of early-stage cancers and risk assessment.

Some of the key features of our program which makes it well suited for the EDRN program include:

1. High representation of disparately affected African American men
2. Equal access to DOD Military Healthcare System,
3. Long clinical follow-up (up to 25 years)
4. CAP accredited biospecimen bank
5. Committed long term personnel
6. State of the art technology.

The CPDR Biospecimen bank has high quality and well annotated biospecimens, including both formalin-fixed paraffin-embedded (FFPE) and frozen prostate cancer tissue specimens, as well as urine and blood biofluids that contain circulating tumor cells (CTC), circulating cell free DNA (cfDNA) and exosomes. We use innovative methods such as next generation sequencing (NGS), fluorescent in situ hybridization (FISH), NanoString, Droplet Digital PCR (ddPCR) to study the genomic and transcriptomic profiles, and immunochemistry (IHC), multiplexed Enzyme Linked Immunosorbent Assay (ELISA) and mass spectrometry to evaluate proteomic spectrum of these biospecimens. The CPDR biospecimen derived molecular database developed over the years (WGS, RNA Seq, Proteome, Lipidome, Metabolome, Oncoarray SNP, Digital images) has been linked with the CPDR National Clinical database, which places CPDR in a unique setting under the EDRN infrastructure.

The specific tasks described under the current IAA are as follows:

1. Provide molecular pathology resource for tissue-based validation of EDRN discoveries of key molecular alterations
2. Enrichment of prostate cancer epithelial cells and other cell types by Laser Capture Microdissection (LCM) and manual microdissection to support the development of antibody free mass spectrometry assays (SRM-MS and PRM-MS) for fast and efficient

identification and verification of candidate biomarkers

3. Support EDRN GU group's collaborative projects by leveraging the unique biospecimens and database resources from the longitudinally followed and racially diverse DOD patient cohort
4. Evaluation of race and ethnicity optimized prostate cancer gene panels and assays

USU-CPDR as a reference laboratory under EDRN is collaborating with other EDRN laboratories by providing unique biospecimens and database resources. Details of our ongoing collaborations under EDRN are listed in following table.

| **Collaborating Institution** | **Project** |
|---|---|
| **Pacific Northwest National Laboratory (DOE)** | |
| Dr. Karin Rodland, Dr. Tao Liu | Antibody free mass spectrometry assays (SRM-MS and PRM-MS) for the early identification of biomarkers for aggressive prostate cancer |
| **Johns Hopkins University** | |
| Dr. Hui Zhang | Prostate cancer glyco-protein markers for early detection of aggressive prostate cancer at diagnosis using urine specimens |
| Dr. Daniel Chan | Serum marker validation study: Early prostate cancer diagnostic and prognostic marker panels using serum specimens |
| **University of Texas Health Science Center San Antonio** | |
| Dr. Robin Leach | Predictive prognostic markers for differentiating indolent vs. progressive disease using tissues |
| **University of Michigan** | |
| Dr. John Wei | Urine marker pre-validation study: Early prostate cancer diagnostic and prognostic marker panels using urine |
| **National Institute of Standards and Technology** | |
| Dr. Kenneth Cole | Development of cfDNA based reference biomarker |
| Dr. Lili Wang | Characterization of Extracellular vesicles in clinical specimens |
| **Joint Pathology Center (DOD)** | |
| Dr. Isabell Sesterhenn, Dr. Joel T. Moncur | Molecular pathology resource for tissue-based validation of EDRN discoveries of key molecular alterations |

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**Category:** Biomarker **Color:** Purple **Poster Board #:** 10

**Non-Small Cell Lung Cancer and Peripheral Immunity: Mining the Tumor Macroenvironment**

Andrew V. Kossenkov, PhD, The Wistar Institute, Rehman Qureshi, PhD, The Wistar Institute, Kiranmai Gumireddy, PhD, The Wistar Institute, Jayamanna Wickramasinghe, PhD, The Wistar Institute, R., Sonali Majumder, MS, The Wistar Institute, Sandy Widura, MS, The Wistar Institute, Qin Liu, PhD, The Wistar Institute, Harvey Pass, MD, VA New York Harbor Healthcare System, Sai Yendamuri, MD, Roswell Park Cancer Institute, Anil Vachani, MD, University of Pennsylvania Perelman School of Medicine, Thomas Bauer, MD, present address Meridian Health, N.J., Brian Nam, MD, Helen F. Graham Cancer Center, William Rom, MD, NYU Langone Health, Jun-Chieh Tsay, MD, VA New York Harbor Healthcare System, Michael K. Showe, PhD, The Wistar Institute, Louise C. Showe, PhD, The Wistar Institute

## Abstract

We recently established that gene expression in PAXgene stabilized blood RNA distinguishes benign (BN) and malignant (MN) pulmonary nodules in high risk candidates (AUC 0.84) based on a biomarker panel designed using cohort of 283 patients. We now expand our studies to include PAXgene expression data from 514 high risk patients with benign or malignant pulmonary nodules and focus on the immunological and physiological differences associated with presence of a benign or malignant pulmonary nodule. This study identifies significant increases in expression of genes promoting cell death and significant decreases in expression of genes with functions promoting transcription, lymphogenesis and cell viability are associated with the presence of a malignant lung nodule. Patients with malignant nodules also exhibit a preferential use of oxidative phosphorylation and a gene signature associated with mitochondrial dysfunction. *In silico* flow cytometry using CIBERSORT, identifies differences in proportions of γδT-cells, M0 macrophages, NK cells, B cells and exhausted CD8 T-cells in the periphery of patients with malignant compared to benign lung nodules. The study also identifies differences associated with a number of demographic factors including gender, race, nodule type and cancer stage. Most notable are differences associated with race showing lower numbers of M0 macrophages and neutrophils and proportionally more CD8 and naïve CD4 T cells in African American patients. Although the findings are based on a relatively small patient subset, they may be relevant to poorer outcomes, despite reduced tobacco exposure, in AA's. We also find that naïve CD4 T cells are more numerous in females while monocyte numbers are proportionately lower. Overall, Naïve CD4 T cells decreased with age while resting NKs increased.

**Category:** Biomarker **Color:** Green **Poster Board #:** 3

# High Performance in Risk Stratification of Intraductal Papillary Mucinous Neoplasms by EUS-guided Confocal Laser Endomicroscopy

Somashekar G. Krishna, MD, MPH, The Ohio State University Wexner Medical Center; Wei-Lun Chao, PhD; David E. Carlyn, BS; Tai-Yu Pan, MS; Kyle Porter, MS; Department of Computer Science and Engineering, College of Engineering, The Ohio State University, Phil A. Hart, MD; Georgios I. Papachristou, MD, PhD; Zobeida Cruz-Monserrate, PhD; Darwin L. Conwell, MD, MS The Ohio State University Wexner Medical Center, Columbus, Ohio

## Abstract

**Background and Aims:** Intraductal papillary mucinous neoplasm (IPMNs) are the most common precancerous pancreatic cystic neoplasms. Current diagnostic modalities are suboptimal in risk stratification of IPMNs. Accurate detection of high-grade dysplasia/adenocarcinoma (HGD-Ca) and surgical resection of such cysts is critical to prevent progression of IPMNs to pancreatic cancer. In a derivation study, we have previously shown that endoscopic ultrasound-guided needle-based confocal laser endomicroscopy (EUS-nCLE) in vivo image-guided quantification of papillary epithelial width and darkness identified high-grade dysplasia/adenocarcinoma (HGD-Ca) in intraductal papillary mucinous neoplasm (IPMNs) with high accuracy. However, manual real-time in vivo risk stratification of IPMN during EUS-nCLE requires expertise in interpretation and is labor-intensive. Hence, we sought to derive predictive computer-aided diagnosis (CAD) and artificial intelligence (AI) algorithms to facilitate accurate diagnosis and risk stratification of IPMNs.

**Methods**: A post-hoc analysis of a single-center prospective study evaluating EUS-nCLE (2015-2019; INDEX study; NCT02516488) was conducted using 15,027 video frames from 35 consecutive subjects with histopathologically proven IPMNs (18 with HGD-Ca). We designed a CAD-convolutional neural network (CNN) algorithm - a reasonably agnostic holistic-based model where the CNN-AI system automatically extracted nCLE features for risk stratification. For the detection of HGD-Ca in IPMNs, the diagnostic performance of the CNN-CAD algorithms was compared to that of American Gastroenterological Association (AGA) and revised (2017) Fukuoka international consensus guidelines.

**Results:** Applying the Fukuoka high-risk criteria to detect HGD-Ca, we found a sensitivity of 55.6% (95% CI, 31-79%), specificity of 94.1% (95% CI, 71-100%), and accuracy of 74.3% (95% CI, 57-88%). The sensitivity (55.6%; 95% CI, 31-79%) of AGA criteria was similar to Fukuoka guidelines; however, the specificity (82.4%; 95% CI, 57-96%), and accuracy (68.6%; 95% CI, 51-83%) were lower. Compared to standard of care guidelines, the n-CLE-image (video) CNN-CAD algorithms yielded higher sensitivity (83.3%, 95% CI, 59-96%) and accuracy (85.7%, (95% CI, 67-95%) for diagnosing HGD-Ca, with comparable specificity 88.2%, (95% CI, 64-99%).

**Conclusion:** EUS-nCLE based CNN-CAD algorithms can accurately risk stratify IPMNs. Future multicenter validation studies and AI model improvements could enhance the accuracy and fully automatize real-time in vivo interpretation during EUS evaluation of pancreatic cysts.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 2

# Identification of Autoantibody Biomarkers against Post-Translationally Modified Proteins for the Early Detection of Breast Cancer

Lusheng Song[1], Kailash Karthikeyan[1], Jin Park, PhD[1], Karen S. Anderson, MD, PhD[1], Ji Qiu, PhD[1], Joshua LaBaer, MD, PhD[1]
[1]Center for Personalized Diagnostics, The Biodesign Institute, Arizona State University, Tempe, Arizona.

## Background

Blood-based biomarkers that are sensitive and specific for early and determinate diagnostic, which would improve cancer detection, are urgently needed. Our breast cancer autoantibody (AAb) biomarkers that target tumor antigens produced by cancer cells, have been licensed and integrated into Videssa™ Breast that is now available as CLIA-certified test. These AAbs are targeted native proteins. We hypothesize that aberrant protein glycosylation, a hallmark of breast cancer, will induce glycoprotein-specific tumor AAb to be measured as specific serum biomarkers for breast cancer.

## Methods

We have developed a contra capture protein array (CCPA) platform that could display thousands of proteins. A total of 1,600 breast cancer-related glycoproteins comprising 1) membrane and secreted proteins highly expressed in breast cancer; 2) previously reported O-glycosylated proteins; 3) all mucins; and 4) previously reported autoantigens in breast cancer are cloned and displayed on CCPA. These proteins displayed on CCPA are either kept as native (un-modified), or Tn, sTn, core3 glycosylated, and IgG and IgM AAbs are assessed using fluorescence-based array analysis. We collect a discovery sample set, 40 pairs of sera from breast cancer patients and controls with benign breast disease, both with BIRADS 3/4 mammography. The candidate biomarkers found are validated for each glycosylation sets using a validation sample set, an independent set of 140 cases, and 40 controls.

## Results

Native CCPA arrays with 1,600 breast cancer-related glycoproteins (described in methods) are successfully produced, and a following generated Tn, STn, and Core3 glycosylated protein on CCPA are developed and verified. We assess the serology response of 40 paired breast cancer and benign breast disease sera, both with BIRADS 3/4 mammography, against all four sets of glycoproteins. For each protein sets, there are AAbs, candidate biomarkers, that could differentiate cancer and control: Native (45 IgG, 132 IgM), Tn (34 IgG, 79 IgM), sTn (25 IgG, 53 IgM), and Core3 (35 IgG, 88 IgM). These AAbs are not overlapped between IgG and IgM of the same protein modification, nor overlapped between all four sets of proteins. We will validate these proteins/glycosylations using the validation sample sets against CCPA with candidate biomarkers' targets.

## Conclusions

We found a significant amount of AAbs difference between breast cancer and benign disease patients' serum. The AAbs that target native proteins we found here are similar to previous studies. Interestingly, we discovered three sets of AAbs that target Tn, sTn, or Core3 glycosylated proteins. These AAbs are not highly overlapped between all four protein sets, which echoes our hypothesis that glycosylated protein may be another candidate biomarker

for a breast cancer diagnostic. Our CCPA is a unique platform that could study both the native and modified protein in a high-throughput format, which would open up opportunities for studies of other modifications in breast and other cancers.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 19

**Validation of Early Detection Biomarkers for Colorectal Cancer**

Jon J Ladd, PhD, Fred Hutchinson Cancer Research Center, Seattle, WA
Ib Christensen, PhD, Department of Surgical Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark
Dave Shelley, MS, Fred Hutchinson Cancer Research Center, Seattle, WA
Hans J Nielsen, MD, Department of Surgical Gastroenterology, Hvidovre Hospital, Hvidovre, Denmark
Yuzheng Zhang, MS, Fred Hutchinson Cancer Research Center, Seattle, WA
Christopher Li, MD, Fred Hutchinson Cancer Research Center, Seattle, WA
Paul D. Lampe, PhD, Fred Hutchinson Cancer Research Center, Seattle, WA

## Abstract

Colon cancer is the second leading cause of cancer death worldwide. Approximately 40% of adults over the age of 50 do not participate in adequate colorectal cancer screening (e.g., Colonoscopy, Sigmoidoscopy, Fecal Occult Blood Test (FOBT)), even though it is well-established that early detection of colon cancer can save lives. Development of minimally invasive, blood-based biomarkers for colorectal cancer may help replace or augment current screening guidelines and increase compliance. High-density antibody arrays are an effective method to unbiasedly discover and validate markers in samples due to the high affinity and specificity of printed antibodies. Here we show results from a panel of five proteomic markers and glycan modifications on two of those markers in multiple diagnostic and prediagnostic cohorts including colorectal cancers and adenomas. Due to limitations on the availability of the commercial antibodies for some of these markers, epitope mapping was performed, and replacement monoclonal antibodies were produced, screened, and selected. These antibodies were then incorporated into more clinically relevant sandwich ELISA and Chemiluminescence assays. These assays were then performed on a large (~4,700 samples) screening cohort from Hvidovre Hospital in Denmark. A subset of preliminary results is reported here, showing individual marker performance in the screening cohort.

**Category:** Biospecimens **Color:** Blue **Poster Board #:** 5

# Establishing a Cohort and a Biorepository to Identify Biomarkers for Early Detection of Lung Cancer: The Nashville Lung Cancer Screening Trial Cohort

Dhairya A. Lakhani, MD[1,2,3], Sheau-Chiann Chen, PhD[4], Sanja Antic, MD[2], Anel Muterspaugh MPH.[2], Christine Cook, MD[1], Nancy Liu, BS[1], Hina Shujat, MD[2], Sophie Jouan, BS[2], Brandon Winston, BS[2], Kim Fields, BA[2], Janelle Wenstrup, BS[2], Sara Block, MS[5], Amy Hinton, RN[6], Alexandra Miller, BS[6], Sarah Atmajoana, BS[2], John Helton, RN[6], Khushbu Patel, MD[2], Aneri Balar, MD[2], Katrina Brewer, MS [2], Subodh Nag, PhD[7], Rajbir Singh, MD[7], Anthony Disher, MD[7], Luis Huerta, MD[7], Richard Fremont, MD[7], Otis Rickman, DO[1,2], Heidi Chen, PhD[4], Rosana Eisenberg, MD[8], Kim Sandler, MD[9], Alexis Paulson, APRN[9], Ronald Walker, MD[6,9], Chirayu Shah, MD[6,9], Gary Smith, MD[6,9], Bennett Landman, PhD[9,10], Stephen Deppen, PhD[1,2,6,11], Eric Grogan, MD[6 11], Melinda C. Aldrich, MPH, PhD[1,5], Pierre P. Massion, MD[1,2,5,6,9]

[1]Department of Medicine; [2]Division of Allergy, Pulmonary and Critical Care, Vanderbilt University Medical Center; [3]Department of Radiology, West Virginia University; [4]Department of Biostatistics; [5]Vanderbilt Ingram Cancer Center; [6]Veterans Affairs Tennessee Valley Health Care System, Nashville, TN; [7]Meharry Medical Center, Nashville TN; [8]Division of Pathology; [9]Department of Radiology and Radiological Sciences; [10]Department of Electrical Engineering; [11]Department of Thoracic Surgery, Vanderbilt University Medical Center, Nashville, TN.

## Abstract

**Rationale:** A prospective longitudinal cohort of individuals at high-risk of developing lung cancer was established to build a biorepository of carefully annotated biological specimens and low-dose computed tomography (LDCT) chest images for derivation and validation of candidate biomarkers for early detection of lung cancer. The goal of this study is to characterize individuals with high-risk for lung cancer, accumulating valuable biospecimens and LDCT chest scan longitudinally over five years.

**Methods:** Participants 55-80 years of age and with a 5-year estimated risk of developing lung cancer greater than 1.5% were recruited and enrolled from clinics at Vanderbilt University Medical Center, the Veteran Affairs Medical Center, and Meharry Medical Center. Individual demographic characteristics were assessed via questionnaire at baseline. Participants underwent a LDCT scan, spirometry, sputum cytology, and research bronchoscopy at the time of enrollment. Participants will be followed yearly for five years. Positive LDCT scans are followed-up according to standard of care. The clinical, imaging and biospecimen data are collected prospectively and stored in a biorepository.

**Results:** A total of 480 participants were enrolled at study baseline and consented to sharing of their data and biospecimens for research. Participants are followed with yearly clinic visits to collect imaging data and biospecimens. We report 409 prevalent nodules at baseline among 223 individuals with a median maximum diameter of 6 mm for the benign nodules and 14 mm for the cancers. To date, a total of 19 cancers (13 adenocarcinomas, 4 squamous cell carcinoma, 1 large cell neuroendocrine and 1 small cell lung cancer) have been identified. Among these cancers, 64% are early stage. Ten were diagnosed at study baseline and 9 were incident cancers. Three participants had a lung-RADS 1 and went on to develop lung cancer during the study. A total of 4298 biospecimens were collected at baseline including bronchial samples from 212 participants.

**Conclusion:** We established a unique prospective cohort of individuals at high-risk for lung cancer, enrolled at three institutions for which full clinical data, well-annotated LDCT and biospecimens are being collected longitudinally. This repository will allow for the derivation of independent validation of clinical, imaging and molecular biomarkers of risk or diagnosis of lung cancer. Clinical trial registered with ClinicalTrials.gov (NCT01475500).

**Category:** Biomarker **Color:** Green **Poster Board #:** 13

# Deletion of Either *CHD1* or *LSAMP* Genes Indicates Rapid Disease Progression in African American Prostate Cancer Patients

Hua Li[1], Denise Young[1], Jiji Jiang[1], Indu Kohaar[1], Isabell Sesterhenn[1,2], Gregory Chestnut[1,3], Gyorgy Petrovics[1] and Albert Dobi[1]

1 Center for Prostate Disease Research, John P. Murtha Cancer Center, Department of Surgery, Uniformed Services University of the Health Sciences and the Walter Reed National Military Medical Center, Bethesda, Maryland, USA

2 Genitourinary Pathology, Joint Pathology Center, Silver Spring, Maryland, USA

3 Urological Services, Walter Reed National Military Medical Center, Bethesda, Maryland, USA

USU-CPDR continues to serve as NCI-EDRN Biomarker Reference Laboratory (BRL) by evaluating biologically significant prostate cancer gene alterations and promising biomarker candidates in specimens from defined CPDR and EDRN patient cohorts using innovative molecular assays. One of our major tasks is to evaluate chromosomal alterations (e.g., in *CHD1*, *LSAMP)* by FISH assay, and determine the prognostic associations of these molecular aberrations in prostate cancer. This approach is based on the rationale that carefully designed FISH assays against the right candidate biomarkers can be rapidly translated to detect and stratify prostate tumors for effective management of the disease.

Here, we report that a panel of *LSAMP* or *CHD1* deletion captures 66% of African American (AA) prostate cancer patients with BCR events. We further evaluated the associations between *LSAMP* and *CHD1* deletions with aggressive clinico-pathologic features including metastasis, and higher pathology stages and Gleason scores in a cohort of 42 AA and 59 Caucasian American (CA) patients. We found that the chromosomal alterations in these genes are significantly more frequent in AA men as compared to CA men (65.5% vs 34.5%). Additionally, these deletions are strongly associated to higher pathological grade group, pathology T stage and Biochemical Recurrence (BCR) outcome. Kaplan-Meier analysis for time to BCR as a function of genomic deletions in *LSAMP* or *CHD1* indicated that there is a significant association (log-rank p value = 0.0001) between deletions in *LSAMP* or *CHD1* regions and BCR over 25 years of clinical follow up after radical prostatectomy in prostate cancer patients, particularly AA men. Multivariate Cox proportional hazard analysis after adjusting for the co-variables found that *LSAMP* and *CHD1* deletions are highly associated with higher pT stage (p= 0.001; HR 6.9) and an independent indicator of BCR (p= 0.01; HR 3.27).

Unlike CA patients, the incidence and mortality rates of prostate cancer in much higher in AA men. However, lack of prognostic and therapeutic targets in AA patients further hinders the development of efficient early prognostic evaluation strategies. Our progress further highlighted the importance and urgency to identify specific biomarkers in diverse populations for early detection and prognostic evaluation of prostate cancer patients.

**Disclaimers:** The contents of this publication are the sole responsibility of the author(s) and do not necessarily reflect the views, opinions or policies of Uniformed Services University of the Health Sciences (USUHS), the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., the Department of Defense (DoD) or the Departments of the Army, Navy, or Air Force. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. Government.

**Category:** Biomarker **Color:** Green **Poster Board #:** 17

# Characterization of Primary and Metastatic Prostate Cancer Using Proteomics and Transcriptomics Reveals Reduced Expression of Proteinases and Accumulation of Protease DPP4 Substrate Peptide in Aggressive Tumors

Qing Kay Li[1,2], MD, PhD; Jing Chen[1], PhD; Yingwei Hu[1], PhD; Naseruddin Höti[1], PhD; Stefani N. Thomas[1], PhD; Li Chen[1], PhD; Punit Shah[1], PhD; Lijun Chen[1], PhD; G. Steven Bova[3], MD; Bai Zhang[1], PhD; and Hui Zhang[1,2], PhD.

1. Department of Pathology, Johns Hopkins University, Baltimore, MD, USA
2. Department of Oncology, Sidney Kimmel Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD, USA
3. Faculty of Medicine and Health Technology, Tampere University and Tays Cancer Center, Tampere, FI-33014, Finland

## Abstract

Our previous study has indicated a critical role of certain proteinases in prostate cancer (PCa). To better understand protein signature and the functional consequences of tumor progression, and the useful features to separate aggressive PCa (AG) from non-aggressive PCa (NAG) PCa, we analyzed 96 PCa tumor tissues (including 48 primary and 48 metastatic cancers) and 12 normal prostate tissues using SWATH–MS proteomic platform. Among 4,415 proteins identified and quantified, 138 up-regulated and 52 down-regulated proteins were detected in AG PCa.

Analysis of tumors-associated proteins using proteomics, transcriptomics, and bioinformatics revealed that the expression of several proteinases, including dipeptidyl peptidase 4 (DPP4), carboxypeptidase E (CPE) and prostate specific antigen (KLK3), was significantly reduced in both AG and metastatic PCa. The decreased expression of DPP4 in AG was further validated using the immunochemical stains. A targeted re-analysis using SWATH-MS spectra further identified that the decreased expression of DPP4 was correlated with the accumulation of DPP4 substrate peptide, neuropeptide Y (NPY), and the reduction of the ratio of corresponding DPP4 cleavage peptide to DPP substrate.

Our study is the first large-scale integrated analysis of primary and metastatic prostate cancer. The SWATH-MS spectrum maps and protein profiles are used to investigate potential mechanisms of protease activities involved in the PCa progression using targeted spectrum-searching approach. Our findings not only provide an interrogative data of PCa, but also indicate the association of protease activities in PCa progression, our findings can be used for the development of a clinical tool to detect protease activities for distinguishing aggressive PCa from indolent tumors.

**Category:** Biomarker **Color:** Green **Poster Board #:** 12

**Development Of Parallel Reaction Monitoring (PRM) Assays for the Detection of Aggressive Prostate Cancer Using Urinary Glycoproteins**

Tung-Shing M. Lih, Ph.D., Johns Hopkins School of Medicine, Mingming Dong, Naseruddin Höti, Shao-Yung Chen, Sean Ponce, Alan Partin, Hui Zhang, Johns Hopkins School of Medicine

## Abstract

Currently, there are unmet clinical needs for non-invasive and robust tests for distinguishing aggressive (Gleason ≥ 8) and non-aggressive (Gleason score = 6) prostate cancer (PCa) to guide risk stratification and treatment selection. We have recently found two urinary glycoproteins, prostatic acid phosphatase (ACPP) and clusterin (CLU) combined with serum prostate specific antigen (PSA) can serve as a three-signature panel for detecting aggressive PCa based on a quantitative glycoproteomic study. To facilitate the translation of candidates into clinically applicable tests, robust and accurate targeted parallel reaction monitoring (PRM) assays that can be widely adopted in multi-labs were developed in this study. We developed and characterized the targeted PRM assays for the urinary glycopeptides, FLN*ESYK from ACPP and EDALN*ETR from CLU. The PRM assays were further applied to the quantitative analysis of urine specimens collected from 69 non-aggressive and 73 aggressive PCa patients to assess performance of assays in differentiating the two groups. The results elucidated that when ACPP combined with CLU, the discrimination power was improved from an AUC of 0.66 to 0.78. Notably, when ACPP, CLU and serum PSA were combined together to form a three-signature panel, the AUC was further improved to 0.83 (84.9% sensitivity and 68.1% specificity). Since serum PSA test itself only produced an AUC of 0.68, our results demonstrated that the new urinary glycopeptide PRM assays can serve as an adjunct to serum PSA test to achieve better predictive power toward aggressive PCa. In summary, our developed PRM assays for urinary glycopeptides were successfully applied to clinical PCa urine samples with promising performance in aggressive PCa detection.

**Category:** Methods **Color:** Orange **Poster Board #:** 2

**Feasibility of Targeted Proteomics Analysis of Prostate Cancer Progression Biomarkers in FFPE Biopsy Specimens**

Tao Liu, PhD, Pacific Northwest National Laboratory, Yuqian Gao, PhD, Yi-Ting Wang, PhD, Tujin Shi, PhD, Athena Schepmoes, BS, Thomas Fillmore, BS, Pacific Northwest National Laboratory, Robert Hudson, BS, University of Texas Health San Antonio, Jacob Kagan, Sudhir Srivastava, PhD, National Cancer Institute, Robin Leach, PhD, University of Texas Health San Antonio, and Karin Rodland, PhD, Pacific Northwest National Laboratory

## Abstract

A critical unmet need facing the physician and patient is to distinguish early between indolent and aggressive prostate cancer, so that the proper treatment can be selected while overtreatment of indolent disease can be avoided. To address this critical need, we have recently developed a tissue-based proteomic classifier, detectable in FFPE radical prostatectomy (RP) samples from men with organ-confined prostate cancer, that improves the ability to stratify patients for risk of progression, e.g., biochemical recurrence (BCR) after prostatectomy or distant metastasis (DM). An immediate objective is to validate the tissue-based proteomic classifier in an independent clinical cohort. A second objective is to address the problem of over-diagnosis of prostate cancer by testing classifier on biopsy samples, to reduce the rate of unnecessary radical prostatectomies.

As a first step of this continuing work, we determined the feasibility of effectively measuring the protein biomarker candidates in diagnostic biopsy specimens using a highly sensitive, antibody-independent targeted mass spectrometry method, namely high-pressure, high-resolution separations coupled with intelligent selection and multiplexing-selected reaction monitoring (PRISM-SRM). A total of nine FFPE biopsy specimens (18-Gauge needle biopsy, 1-cm in length; Gleason score ranges from 3+3 to 5+5) were processed and analyzed using the PRISM-SRM assays for the 16 top-ranked protein biomarker candidates that were previously determined as predictive of adverse prostate cancer progression (DM or BCR). We found that: 1) proteotypic peptides of sufficient quality for successful targeted mass spectrometry analysis can be extracted from clinical FFPE biopsy specimens; 2) peptide yields from biopsy samples range from 8% to 55% of that obtained from paired RP specimens; 3) previously identified peptides predictive of prostate cancer progression can be detected in biopsy specimens; and 4) reliable detection can be achieved in the biopsy samples for most of the top-16 proteins, including four (KLK3, TGFB1, SPARC, and CAMKK2) out of the five proteins in the proteomic classifier; the fifth protein, FOLH1, had a tendency to drop out in lower input samples, and the ability to detect FOLH1 in biopsies did not correlate with the level of FOLH1 expression in RPs.

The results from this due diligence study confirm the feasibility of targeted proteomics measurement of the protein biomarker candidates in FFPE biopsy specimens, paving the way for validating the proteomic classifier, which was initially developed via analysis of RP specimens, for even earlier detection of aggressive prostate cancer.

**Category:** Technology **Color:** Red **Poster Board #:** 6

**Enrichment of Rare Circulating Cells using Microbubble-based Preparation**

Carolina Livi, PhD, Southern Oregon University, Ed Grimley, PhD, Jon Roussey, PhD, Brandon McNaughton, PhD, Akadeum Life Sciences

## Abstract

Circulating Tumor Cells (CTCs) are a useful biomarker for cancer detection and monitoring. Enriching for rare cells from blood or tissues presents significant sample preparation challenges. Current methods for CTC preparation leave behind red blood cells (RBCs) as well as other nucleated cells affecting their downstream analysis or are harsh often damaging the cells of interest.

Functionalized microbubbles can be used for Buoyancy Activated Cell Separation (BACS) using positive or negative selection workflows by capturing and separating cells by flotation. In this case, we show improvement of isolation of CTCs from RBCs in the form of depletion microbubbles from Akadeum Life Sciences covered with antibodies to human erythrocytes using cultured human pancreatic cancer cells (line CFPAC-1, ATCC CRL 1918) spiked into standardized samples ($10^6$ RBC : $5x10^4$ WBC : $1.5x10^2$ tumor cells).

Microbubbles bearing antibodies to human erythrocytes were added at various Bubble:RBC ratios and mixed via trituration for 1 minute. Samples were then centrifuged, the microbubble-captured RBCs removed, and the remaining pelleted cells analyzed for the fraction of RBCs removed and the extent of off-target depletion of tumor cells. Cells were quantified with an automated cell counter, and all experimental conditions were performed in at least triplicate. RBC removal rose with increasing Bubble:RBC ratio ($p < 0.001$). At a ratio of 1:1, 92 +/- 1% of RBCs were removed with one minute of mixing; at a ratio of 1.5:1, 97 +/- 1% of RBCs were removed. Off-target binding of CFPAC-1 cells was minimal at a Bubble:RBC ratio of 1:1, with 0 +/- 6% of tumor cells captured. Off-target capture increased at a Bubble:RBC ratio of 2:1, with 12 +/- 9% tumor cell loss from the experimental samples. Using a standardized blood sample with a clinically relevant low concentration of cultured tumor cells, microbubbles targeting RBCs and dosed at a ratio of one bubble per target erythrocyte were able to achieve > 90% removal without statistically detectable loss of a cultured cancer line. Higher bubble concentrations resulted in more exhaustive RBC removal, but with some loss of cultured cells.

Microbubble technology can remove up to 99% of contaminant RBCs in less than 10 minutes start-to-finish. By binding to the surface markers identified by specific antigens in the bubbles, the removal of RBCs, platelets, and other unwanted cells becomes quick and simple. Microbubble have been described as an alternative or adjunct to magnetic beads or microfluidic methods for CTC sample preparation. This technology may find particular value in applications where mechanical force needs to be minimized or magnetization of cells needs to be avoided.

**Category:** Methods **Color:** Orange **Poster Board #:** 5

# Nucleic Acid Extraction with Microbubbles from Saliva Enables Enrichment from Larger Sample Volume

Carolina Livi, PhD, Southern Oregon University, Ed Grimley, PhD, Jon Roussey, PhD, Brandon McNaughton, PhD, Akadeum Life Sciences

## Abstract

Saliva presents an excellent option for self-collected samples for screening studies. Previous research suggests using saliva to quantifiably measure analytes is predictive of disease presentation. Still, technical hurdles have prevented efficient and cost-effective scaling that would allow widespread adoption of saliva testing. In particular, the realization of the full potential of saliva testing has been impeded by the inherent limitations of magnetic technologies. Since magnetic bead-based separation methods are only able to process a small fraction of the overall sample, most of the sample and any remaining biomarkers contained within it are wasted. This inability to assess the sample in its entirety needlessly impairs disease detection.

Akadeum's functionalized buoyant microbubbles overcome the limitations of magnetics and enable quantitative large-volume saliva testing. In this presentation, we demonstrate the power of the microbubble technology using an example based on current viral testing needs, but microbubbles can be used to enrich cells, proteins, or nucleic acid analytes. Here proprietary microbubbles are specifically functionalized to capture nucleic acids, including SARS-CoV-2 viral RNA from saliva and pooled saliva samples. Unlike magnetic separation methods, microbubbles do not have the same volume and equipment restrictions. The microbubbles are able to interrogate the full sample volume, which in a pooled saliva application is critical for overcoming the effects of dilution.

In head-to-head comparisons with the MagMAX™ Viral RNA Isolation Kit (ThermoFisher Scientific) Akadeum's microbubble-based nucleic acid extraction protocol routinely achieved 10-25 times more viral RNA detected by downstream qPCR. This enhancement in sensitivity was shown to be robust and reproducible and translated into greater concordance with NPS tests. In a set of patients with paired positive NPS tests, a CLIA certified commercial lab was only able to detect SARS-CoV-2 RNA in 1 of the 4 patient saliva samples using a magnetics protocol while the Akadeum's novel microbubble workflow led to detection in 4 out of 4 patient samples. In addition to a manual isolation protocol, we have also developed an automated protocol that enables higher throughput processing of samples. With a limit of detection of 50 copies/mL of SARS-CoV-2 RNA with our manual protocol and 140 copies/mL with our automated protocol, Akadeum microbubbles represent the next generation of isolation technology and offer the tools needed to identify patients early in disease, those with low viral load, or positive samples diluted in a pooled saliva setting.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 4

**Discovery of Multi-Marker Algorithms for Ovarian Cancer Risk**

Anna Lokshin PhD, University of Pittsburgh, Pittsburgh PA, Michael Tainsky, Karmanos Cancer Institute, Detroit MI, Daniel Cramer, Harvard University, Moston MA, Robert C Bast, MD Anderson Cancer Center, Houston TX

Auto-antibodies (AAbs) against mutant or overexpressed proteins are promising class of biomarkers for early detection of cancer. In our search for biomarkers for early detection of ovarian cancer (OC), we have generated a multiplex bead-based protein array for detection of AAbs (IgG and IgM) against following antigens: H-Ras, UCHL1, SURV, KRT19, TP53, ARID1A, CA125, K-Ras, IL8, T71-2A, HARS70-1, NY-ESO-1, PDGF-BB, VEGF, 4B7, c-Myc, OVX1, LDHA, and HE4. Antigens were selected based on our previous studies and published observations.

The array was applied for analysis of pre-diagnostic serum samples prospectively collected 0-84 months before diagnosis in the course of the NIH Prostate, Lung, Colorectal, Ovarian (PLCO) study. Cases were stratified by collection time prior to diagnosis (Months To Diagnosis, MTD) as 6-18 (N=36), and 18-84 (N=28). We analyzed 200 matched healthy controls. Cases collected <6 MTD were not considered. IgG AAbs to TP53 and NY-ESO-1 showed highest individual SN of 14% at 98%SP with the lead time of 6-18 MTD. At 18-84 MTD, aCA125 and aT71-2A IgG offered highest diagnostic power at 95%SP of 21% and 25%.

We additionally analyzed several non-Ig biomarkers, FSH, fibrinogen (FBG), PF4, CCL18, and Lumican (LUM). We used CA125 data provided by PLCO, and HE4 data provided by Dr. Cramer. At 6-18 MTD, FSH had highest classification power with 22%SN at 98%SP. CA125 offered 8%SN at 98%SP. At 18-84 MTD, only FBG offered appreciable classification power of 16%SN at 95%SP. CA125 had 3% SN at 95%SP

In the 6-18 MTD interval, best performing classification algorithm, was comprised of FSH, HE4, LUM and had 51%SN at 98%SP. In the18-84 MTD interval, the combination of FBG, aT71-2A, aNY-ESO1, aCA125 IgG offered 50%SN at 95%SP or 25%SN at 98%SP. These two classification algorithms for pre-diagnostic OC offered the best power reported so far for lead time 6-18 months. Clinical utility of algorithm performance has to be further discussed.

**Category:** Imaging **Color:** Orange **Poster Board #:** 4

**AI and Methodology Transfer for Early Detection of Tumors**

Ashish Mahabal[1], Daniel Crichton[2], Heather Kincaid[2], Luca Cinquini[2], Victoria Seewaldt[3], David Frankhouser[3], Radka Stoyanova[4], Isaac Xu[4], Sanoj Punnan[4], Alan Pollack[4], Ellie Kuang[1], Xiangfeng Dai[7], Denise Aberle[5], Matthew B. Schabath[6], Jin Qi[6], Alphan Altinok[2], Kristen Anton[7], Maureen Ryan Colbert[7], David Liu[2], Sean Kelly[2], Asitang Mishra[2], Christos Patriotis[8], Sudhir Srivastava[8]

[1]California Institute of Technology, Pasadena, CA
[2]NASA Jet Propulsion Laboratory, Pasadena, CA
[3]City of Hope, Duarte, CA
[4]University of Miami, Miami, FL
[5]UCLA, Los Angeles, CA
[6]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL
[7]University of North Carolina, Chapel Hill, NC
[8]National Cancer Institute, Rockville, MD

Over the last few decades instruments capturing data that aid cancer diagnosis have consistently improved. More diagnostics and bigger samples are available at higher resolutions. Coupled with the availability of faster computing and broader availability of well-tuned machine learning libraries, the data processing workflows have become more accessible. We summarize methods we are applying to datasets in breast cancer, prostate cancer, and lung cancer to show the potential of applying methods from diverse fields combined with labeling and machine learning to detect smaller tumors with greater accuracy. We show how there exist certain parallels with large scale structure in cosmology, multi-color images of the sky that can be utilized for looking at these data through different techniques, and how citizen science techniques can be used to collect well-curated labeled datasets that are crucial for effective machine learning.

**Category:** Imaging **Color:** Orange **Poster Board #:** 2

**Combining Automated Image Measurements, Blood Biomarkers, and Clinical Data for Improved Decisions in Diagnostic Mammography**

Duke University: Jeffrey R. Marks, PhD; Joseph Lo, PhD; Lars Grimm, MD
Moffitt Cancer Center: John Heine, PhD; Erin Fowler, MPH; Emma Hume, MPH; Jared Weinfurtner, MD
Creatv MicroTech: Cha-Mei Tang, PhD; Daniel L. Adams, BS
NASA Jet Propulsion Laboratory: Luca Cinquini, PhD; Heather Kincaid; Ashish Mahabal, PhD
Fred Hutchinson Cancer Research Center: Data Management and Coordinating Center

## Abstract

In breast cancer (BC) screening, approximately 2-3% of the women are classified as Breast Imaging Reporting and Data System (BI-RADS) 4 by the radiologist's interpretation. These women are recommended for biopsy and about 80% are found to be BC negative. This is a strong indication that computerized decisions could lead to improved specificity for this subset of women.

This is a network collaborative project conducted by the BDL at Duke University (Marks et al.) and the CVC at Moffitt Cancer Center (Heine et al.) that targets BI-RADS 4 women prospectively (n = 1050). This study is comprised of two parts: (1) developing an EDRN central data repository resource supported by the DMCC and JPL; and (2) developing AI based decision models by considering a wide set of variables and automated image analyses to reduce biopsy procedures. The repository includes mammograms (close to the time of diagnosis), blood samples taken at the time of biopsy, an array of data variables (i.e. pathological, radiological, clinical, and demographical data), and complete image annotation. Blood samples are analyzed in *real-time* for rare cell detection (Creatv MicroTech) and archived for future EDRN use. Image annotation includes translating findings in the radiology report and mammogram to fully annotated overlay images, defined as the truth-files (TFs) at the individual level. Findings for a given woman are annotated by scribing the lesion boundaries that are saved in TFs. In this way, automated image analyses can target lesion areas with confidence. The automated decision modeling includes exploring the measures defined above with sophisticated AI techniques.

Progress to date includes patient/data accrual (n = 909), annotation, and developing baseline (BL) models, during the data collection interim, without automated image measurements or blood markers; this provides BL performance metrics for comparison with more sophisticated modeling techniques using wider sets of variables. Performance metrics are cited with 95% confidence intervals (CIs) parenthetically. For women with masses ($n_{positive}$ = 47 and $n_{negative}$ = 218), a three variable logistic regression model with age, BI-RADS margin descriptors, and BI-RADS 4 (a, b and c) ordinal ratings produced significant odds ratios (ORs): OR = 2.10 (1.34, 3.28) for age; OR = 1.67 (1.11, 2.52) for margins; and OR = 4.67 (2.55, 8.55) for the ordinal ratings. The corresponding area under the receiver operating characteristic curve (Az) was Az = 0.89 (0.82, 0.95). Including BI-RADS shape descriptors in a similar model produced two significant measures: OR = 2.09 (1.26, 3.46) for age; and OR = 4.53 (2.33, 8.79) for shape. Although both margins (OR = 2.01) and BI-RADS ratings (OR =1.40) lost significance, the model's predictive capability increased giving Az = 0.94 (0.89, 0.99). These preliminary findings are based on a small

sample size as noted in the wide CIs. The shape finding has not been noted in our work previously.

Although preliminary, these simple BL findings suggest modeling with more sophisticated techniques that includes both automated image measurements and blood markers may produce a viable automated tool for further testing in the clinical environment. The repository development has many user-friendly attributes (e.g., querying methods, documentation, data structure, and image file naming conventions), incorporated into its design, in addition to the wide array of data elements, representing a unique EDRN resource.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 16

**Free Hemoglobin-Alpha (Hbα) and Colitis-Associated Colorectal Cancer: Can It Be Mitigated?**

Maya Bragg, B.S., Medical Student and Amosy M'Koma, M.D., M.S., Ph.D., Associate Professor, Meharry Medical College School of Medicine

**Abstract**

Colorectal cancer (CRC) is often described as the "disease no one has to die from." Despite this, patients with inflammatory bowel disease (IBD) are at increased risk for development of CRC. Currently, colonoscopy surveillance serves as the gold standard for prevention. However, it has proved to be relatively inadequate for several reasons and further research needs to be conducted to ascertain the molecular pathogenic relationship between neoplasia and chronic inflammation: more specifically, Fenton chemistry and its relationship with free hemoglobin-α (Hb-α), hydroxyl radical formation, DNA damage, and subsequent tumor formation. Our central hypothesis is that Hb-α induces oxygen free radical-mediated DNA damage through increased ROS (exacerbated by decreased antioxidant defenses). The focus is that if the Fenton reaction was mitigated via pharmaceutical therapy (haptoglobin, deferoxamines, and flavonoids), then this would reduce the ROS and apoptosis, promote DNA repair, and prevent incidence of colitis associated colon cancer (CACRC). To test our hypothesis, a literature review was conducted to determine the efficacy of pharmaceutical therapy on Fenton reaction mitigation. According to our review, haptoglobin's rate of clearance from plasma is inversely correlated with its size, the smaller the size, the faster the clearance. Thus, administration of Hp1-1 may prove to be beneficial. Furthermore, deferoxamine's hydrophilic structure limits its ability to cross cell membranes and it may be beneficial if administered intracellularly to avoid the higher concentrations and longer incubation periods associated with extracellular administration. Finally, the effectiveness of flavonoids was analyzed with natural herbs. It was determined that the strength of their antioxidant activity was associated with high reactivities of hydroxyl substituents. Despite our review, most articles performed experiments on cell lines outside of the GI tract and more information needs to be collected from our area of study. In the future, we would like to assess Hbα-induced ROS by exposing normal human colon epithelial cell lines to Hb-α and then treat the cell lines with haptoglobin, DFO, and flavonoids to separate free Hb-α and measure hydroxyl radical formation. This project was approved by Meharry Medical College (IRB#:100916AM206) and Vanderbilt University Medical Center (IRB#s: 080898 and 100581). **Support**: Pilot Project - VICC GI SPORE P50CA236733.

**Category:** Biomarker **Color:** Green **Poster Board #:** 1

**Massively Parallel Sequencing of Esophageal Brushings Enables an Aneuploidy-Based Classification of Patients with Barrett's Esophagus**

Christopher Douville*, PhD, Department of Oncology, the Sidney Kimmel Cancer Center, the Ludwig Center, the Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine; Helen R. Moinova*, PhD, Department of Medicine, Case Western Reserve University, Prashanthi N. Thota, MD, Cleveland Clinic Foundation, Nicholas J. Shaheen, MD, Center for Esophageal Diseases and Swallowing, University of North Carolina, Prasad G. Iyer, MD, Mayo Clinic, Marcia Irene Canto, MD, MHS, Department of Oncology, the Sidney Kimmel Cancer Center, Department of Medicine, Johns Hopkins Medical Institutions, Jean S. Wang, MD, PhD, Washington University in St Louis, John Dumot, DO, Department of Medicine, Case Western Reserve University, Ashley Faulx, MD, Department of Medicine, Case Western Reserve University, Kenneth W. Kinzler, PhD, Department of Oncology, the Sidney Kimmel Cancer Center, the Ludwig Center, the Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Nickolas Papadopoulos, PhD, Department of Oncology, the Sidney Kimmel Cancer Center, the Ludwig Center, the Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, Johns Hopkins University School of Medicine, Bert Vogelstein, MD, Department of Oncology, the Sidney Kimmel Cancer Center, the Ludwig Center, the Sol Goldman Pancreatic Cancer Research Center, The Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Sanford D. Markowitz, MD, PhD, Department of Medicine, Case Comprehensive Cancer Center, Case Western Reserve University, Seidman Cancer Center, University Hospitals Cleveland Medical Center, Chetan Bettegowda[#], MD, PhD, Department of Oncology, the Sidney Kimmel Cancer Center, the Ludwig Center, the Sol Goldman Pancreatic Cancer Research Center, Department of Neurosurgery, Johns Hopkins University School of Medicine, Joseph E. Willis[#], MD, Case Comprehensive Cancer Center, Department of Pathology, Case Western Reserve University, Seidman Cancer Center, University Hospitals Cleveland Medical Center, Amitabh Chak[#], MD, Case Comprehensive Cancer Center, Department of Medicine, Case Western Reserve University, Seidman Cancer Center, University Hospitals Cleveland Medical Center

*Denotes Equal Contribution, [#]Denotes Equal Contribution

## Abstract

**BACKGROUND & AIMS:** Aneuploidy has been proposed as a tool to assess progression in patients with Barrett's Esophagus (BE), but has heretofore required multiple biopsies. We assessed whether a single esophageal brushing that widely sampled the esophagus could be combined with massively parallel sequencing to characterize aneuploidy and identify patients with disease progression to dysplasia or cancer.

**METHODS:** Esophageal brushings were obtained from patients without BE, with non-dysplastic BE (NDBE), low-grade dysplasia (LGD), high grade dysplasia (HGD), or adenocarcinoma (EAC). To assess aneuploidy, we employed RealSeqS, a technique that uses massively parallel sequencing to interrogate ~350,000 genome-spanning regions and identify specific chromosome arm alterations. A classifier to distinguish NDBE from EAC was trained on results from 79 patients. An independent validation cohort of 268 subjects was used to test the classifier in distinguishing patients at successive phases of BE progression.

**RESULTS:** Aneuploidy progression was associated with gains of 1q, 12p, and 20q and losses on 9p and 17p. The entire chromosome 8q was often gained in NDBE, whereas focal gain of 8q24 was often found in dysplasia. Among validation subjects, a simple classifier incorporating these features with a global measure of aneuploidy scored positive in 96% of EAC, 68% of HGD, but only 7% of NDBE.

**CONCLUSIONS:** RealSeqS analysis of esophageal brushings provides a practical and sensitive method to determine aneuploidy in BE patients. It identifies specific chromosome changes that occur early, in NDBE, and others that occur late and mark progression to dysplasia. The clinical implications of this approach can now be tested in prospective trials.

**Category:** Biomarker **Color:** Green **Poster Board #:** 6

**Diagnostic Significance of Mucin (MUCs) for Early Detection of Pancreatic Cancer**

Gopalakrishnan Natarajan, PhD, University of Nebraska Medical Center, Pranita Atri, MTech, University of Nebraska Medical Center, Lynette Smith, PhD, University of Nebraska Medical Center, Randall E. Brand, MD, University of Pittsburgh School of Medicine, Surinder K. Batra, PhD, University of Nebraska Medical Center, Sukhwinder Kaur, PhD, University of Nebraska Medical Center.

Pancreatic Cancer (PC) patient's survival does not improve considerably over the years due to the lack of decidedly specific and sensitive Biomarkers. In our previous studies, MUC4 and MUC5AC emerged as potential markers combined with Gold-standard CA19.9 for differentiating PC from chronic pancreatitis (CP) and other benign controls. However, the diagnostic significance of other mucins and mucins-associated carbohydrate antigens (DUPAN2) remains unexplored. Unsupervised clustering analysis *via* k-medoid identified three distinct clusters of mucins across in TCGA dataset for pancreatic tumors. Notably, MUC1, MUC5B, MUC16, and MUC13 emerged as key components of two distinct clusters. Considering the high stability of mucin-based markers along with their differential expression across distinct patient groups in the global RNA-seq studies, we hypothesized that the development of a multi-mucin marker panel could improve the sensitivity for early detection of pancreatic cancer. Using in silico analyses of TCGA, GTEX, and GEO datasets, a differential expression of MUC1, MUC4, MUC5AC, MUC5B, MUC13, and MUC16 (p-value <0.01) was observed in early (till stage 2B) and late-stage (stage 3 and 4) pancreatic cancer in comparison to healthy control groups. MUC1, MUC5AC, MUC5B, and MUC16 expression were associated with poor patient survival in PDAC. We next focused on developing uniplexed and multiplex immunoassay for mucin detection. Differential expression of MUC1, MUC5B, MUC13 was observed at mRNA and protein levels in various PC cell lines and the early and late stages of pancreatic tumor tissues. Notably, expression was maintained across pancreatic tumors METs from lung, liver. Next, we assessed the combined circulating levels of MUC1, MU5AC, MUC4, MUC16 along with CA19.9 in the training set comprised of benign controls, CP, early and late-stage PC cases. At serum levels, these markers remarkably elevated in early and late-stage cancer cases. We observed Area under the ROC Curve (AUC) ranging from 0.47 to 0.83 for differentiating benign control and CP cases from early and late-stage PC cases for these markers. Next, levels of these markers were evaluated in combination with MUC5AC. The differentially overexpressed mucin, MUC5AC in combination with MUC1 and CA19.9 significantly improved the accuracy of CA19.9 for differentiating mucinous tumors from benign groups, healthy control (improved AUC from 0.7 to 0.75); benign biliary obstruction (improved CA19.9 AUC from 0.74 to 0.875); chronic diabetes (improved CA19.9 AUC from 0.71 to 0.745); and CP (improved CA19.9 AUC from 0.87 to 0.93) cases. In our studies, MUC4 performed exceptionally well in combination with CA19-9 in differentiating mucinous tumors from healthy control (improved CA19.9 AUC from 0.7 to 0.786); benign biliary obstruction (improved CA19.9 AUC from 0.75 to 0.83); CP (improved CA19.9 AUC from 0.88 to 0.94). Overall, these biomarkers in combination provide improved CA19-9 accuracy for differentiating control cases from cancer cases. In the future, we aim to evaluate further this panel's clinical potential in pre-diagnostic cohorts of PC.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 15

**Intermolecular Profiling of Epigenetic Heterogeneity of Rare Biomarkers**

Christine O'Keefe, Ph.D., Johns Hopkins University, Thomas Pisanic, Ph.D., Johns Hopkins University, Jeff Wang, Ph.D., Johns Hopkins University

Trace amounts of tumor-derived methylated DNA molecules can be found non-invasively in blood samples, or "liquid biopsies," but at very low fractions compared to healthy molecules (<0.01%). Recently, studies have shown that cancer-precursor tissue exhibits highly variable methylation patterns. Current techniques, such as sequencing and droplet digital PCR (ddPCR), have technical limitations precluding their ability to profile methylation patterns of rare molecules. Sequencing, although comprehensive, has limited sensitivity, undermining its utility for routine detection of fractions below 0.1%. ddPCR can achieve high sensitivity, but is limited to known sequences, thus cannot provide comprehensive analysis of molecular heterogeneity.

Such limitations may be overcome by high resolution melt (HRM), which is a thermodynamics-based approach to detecting molecular variability by observing the sequence-specific release of a DNA-intercalating dye under a thermal ramp. HRM can discriminate methylation patterns on CpG-by-CpG basis, but has limited applicability in a low-throughput microtiter plate system. Microfluidic arrays offer a potential solution; however, the current generation of commercially-available technologies are not readily suitable for highly parallelized HRM-based profiling due to the numerous technical challenges.

This work presents a digital PCR and HRM microfluidic platform for the absolute quantification and comprehensive assessment of intermolecular heterogeneity. A microfluidic device digitizes the sample into 4096 1-nL well chambers for single-molecule detection and analysis. The digital HRM (dHRM) platform discriminates small sequence variations within the amplicons to assess sample heterogeneity at a target to background ratio as low as 0.0005%. The sensitivity of the platform marks a significant step towards rare biomarker detection in challenging samples such as liquid biopsies. This all-in-one molecular profiling and analysis platform provides simple fabrication and operation, robustness and flexibility to multiple assays, and the practicality necessary for routine use.

**Category:** Methods **Color:** Orange **Poster Board #:** 1

**Blood and Fibulin 3: Be Careful How and What You Collect**

Pass H, NYU Langone Health; Viapiano M, SUNY Upstate Medical University; Cengel K, University of Pennsylvania Medical Center; Vachani A, University of Pennsylvania Medical Center; Carbone M, Yang H, University of Hawaii; Johnen G, Institute of the Ruhr University; Goparaju C, NYU Langone Health

**Objectives**: Fibulin 3 (FBLN3) is a controversial diagnostic blood based biomarker for mesothelioma (MPM) with validations in North America, Turkey, China, and Egypt, and failures in Europe, UK, and Australia. The lack of proper ELISAs has been partly blamed, along with suspicion of cohort impropriety; however, a prognostic signal in pleural fluid *has* been validated in the US, Australia, and Europe. We present a blinded analysis from UPenn that validated FBLN3; however, FBLN3 once again failed in a blood based study investigating Calretinin and FBLN3 with mutual exchange of blinded specimens. The possible reasons for the FBLN3 9-year controversy were investigated. **Methods:** We report three studies. *UPENN validation*: From an NIEHS SRP grant, blinded samples of plasma from 34 pretreatment MPMs with 27 matched postoperative plasmas along with 34 individuals with documented asbestos exposure had FBLN3 quantification using a novel NYU-constructed ELISA using mAB428.2. *Calretinin-FBLN3 Exchange Study*: Blinded NYU plasma samples from 120 MPMs, 50 asbestos exposed (AE) individuals, and 30 individuals with non-MPM effusions were sent to German investigators for Calretinin determination. In turn, NYU received blinded plasma samples from 80 MPM patients and 75 AE individuals for mAB428.2 ELISA from Germany. Analyses were performed via ROC curves. *Vehicle comparison experiment:* 60 cc of fresh blood from a "normal" volunteer was divided into a 30 cc aliquot with recombinant FBLN3 spiked in for a concentration of 600ng/ml and a 30cc aliquot without spike in. Each of these aliquots were then drawn into either a K2 monovette with polypropylene gel (Germany), K2 EDTA polypropylene spray, or K3EDTA liquid in glass tubes. These tubes were then immediately processed in an identical fashion for subsequent FBLN3 determination. Non-reducing western gels were performed on all samples with positive control lanes of recombinant FBLN3. **RESULTS:** An AUC of 0.97 was recorded for the NYU plasma cohort of 129 MPMs compared to that of 172 AE and 172 non-MPM effusions. Blinded validation with UPENN revealed an AUC of 0.89. The NYU cohort samples sent to Germany validated the Calretinin assay, insuring the robustness of the NYU cohort. The FBN3 assay, however, failed with German samples that had worked perfectly for the Calretinin assay in Germany. Since blood collection methods were different between Germany and US, the vehicle comparison experiment was performed, and the results seen in Table 1. While K3 glass revealed "normal" FBLN3 levels for the non-spike-in and expected spike-in levels, the German-used monovette and the universal K2 tube levels were non-sensical. Western blots of selected German samples were characterized by either di-trimers or degradation, not seen in the K3 glass NYU study. Western blot of the vehicle comparison plasmas revealed expected bands with K3, while K2 spray and monovette samples were degraded. Of note, 50 NYU matched serum and plasma FBLN3 levels correlated ($r=0.9329$, $p<0.0001$). **Conclusions:** FBLN3 collection vehicles will influence ELISA determinations. Serum levels may be the most convenient/ accurate to insure concordance between geographic sites.

*Table 1: Vehicle Comparison Experiment*

| | K3 EDTA Glass Liquid | K2 EDTA Polypropylene, spray | K2 monovette, polypropylene gel |
|---|---|---|---|
| | FBLN3 concentration in ng/ml | | |
| No Spike in | 80 | 212 | 227 |
| FBLN3 600 ng/ml | 516 | 316 | 313 |

**Category:** Imaging **Color:** Orange **Poster Board #:** 5

**Volume Doubling Time and Radiomic Features Predict Tumor Behavior of Screen-Detected Lung Cancers**

Jaileene Pérez-Morales[1], Hong Lu[2], Wei Mu[2], Ilke Tunali[2, 3], Steven A. Eschrich[4], Yoganand Balagurunathan[4,] Robert J. Gillies[2], Matthew B. Schabath[1,5]

[1]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; [2]Department of Cancer Physiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; [3]Institute of Biomedical Engineering, Bogazici University, Istanbul, Turkey; [4] Department of Bioinformatics and Biostatistics, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA; [5]Department of Thoracic Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

**Background**: Utilizing non-invasive image-based biomarkers could have translational implications by characterizing tumor behavior lung cancers diagnosed in the lung cancer screening setting. In this study, peritumoral and intratumoral radiomics and volume doubling time (VDT) were used to identify high-risk subsets of lung patients diagnosed in lung cancer screening that are associated with poor survival outcomes.

**Methods:** Patient data and LDCT images were acquired from incidental lung cancer patients from the National Lung Screening Trial. VDT was calculated between two consequent screening intervals ~1 year apart and peritumoral (N=109) and intratumoral (N=155) radiomic features were extracted from the baseline screen. Overall survival (OS) was the main endpoint. Classification and Regression Tree (CART) analyses were used to identify the most predictive models to classify patient outcomes.

**Results:** Decision tree analysis stratified patients into three risk-groups (low, intermediate, and high) based on VDT and one radiomic features (compactness). Patients in the high-risk patients had extremely poor survival outcomes (HR= 8.15; 25% 5-year OS) versus the low-risk patients (HR= 1.00; 83.3% 5-year OS). Using the same decision tree and restricting the analysis to early-stage lung cancers (Stages I and II), high-risk patients had poor survival outcomes (HR= 9.07; 44.4% 5-year OS) versus the low-risk group (HR= 1.00; 90.9% 5-year OS; C-index = 0.69). For the VDT variable, the decision tree analysis identified a novel cut-point of 279 days and using this cut-point VDT alone discriminated between aggressive lung cancers (HR = 4.18; 45% 5-year OS) versus indolent/low-risk cancers (HR= 1.00; 82.8% 5-year OS). Because of the clinical and statistical importance of VDT, analyses were conducted to reveal the biological underpinnings of VDT. Two peritumoral features, non-uniformity in gray level size zone non-uniformity [GLSZM ZNU] and average 3D run length non-uniformity [RLN], were both predictive of VDT. *Nicotinamide N-methyltransferase* (*NNMT*) was significantly associated with the GLSZM ZNU radiomic feature and *phosphoinositide-3-kinase regulatory subunit 5* (*PIK3R5*) was found to be significantly associated with the RLN radiomic feature.

**Conclusion**: In this study we utilized peritumoral and intratumoral radiomic features and VDT to generate a model that to identify a high-risk group of screen-detected lung cancer patients that is associated with poor survival outcomes. These vulnerable subset of screen-detected

lung cancers may be candidates for more aggressive surveillance/follow-up and treatment, such as adjuvant therapy.

**Category:** Methods **Color:** Orange **Poster Board #:** 4

# Developing Targeted Mass Spectrometry Assays for Candidate Glycoproteins Using Data-Dependent Acquisition, Data-Independent Acquisition, and Parallel Reaction Monitoring and Evaluating Their Analytical Performance on Q Exactive, Lumos, and Exploris 480 Orbitrap Instruments

Sean Ponce and Hui Zhang, Johns Hopkins University

## Abstract

Prostate cancer (PCa) is the second leading cause of cancer death in American men. Current screening techniques have limited ability to detect PCa nor whether PCa is aggressive. Using quantitative glycoproteomics, we have analyzed 142 urine samples from aggressive and non-aggressive prostate cancer and identified candidate glycoproteins, LRG1, ANPEP, CD63, CLU, ACPP and PTGDS, associated with aggressive prostate cancer. Quantifying these candidates with sufficient sensitivity and precision would help the validation of the candidate urianry glycoprotein biomarkers identified in discovery phase for the detection of aggressive prostate cancer. To facilitate the validation, we developed targeted assays using different mass spectrometry data acquisition techniques: Data Dependent Acquisition (DDA), Data Independent Acquisition (DIA), and Parallel Reaction Monitoring (PRM), and evaluating their performance using synthetic peptide standards on three High-Resolution/Accurate-Mass instrument platforms: Q Exactive, Lumos, and Exploris 480. The data was analyzed using the bioinformatics software tools: Skyline for PRM and DIA and Pycloud for DDA, with statistical analysis for determining the analytical performance. Results showed that without using stable heavy-isotope labeled peptides as internal standards, the Lumos and Exploris 480 instruments showed median Coefficient-of-Variation (CV) (<5% for most peptides) at the low median limit-of-detection (LOD) of 200 fmol. The Q Exactive showed the median CV of 17% at the LOD of 500 fmol. It was also found that peptide intensities and assay linearities were instrument specific, with poor performing peptides being good performers on a different instrument. Per instrument, peptides showed consistent trends in quantitation between DIA and PRM acquisition. However, DDA method using TMT label resulted in the lowest dynamic range. The developed targeted assays are used for the validation of urinary glycoprotein biomarkers for prostate cancer. This work sets the foundation for highly reproducible, sensitive, and selective targeted analysis of candidate biomarkers in clinical urine samples for aggressive PCa detection.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 11

**Validation of a Proteomic Signature of Lung Cancer Risk from Bronchial Brushings**

S.M. Jamshedur Rahman[1], Yuqian Gao[2], Athena A. Schepmoes[2], Yi-Ting Wang[2], Tujin Shi[2], Sheau-Chiann Chen[3], Heidi Chen[3], Karin D. Rodland[2], Tao Liu[2], Pierre P. Massion[1,4]

[1]Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, [2]Bioloigcal Sciences Division, Pacific Northwest National Laboratory, Richland, WA, [3]Division of Cancer Biostatistics, Vanderbilt University, Nashville, TN, [4]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN

A major challenge in lung cancer prevention and cure hinges on identifying the at-risk population who ultimately develops lung cancer. Previously we reported proteomic alterations in the cytologically normal bronchial epithelial cells collected from the bronchial brushings of individuals at-risk for lung cancer. Proteins were identified by shotgun proteomics using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and selected candidate biomarkers of risk were validated in an independent sample cohort by parallel reaction monitoring. The purpose of the current study is to validate, in independent cohorts from Vanderbilt University Medical Center, a selected list of 54 candidate proteins associated with risk for lung cancer with sensitive targeted proteomics using selected reaction monitoring (SRM) at the Pacific Northwest National Laboratory. We tested proteins from 94 bronchial brushings collected in two batches from individuals at low risk (batch 1, n=10; batch 2 n=20) and high risk (batch 1, n=10; batch 2 n=24) for developing lung cancer as well as patients with lung cancer (batch1, n=10 and batch 2, n=20). Multiplexed LC-SRM assays were developed for all 54 proteins. Wilcoxon rank sum test were performed for pairwise comparison. When compared between the high risk group and the low risk group, ALDH3A1 was found to be overexpressed in the high risk group in both batch1 and batch 2 brushings specimens. Among the proteins overexpressed in the cancer group compared to the low risk group, ANXA2 demonstrated significance in both batches. In comparison to the low risk group, ALOX15 was overexpressed in the cancer group in batch 1 and in the high risk group in batch 2. Expression of the proteins were not significantly different between high risk group and the cancer group except LGALS7B in batch 1 and BRCA2 in batch 2. In Batch I, LEG7 and PYGB proteins were selected as potential predictors of cancer using lasso logistic regressions. The original area under curve (AUC) with 95% CI is 0.83 (0.67, 0.99). The AUC with bootstrapping for internal validation is 0.74 (0.37,1).

In conclusion, these SRM results revealed promise of selected candidate proteins to stratify the at-risk population for lung cancer. Currently, validation efforts to test the promising candidates in a larger independent cohort is ongoing. Methods with increased sensitivity for analysis of the previously undetected low abundant protein candidates and carrier-assisted SRM approaches for analysis of very small-sized samples will be employed. Ultimately, we hope to deliver a signature of risk that may provide the basis for lung cancer risk assessment and possibly novel future prevention strategies. This work is supported by UO1CA152662.

**Category:** Biomarker **Color:** Green **Poster Board #:** 14

# Use of the Myprostatescore (MPS) Test for Risk Stratification in Men with a Previous Negative Biopsy: Initial Validation of a Straightforward Testing Approach

Michael Sessine, Todd Morgan, John Wei, Simpa Salami, Ganesh Palapattu, University of Michigan, Bruce Trock, Johns Hopkins University, Ashley Ross, Northwestern University, Joshua Cabral, Howard University, Gary Longton, Yingye Zheng, Fred Hutchinson Cancer Research Center, Scott Tomlins, Lakshmi Kunju, Javed Siddiqui, Yashar Niknafs, Arul Chinnaiyan, Jeff Tosoian, University of Michigan

## Abstract

**Introduction and Objective:** The MyProstateScore (MPS) test incorporates prostate cancer antigen 3 (PCA3) and the TMPRSS2:ERG gene fusion (T2:ERG) with serum PSA to better define risk of Grade Group (GG) ≥2 cancer on biopsy. We previously validated use of MPS as a rule-out test with 98% negative predictive value (NPV) in biopsy-naïve men. We sought to identify a straightforward testing approach for risk stratification in patients with a previous negative biopsy.

**Methods:** All patients provided post-digital rectal examination urine prior to biopsy. MPS was calculated using the validated, locked multivariable model including serum PSA, urinary PCA3, and urinary T2:ERG and reported on a continuous scale from 0 (very unlikely to detect GG≥2 cancer) to 100 (very likely to detect GG≥2 cancer). The MPS values approximating ≤5% risk of GG≥2 cancer (lower threshold) and ≥20% risk of GG≥2 cancer (upper threshold) were identified in a primary cohort, and performance was measured in an external validation cohort.

**Results:** The primary cohort included 422 men of median age 66 years (IQR 61-72), PSA 6.4 ng/ml (4.3-9.1), and MPS 23.1 (13.0-38.4). On repeat biopsy, 58 men (14%) were found to have GG≥2 cancer. MPS thresholds of 15 and 40 were identified in this cohort and applied to the external validation cohort of 268 men of median age 64 years (59-69), PSA 7.6 ng/ml (5.5-10.6), and MPS 28.9 (16.9-49.2). Twenty-five men (9.3%) in the validation cohort were found to have GG≥2 cancer. Test performance and clinical outcomes based on applying each threshold to the validation cohort are listed in Table 1. Notably, use of MPS≤15 to rule out biopsy would have avoided 21% of biopsies with 100% NPV. Meanwhile, MPS≤40 would have avoided 67% of biopsies with 95% NPV.

**Conclusions:** Men with persistently elevated PSA following negative biopsy present a clinical challenge. We herein identified and validated MPS thresholds to accurately rule-out the need for repeat biopsy. Given the limited prevalence of GG≥2 cancer in the repeat-biopsy population, additional data are needed to confirm these findings and to characterize use of MPS in combination with diagnostic imaging such as mpMRI.

| | **Sn** | **Sp** | **NPV** | **PPV** | **N (%) bx avoided** | **N(%) unnecessary bx avoided** | **N (%) GG≥2 diagnoses missed** |
|---|---|---|---|---|---|---|---|
| Validation (n=268) | | | | | | | |
| MPS ≤15 (n=55) | 100% | 23% | 100% | 12% | 55 (21%) | 55 (23%) | 0 (0%) |
| MPS ≤40 (n=179) | 68% | 70% | 95% | 19% | 179 (67%) | 171 (70%) | 8 (32%) |

**Category:** Biomarker **Color:** Purple **Poster Board #:** 5

**Identification of Serum Protein Biomarkers Complementary to HE4 Derived from Proteomic and Genomic Analyses of Proximal Biospecimens for the Early Detection of Ovarian Cancer.**

Yiling Liu[1], Natalie L. Waldron[1], Eric Kuhn[2], Michael A. Gillette[2], Michael Birrer[3], Steven J. Skates[1]

[1]Biostatistics Center, Massachusetts General Hospital, Boston, MA, [2]Proteomics Platform, Broad Institute, Cambridge MA, [3]University of Arkansas for Medical Sciences, Little Rock, AS.

**Background:** Standard serum biomarkers for ovarian cancer include CA125 (MUC-16) and HE4 (WFDC2). These two markers have a large overlap of ovarian cancers for which both are often positive. Identifying serum biomarkers providing complementary sensitivity has proven difficult, but if achieved, such biomarkers could add to the spectrum of ovarian cancers identified by screening with early detection algorithms based on regular blood tests including such complementary biomarkers.

**Methods:** Mass spectrometry analyses of two proximal biospecimens, ovarian cyst fluid (OCF) and tissue interstitial fluid (TIF), and transcriptomic analyses of ovarian surface epithelium (OSE) and fallopian tube epithelium (FTE), identified 50 high probability protein candidates either present in OCF or TIF, or overexpressed transcripts with protein products likely to be secreted, and therefore likely to be present in the general circulation. Five tryptic peptides per candidate protein were selected and synthesized for development of rabbit peptide antibodies with the two best antibodies selected for immuno-MRM (multiple reaction monitoring). Of the 100 iMRMs, 84 were successfully developed, and evaluated in triplicate in serum from 73 ovarian cancer cases and 36 benign pelvic mass controls using heavy spiked-in peptides as reference concentrations for the endogenous light target peptides.

**Mixture Models**: Statistical analyses of the light-to-heavy ratios (log scale), directly related to the endogenous peptide concentration and thus serum protein concentration, employed a two-component mixture distribution for the cases. The first component over-expresses the protein compared to controls as a result of the malignancy and the second component expresses it at the same level as in controls. Because the replicates were run months apart, there was the potential for assay drift and assay variability to affect the results. We combined replicates when the variation between control subjects was less than 1.96-fold different between the three replicates as measured by the univariate mixture model. For each peptide that passed this criterion, we combined replicates by allowing for assay shift in the mean by holding constant across replicates the difference between the control mean and the mean of the over-expressing component for the cases, with the estimates for the variance of the first component for cases, the mixture fraction, and variance of controls, combined across the three replicates. The sensitivity at 98% specificity was estimated from the mixture model for cases and a t-distribution for controls providing robustness of estimation to outliers.

To estimate the additional sensitivity provided by other serum proteins we developed a bivariate mixture model with HE4 (best peptide DQCQVDSQCPGQMK) and selected 15 peptides from the univariate analysis for their high sensitivity at 98% specificity. A protein was over-expressed in a fraction of cases but not in the rest of the cases; for two proteins, this results in four components: both are over-expressed, one is over-expressed but not the other and vice-versa, and neither are over-expressed. Each component is a bivariate $t_5$ distribution. To combine replicates, we held constant the difference between the means for the component over-expressing both markers and the means for the controls, analogous to the univariate mixture model. The HE4 sensitivity at 98% specificity of WFDC2 alone was calculated and compared to the estimate from the bivariate model. The complementarity provided by the second protein was the increase in sensitivity estimated in the bivariate model compared to the univariate model for HE4, both estimated at 98% specificity.

**Results:** The top five peptides complementing HE4 (WFDC2) best increased the serum HE4 sensitivity from 58% to a maximum of 67% with a range of increase from 4% to 9%. These proteins are different from the proteins ranked by their sole sensitivity (at 98% specificity).

**Conclusions:** Identifying complementary serum proteins to HE4, and likely to CA125, are rare and difficult, but with a systematic approach and careful bivariate modeling, such protein biomarkers can be identified as an important step in maximizing the sensitivity of a panel of biomarkers for the early detection of ovarian cancer.

**Category:** Biomarker **Color:** Green **Poster Board #:** 10

# A Panel of Selected Serum Protein Biomarkers for the Detection of Aggressive Prostate Cancer**

Jin Song, MD, PhD[1,*], Shiyong Ma, PhD[1,*], Lori J. Sokoll, PhD[1,2,3], Rodrigo V. Eguez[1], Naseruddin Höti, PhD[1], Hui Zhang, PhD[1,2], Phaedre Mohr[1], Renu Dua[1], Dattatraya Patil[5], Kristen Douglas May[5], Sierra Williams[5], Rebecca Arnold[5], Martin G. Sanda, MD[5], Daniel W. Chan, PhD[1,2,3,4,#], Zhen Zhang, PhD[1,2,#]

[1]Center for Biomarker Discovery and Translation, Department of Pathology, Departments of [2]Oncology and [3]Urology and [4]Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, 21287, USA. [5]Department of Urology, Emory University School of Medicine, Atlanta, GA, 30322, USA

*These authors contributed equally to this work.

[#]Corresponding authors with equal contributions.

** This study was conducted as part of the EDRN BRL developmental project at JHU (Daniel W. Chan, PI)

## Abstract

Background: Current PSA-based tests used to detect prostate cancer (PCa) lack sufficient specificity, leading to significant overdetection and overtreatment. Our previous studies showed that serum fucosylated PSA (Fuc-PSA) and soluble TEK receptor tyrosine kinase (Tie-2) had the ability to predict AG PCa. Additional biomarkers are needed to address this significant clinical problem.

Methods: A comprehensive Pubmed search followed by multiplex immunoassays identified candidate biomarkers associated with AG PCa. Subsequently, multiplex and lectin-based immunoassays were applied to a case-control set of sera from subjects with AG PCa, low risk PCa, and non-PCa (biopsy negative). These candidate biomarkers were further evaluated for their ability as panels to complement the prostate health index (phi) in detecting AG PCa.

Results: When combined through logistic regression, two panel of biomarkers achieved the best performance: 1) phi, Fuc-PSA, SDC1, and GDF-15 for the detection of AG from low risk PCa and 2) phi, Fuc-PSA, SDC1, and Tie-2 for the detection of AG from low risk PCa and non-PCa, with noticeable improvements in ROC analysis over phi alone (AUCs: 0.942 vs 0.872, and 0.934 vs 0.898, respectively). At a fixed sensitivity of 95%, the panels improved specificity with statistical significance in detecting AG from low risk PCa (76.0% vs 56%, p=0.029), and from low risk PCa and non-PCa (78.2% vs 65.5%, p=0.010).

Conclusions: Multivariate panels of serum biomarkers identified in this study demonstrated clinically meaningful improvement over the performance of phi, and warrant further clinical validation, which may contribute to the management of PCa.

**Category:** Biomarker **Color:** Green **Poster Board #:** 15

# Development and Characterization of A Rabbit Monoclonal Antibody for the Detection of ETV1 Protein Expression in Human Prostate Tissue Specimens

Shyh-Han Tan, Ph.D.,[1,2], Cara Schafer[1,2], Denise Young[1,2], Sally Elsamanoudi[1,2], Jacob Kagan[3], Sudhir Srivastava[3], Albert Dobi[1,2], Gyorgy Petrovics[1,2], Isabell A. Sesterhenn[4], Gregory T. Chesnut[1].
[1]Center for Prostate Disease Research, Murtha Cancer Center Research Program, Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 20817, USA; [2]Henry M. Jackson Foundation for the Advancement of Military Medicine Bethesda, Maryland, 20817 USA; [3]National Cancer Institute, Bethesda, MD; [4]Joint Pathology Center; Silver Spring, Maryland 20906, USA.

**Background**: Gene fusions involving *ETV-1* that led to its overexpression occur in multiple cancers. In prostate cancer, these events, which are distinct from tumors harboring *TMPRSS2-ERG* fusion or *PTEN* deletion, occur in approximately 5% of cases and are correlated with poor outcome. ETV1 has been reported to synergistically cooperate with KIT as a lineage survival factor in gastrointestinal stromal tumors and it is overexpressed in a subset of sarcomas with *CIC* rearrangements or gene fusions. The availability of a specific ETV1 monoclonal antibody (mAb) will further reveal the role that ETV1 plays in the oncogenic activation of various cancers.
**Methods**: A novel ETV1 mAb was developed by immunizing rabbits with ETV1 peptides followed by hybridoma fusion. In screens using ELISA and immunoblot assays, a clone that produced the most selective antibody that does not cross react with other ETS factors was identified. ETV1 expression on a tissue micro-array (TMA) constructed from radical prostatectomies of 50 African American (AA) and 50 Caucasian American (CA) patients were evaluated by immuno-histochemistry (IHC).The hybridoma was sequenced to identified the complementarity-determining regions (CDRs). Key residues required for Mab binding were probed by ELISA using overlapping peptides and alanine scanning. Affinity measurements using surface plasmon resonance imaging (SPRi) were performed to determine the dissociation constant of the antibody
**Results:** IHC evaluation of a prostate cancer TMA using the using the ETV1 specific mAb identified five cases with ETV1 positive index tumors, of whom four were CA. One of the cases also harbored no-overlapping ERG positive tumor foci. The selective binding of the mAb is validated by the results of the CDR sequencing and dissociation constant measurements. Alanine scanning and epitope mapping by ELISA identified the critical amino acid residues for antibody interaction.
**Conclusions**: This evaluation of the rabbit ETV1 mAb validated its application in IHC assay on human prostate tissue. Initial results from a 100-patient TMA confirmed the reported frequency of ETV1 alteration. Further evaluation of this antibody on tissue specimens from independent patient cohorts are in progress to establish the sensitivity and specificity of the mAb and to validate the utility of ETV1 detection in diagnosis and stratification of prostate and other cancers.

**IRP protocol**: DBS.2020.135 (EDRN2) (Ref #930195),"Discovery of Novel Prognostic and Diagnostic CaP Biomarkers between CPDR and EDRN Partners"
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**Category:** Biomarker **Color:** Green **Poster Board #:** 8

# Urinary MyProstateScore (MPS) to Rule Out Clinically-Significant Cancer in Men with Equivocal (PI-RADS 3) Multiparametric MRI: Addressing An Unmet Clinical Need

Jeffrey J. Tosoian MD MPH[1,2Ψ]*, Udit Singhal MD[1,2Ψ], Matthew S. Davenport MD[1,3], John T. Wei MD[1], Jeffrey S. Montgomery MD[1,2], Arvin K. George MD[1,2], Simpa S. Salami MD MPH[1,2], Stanley G. Mukundi MS[1], Javed Siddiqui[4], Lakshmi P. Kunju MD[5], Benjamin P. Tooke[6], Yoonhee Ryder[6], Sarah P. Dugan[6], Zoey Chopra[6], Rachel Botbyl[4], Yilin Feng[6], Michael S. Sessine[6], Nicholas W. Eyrich[6], Ashley E. Ross MD PhD[7], Bruce J. Trock MPH PhD[8], Scott A. Tomlins MD PhD[4,5], Ganesh S. Palapattu MD[1,2], Arul M. Chinnaiyan MD PhD[1,4,5,9], Yashar S. Niknafs PhD[4], Todd M. Morgan MD[1,2]

**Affiliations:**

[1] Department of Urology, University of Michigan, Ann Arbor, Michigan 48109

[2] Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan 48109

[3] Department of Radiology, University of Michigan, Ann Arbor, Michigan 48109

[4]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, Michigan 48109

[5]Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109

[6] University of Michigan Medical School, Ann Arbor, Michigan 48109

[7] Department of Urology, Northwestern Feinberg School of Medicine, Chicago, Illinois 60611

[8] Department of Urology, Johns Hopkins School of Medicine, Baltimore, Maryland 21287

[9] Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan 48109

*corresponding author

[Ψ] co-first authors

## ABSTRACT

**Objective:** To assess the complementary value of urinary MyProstateScore (MPS) testing and multiparametric MRI (mpMRI) and compare clinical outcomes based on application of MPS and PSA density (PSAD) in patients with equivocal (PI-RADS 3) mpMRI.

**Materials and Methods:** We included patients that underwent mpMRI followed by urine collection and prostate biopsy at the University of Michigan between 2015-2019. mpMRI was evaluated at the patient-level according to the maximum Prostate Imaging Reporting and Data System version 2 (PI-RADS v2) score. MPS values were retrospectively calculated from whole urine specimens using the validated locked model based on serum PSA, urinary PCA3, and urinary TMPRSS2:ERG expression. In the PI-RADS 3 population, the discriminative accuracy of PSA, PSAD, and MPS for GG≥2 cancer was quantified by the area under the receiver operating characteristic curve (AUC). Decision curve analysis was used to assess net benefit of MPS testing relative to PSAD.

**Results:** There were 540 patients that underwent mpMRI and prostate biopsy with valid urinary MPS results. The prevalence of GG≥2 cancer was 13% for PI-RADS 3, 56% for PI-RADS 4, and

87% for PI-RADS 5. MPS was significantly higher in men with GG≥2 cancer [median 44.9, IQR (29.4-57.5)] than those with negative or GG1 biopsy [median 29.2, IQR (14.8-44.2); p<0.001]. This was true overall and within each PI-RADS score. In the PI-RADS 3 population (n=121), the AUC for predicting GG≥2 cancer was 0.55 for PSA, 0.62 for PSAD, and 0.73 for MPS. On decision curve analysis, MPS provided the highest net clinical benefit across all pertinent threshold probabilities. Use of a MPS threshold ≤25 for risk-stratification would have prevented nearly 40% of biopsies while missing only one GG≥2 cancer.

**Conclusions:** In this institutional cohort of patients that underwent mpMRI and prostate biopsy, urinary MPS was significantly associated with GG≥2 cancer across all PI-RADS scores, suggesting a potential additive role of these diagnostic tools. In men with PI-RADS 3 observations, MPS values ≤25 ruled out GG≥2 cancer with high negative predictive value, significantly outperforming PSAD.

**Category:** Biomarker **Color:** Purple **Poster Board #:** 13

**Detection of Lower Airway Dysbiosis in Prognostication of Lung Cancer**

Jun-Chieh (James) Tsay, MD, New York University School of Medicine, Benjamin G. Wu, MD, New York University School of Medicine, Imran Sulaiman, MD, New York University School of Medicine, Rosemary Schluger RN, New York University School of Medicine, Yonghua Li, New York University School of Medicine, Michelle Badri, NYU, James T. Morton PhD Simons Foundation, Nan Shen Icahn School of Medicine at Mount Sinai, Linchen He, New York University School of Medicine, William Moore MD, New York University School of Medicine, William N. Rom MD, MPH, New York University School of Medicine, Harvey I. Pass MD, New York University School of Medicine , Jose C. Clemente PhD, Icahn School of Medicine at Mount Sinai, Huilin Li PhD, New York University School of Medicine, Kwok-kin Wong MD, New York University School of Medicine, Thales Papagiannakopoulos PhD, New York University School of Medicine, and Leopoldo N. Segal MD, New York University School of Medicine

## Abstract

While newer evidence suggest that the lung microbiota is more tightly associated with the local immune phenotype than the gut microbiota, little is known about the effects of the local lung microbiota on the immune surveillance tone. Previously we had shown that lower airway dysbiosis with oral commensals have been shown to be associated with lung cancer. Here we investigate in both a prospective human cohort and preclinical mice model, the association between lung dysbiotic and clinical lung cancer prognosis and progression.

Enrichment of the lower airway microbiota with oral commensals in the lung can trigger host transcriptomic signatures associated with lung carcinogenesis. This lower airway dysbiotic signature was found to be more prevalent in late stage lung cancer and is associated with poor prognosis, compared with early stage disease. It is also associated with worse tumor progression as measured by RECIST scores. There was an upregulation of IL-17, PI3K, MAPK and ERK pathways in airway transcriptome, and we identified *Veillonella parvula* as the most abundant taxon driving this association. In a $Kras^{LSL\text{-}G12D/+}$;$p53^{fl/fl}$ Non-small cell Lung Cancer mice model, innoculation with *V. parvula* led to decreased survival, increased tumor burden, IL-17 inflammatory phenotype and activation of checkpoint inhibitor markers.

In this study, we further identified a pro-tumor host immune phenotype triggered in response to lower airway dysbiosis that contributed to the progression of NSCLC. Further studies of lung dysbiosis may potentially help identify novel biomarker for diagnosis and prognosis in NSCLC.

**Category:** Biomarker **Color:** Green **Poster Board #:** 16

**Addressing Misclassification Bias in Prostate Cancer Early Detection in the P-MRI Study**

**Authors**: Yingye Zheng, PhD, Jacob Kagan, PhD, John T. Wei, MD, Dan Barocas, MD, Christopher Filson, MD, Adam Kibel, MD, Michael Liss, MD, Yair Lotan, MD, Tim McClure, MD, Aria Olumi, MD, Julio Pow-Sang, MD, Sanoj Punnen, MD, Soroush Rais-Bahrami, MD, Geoffrey Sonn, MD, Kara Watts, MD on behalf of P-MRI site PIs

## Abstract

**Background**: Prostate cancer early detection biomarkers have been developed based on pathological diagnosis of ANY cancer. Today, there is an emphasis to develop early detection biomarkers to discriminate clinically significant cancer, e.g. Gleason 7+. However, the pathological diagnosis is almost always based on a prostate needle biopsy, which has known sampling errors including under grading of any given case. We hypothesize that there is significant bias when only the index biopsy is used for prostate cancer biomarker development.

**Methods**: Opportunities for misclassification bias have plagued prior prostate biomarkers development pipelines. In the EDRN MRI prospective cohort (P-MRI) study, several strategies are undertaken to lessen the misclassification. First, we incorporated the use of MRI, which has been found to identify clinically significant cancer, at initial biopsy and then again 2 years after the index biopsy. Second, the study is purposefully designed as a cohort, to capture subsequent diagnostic events. These reclassification events often occur months to years after the index biopsy (e.g., radical prostatectomy, repeat prostate biopsy) and is almost universally excluded in past biomarkers development and validation studies.

**Results**: To date, the P-MRI study has accrued 170 of 350 planned during phase one. Recruitment has been negatively affected this year by COVID but is expected to continue ramping up. A total of 11 sites have now been activated.

**Findings**: The median follow up so far is < 1 year. Due to covid, study sites were at time closed and subjects were reluctant to return for follow up. As clinical sites move more to virtual care, remote consenting has been incorporated into the study. Upon completion of follow up (minimal two years), this study will be able to estimate the misclassification that occurs when only the index biopsy is used for biomarker development.

**Category:** Technology **Color:** Red **Poster Board #:** 1

**Patient Level/Bedside EFIRM Liquid Biopsy (eLB)**

Jordan Cheng DMD, University of California Los Angeles; Hua-Jun PhD, National Institute of Standards and Technology (NIST); Kenneth Cole PhD, National Institute of Standards and Technology (NIST) and David Wong DMD, DMSc, University of California Los Angeles

## Abstract

**Liquid biopsy (LB)** has significantly impacted patient care with lung cancer. Oncologists use liquid biopsy in daily practice to precisely detect activating or resistance mutations in *EGFR*. These tests are performed with plasma DNA and have been approved as companion diagnostics for patients treated with tyrosine kinase inhibitors (TKIs). In addition to *EGFR*, other somatic mutations (*ALK, ROS1, NTRK, MET, BRAF, HER2*) can also be evaluated by liquid biopsy approaches. Liquid biopsy encompasses a biomarker development process of discovery and validation. The validated biomarkers are then deployed at the patient level/bedside for specific and sensitive detection for early detection, treatment monitoring, recurrence, or residual disease detection. Circulating tumor DNA (ctDNA), circulating tumor cells (CTC), epigenetic changes, miRNA and extracellular vesicles have been explored for liquid biopsy. Currently, ctDNA has gained significant momentum for liquid biopsy clinical utilities.

Circulating tumor DNA (ctDNA) are somatic mutations associated with specific tumor shed upon tumor necrosis and/or turn over. *EGFR* ctDNAs (L858R, exon 19del and T790M) are pathopneumonic for NSCLC and also actionable as they are therapeutic targets of TKIs. ctDNA have been extensive discovered and catalogued in the COSMIC database. Circulating free DNA has been explored as liquid biopsy markers for cancer detection. Of particular note is the recent DELFI approach to comprehensive analyzed cfDNA fragment size (fragmentomics) in plasma of tumor and non-tumor subjects and harness fragment size alterations by machine learning to develop fragment profiles for cancer detection.

The ultimate goal of liquid biopsy will be to provide point-of-care early detection of an emerging tumor using ctDNA and/or cfDNA. Bedside liquid biopsy technologies that are has the greatest concordance with tissue genotyping, non-invasive, minimal volume, direct detection will have the greatest clinical value at the patient level for clinical decision/s. Current technologies for patient level liquid biopsy of ddPCR and NGS to detect *EGFR* mutations in plasma of lung cancer patients is 60-70% with tissue genotyping, requiring 10ml blood, DNA extraction. **EFIRM-liquid biopsy has a sensitivity to detect 96% of *EGFR* mutations in 40 uL of plasma and saliva, direct detection. EFIRM preferentially detect ultra-short single-stranded ct/cfDNA (~35bp).**

**Category:** Imaging **Color:** Orange **Poster Board #:** 7

**Enhanced Pattern Mapping (EPM) to Differentiate High vs Low Grade Intraductal Papillary Mucinous Neoplasms (IPMNs): a Multi-Institutional Study**

Mohamed Zaid, MD, MD Anderson Cancer Center, Ezgi Kirimli, MD, MD Anderson Cancer Center, Sophia Hernandez, MS, University of California San Francisco, Julia Douglas, BS, MD Anderson Cancer Center, Aatur D. Singhi, MD, PhD, University of Pittsburgh Medical Center, Anil K. Dasyam, MD, University of Pittsburgh Medical Center, Randall E. Brand, MD, University of Pittsburgh Medical Center, Walter G. Park, MD, Stanford University, Syed Rahmanuddin, MD, City of Hope, Michael H. Rosenthal, MD, PhD, Dana Farber Cancer Institute, Brian M. Wolpin, MD, MPH, Dana Farber Cancer Institute, Natalia Khalaf, MD, Baylor College of Medicine, Ajay Goel, PhD, City of Hope, Daniel D. Von Hoff, MD, Translational Genomics Research Institute, Spencer Behr, MD, University of California San Francisco, Kim Kirkwood, MD, University of California San Francisco, Eric P. Tamm, MD, MD Anderson Cancer Center, Anirban Maitra, MBBS, MD Anderson Cancer Center, and Eugene J. Koay, MD, PhD, MD Anderson Cancer Center

**Objective:** To differentiate the malignant potential of IPMNs using EPM technique on standard of care multiphasic computed tomography (CT) scans.

**Background:** IPMNs are pre-malignant lesions that are commonly detected as incidental imaging findings. Accurate and non-invasive biomarkers to identify the IPMNs that are likely to progress into pancreatic cancer are still lacking. Previously, we described EPM as a method to improve the contrast-to-noise ratio in liver cancers (Park et al, 2020) and radiomics to differentiate IPMNs (Hanania et al, 2016). Here, we used radiomic features extracted from EPM to differentiate high grade (HG) from low grade (LG) IPMNs.

**Methods:** We retrospectively evaluated two cohorts of patients with IPMNs, who underwent surgical resection and histopathological evaluation for the grade of dysplasia: D1, an internal discovery cohort [HG=33, LG=17], and D2 an external multi-institutional validation cohort [HG=17, LG=35]. All patients underwent pre-operative multiphasic CT scans, which we used to create the EPM maps. First, we registered the non-contrast and portovenous phases to the arterial phase, followed by volumetrically contouring the IPMNs. Then, we generated EPM to quantify the root mean square enhancement values for each voxel within the contoured cyst and to generate the enhancement pattern curves, which were mapped back to its originating spatial coordinate, producing the EPM images. Finally, we extracted the first order radiomic statistical features of the IPMNs from these EPM images. Using D1, we conducted a stepwise regression analysis with backward elimination process to select the most significant features. These features were used to build a predictive logistic regression model that we validated using D2. Receiver operating characteristic (ROC) analyses were used to determine the classification ability of the model.

**Results:** Using D1, four radiomic features were identified and used to build the logistic regression model: minimum, maximum, standard deviation and skewness. In D1, the model achieved high performance (area under the curve (AUC) = 0.86, $p<0.0001$) in differentiating HG from LG. When applied to D2, the model demonstrated a similarly high performance (AUC=0.86, $p<0.0001$). In both D1 and D2 the model showed high sensitivity (88% and 94%) and relatively lower specificity (65% and 66%) in predicting the HG IPMNs, with overall accuracy of 80% and 75% respectively.

**Conclusions:** EPM-derived radiomic features may provide a non-invasive means to characterize the malignant potential of IPMNs. Ongoing work includes additional external validation using an EDRN-sponsored imaging repository for pancreatic cancer and integration of clinical correlates, including incorporation of Fukoka criteria.

**Category:** Bioinformatics **Color:** Blue **Poster Board #:** 4

**OmicsCurveNet: Transforming Omics Data to Images for Biomarker Discovery Through Deep Learning**

Shiyong Ma, PhD, Zhen Zhang, PhD, Johns Hopkins University School of Medicine

**Abstract**

Omics data analysis for discovery typically starts with global univariate analysis comparing samples among different phenotypes. It is only afterwards that selected subsets of the original targets are subjected to more complex multivariate analyses. During the initial univariate analysis, correlated co-expression patterns are often ignored, and low-frequency molecular alterations would be deemed insignificant and missed in down-stream analysis.

As a potential remedy, we have previously developed *OmicsMapNet* that rearranges omics expression data of individual samples into 2D images in which molecular features with related functions were mapped spatially into adjacent and patterned locations using a treemap scheme based on hierarchical groupings of molecular functions. The resultant images were used in deep learning to train convolutional neural network (CNN) models to classify the samples into their given phenotypes. The attention maps of the trained CNN were used in turns to identify "hot spots" representing spatial clusters of phenotype-dependent attention features with related molecular functions. These features, individually, might not have sufficient prevalence or magnitudes of changes to be detected in global univariate testing. They could, however, collectively with their functionally related neighboring features, become attention features of the CNN during deep learning and be discovered as potential phenotype-dependent targets.

Recently, we developed a second-generation approach *OmicsCurveNet* that, in addition to color intensity, uses abstract shapes (parameterized curves) to encode expression of molecular features. It produces better visual contrasts in the converted 2D omics data images to further take advantage of CNN's ability to detect high-level visual elements and abstract spatial patterns, and subsequently, improves discovery of biomarkers/targets with subtle yet meaningful phenotype relevant molecular functions.

In this study, we applied OmicsCurveNet to TCGA LGG and GBM data (Ceccarelli *et al*., 2016, PMID: 26824661). Deep learning CNN models were trained to classify between Grade II and Grade IV (GBM) samples, the attention genes that are informative of tumor grades were subsequently identified based on Grad-Class Activation Map algorithm and degree of attention gene significance were calculated based on designed pixel attention statistics.

Supervised and unsupervised analysis using the selected attention genes on Grade III glioma samples identified sub-groups with significantly differences in patient survival. We demonstrated that informative attention genes identified by OmicsCurveNet were distinct from what would be discovered through conventional global significance testing. Our approach therefore could be complementary to existing methods in Omics data analysis and offer a different yet potentially powerful way to facilitate the discovery of biomarkers associated with early (and low frequency) molecular events in cancer initiation and progression.