Emerging Next Generation Clinical Mass Spectrometry for Personalized Medicine and Cancer Moonshot

Report of the 6th Annual 2016 AACC/MSSS Conference: Mass Spectrometry and Separation Sciences for Laboratory Medicine

Developed in cooperation with AACC's Mass Spectrometry and Separation Sciences Division, the AACC Chicago Section and the Chicago Pathology Society

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Report prepared by the Organizing Committee Members and

Officers of the AACC MSSS Division:

Conference Chair, Steven H. Wong, Ph.D. 2014 AACC President, Professor of Pathology, Director of Chemistry and Toxicology, Co-Director of Clinical and Translational Mass Spectrometry Center, Department of Pathology, Wake Forest School of Medicine

AACC MSSS Chair, Victoria Zhang, Ph.D. Vice Chair and Associate Professor of Pathology, Director of Laboratories and Clinical Mass Spectrometry Lab, Department of Pathology and Laboratory Medicine, University of Rochester

Steven Cotten, Ph.D. Assistant Professor of Pathology; Director of Chemistry, Immunology and Toxicology, Ohio State University Wexner Medical Center, Columbus, OH.

Brent Dixon, Ph.D. Director, Bureau of Laboratories, South Carolina Department of Health and Environmental Control

Chicago Pathology Society Liaison – Steven Harris, M.D., Clinical Associate Professor of Pathology at University of Illinois College of Medicine, Chicago, IL

AACC Chicago Section Liaison - Ming Jin Ph.D. Associate Professor of Pathology and Director of Clinical Chemistry, University of Illinois College of Medicine, Chicago, IL

Saeed Jortani, Ph.D. Professor of Pathology and Laboratory Medicine, University of Louisville

Yusheng Zhu, Ph.D. Professor of Pathology, Medical Director of Clinical Chemistry and Toxicology, Co-Director of Pathology Core Reference Laboratory and ATL/SPA, Department of Pathology & Laboratory Medicine, Penn State University Hershey Medical Center.

Mass Spectrometry (MS) and separation sciences are increasingly gaining acceptance for routine assays in clinical laboratories. An increasing menu of MS-based applications in clinical analysis is changing the way many laboratories perform their everyday testing. The goal of this conference was to discuss and shed some light on the issues that have emerged as this technology evolves into a laboratory tool for both routine testing and advanced Personalized/Precision medicine. This intermediate level conference aimed to enable attendees to identify and apply best practices in the use of MS testing for patient-centered care., to discuss why MS-based laboratory tools are a crucial component of clinical decision making in Personalized/Precision medicine programs., to consult with and advise clinicians on the use and interpretation of test results from MS-based lab tools., and to identify new MS-based technologies that are influencing clinical laboratory testing and explain their clinical applications.

After the conferees were welcomed by AACC President Patricia Jones and CAP President Richard Friedberg, the authoritative Open Plenary Lecture was delivered by Amanda G. Paulovich, M.D., Ph.D. of Fred Hutchinson Cancer Research Center. The lecture focused on Translational MS for Proteomics and Peptides. According to Dr. Paulovich, the use of diagnostic biomarkers is essential for the realization of precision medicine and in translational research. Yet, currently there exist biomarker diagnostic assays for less than 600 proteins (LDT and FDA-cleared), a fraction (1-2%) of the total number of proteins encoded by the human genome. MS-based proteomic assays (Multiple Reaction Monitoring, also referred to as Selected Reaction Monitoring) have emerged as a viable platform for protein assays in modern disease management. The lecture presented case studies of this methodology applied to different types of biospecimens for applications ranging from biomarker measurements to pharmacodynamic studies. Ongoing efforts by the Clinical Proteomic Tumor Analysis Consortium (CPTAC) of the National Cancer Institute (NCI) to set standards for MRM-based assay qualification and to make highly characterized assays (assays.cancer.gov) and monoclonal antibodies (antibodies.cancer.gov) available to the community were also discussed.

This was followed by the Expert Panel Round Table discussion on Critical Update on Development and Implementation of MS Based Assays in the Clinical Laboratory, moderated by Dr. Saeed A. Jortani (University of Louisville School of Medicine) and involved the participation of Patricia Jones, PhD (2016 AACC President); Henry Rodriguez, PhD (Director, NCI CPTC program), Julia Lathrop, PhD (Director at the FDA CDRH`); Amanda Paulovich, MD, PhD (physician scientist involved with biomarker discovery for cancer; Charles Root, PhD (Codemap Institute). The discussion began by Dr. Jortani reasserting the need for discovery of novel biomarkers as well as their successful implementation into clinical practice. Many of MS assays if not all of them have been developed clinically under the Laboratory Developed Test (LDT) program and CLIA regulations. The need for LDTs has been recognized for advancement of new knowledge, technology and treatment strategies in clinical practice. Regulatory process is also an important facet of Pathology and Laboratory Medicine. The environment would be when new and current laboratory tests could be offered to clinicians with adequate oversight.

Drs. Paulovich and Rodriguez were engaged with intense efforts in discovery and validation of novel cancer diagnostics using MS. Following the discovery and validation processes, novel cancer diagnostics may and potentially would need to be offered as immunoassays. Dr. Rodriguez' group at the National Cancer Institute (NCI) has started the dialogue with the FDA a while ago in terms of demonstrating which steps have to be taken to take a novel biomarker

through the process of registration with the FDA. Panel members noted that this burden will be on the shoulders of the laboratory which would ultimately offer such tests to the consumers.

Dr. Lathrop outlined the process for the labs, which would need to go through to be compliant with the new proposed regulations. She also stated that the CLSI guidelines as well as other traditionally accepted validation approaches are considered to come up with what steps will be required. The intent for the proposed new registration process with the FDA is to assure the quality of the performed tests which will ultimately be integrated in the decision making process in the care and treatment of patients. The proposed guidelines will consider practical limitations as well as laboratories' capabilities in adhering to such requirements to prevent a situation under which the standard patient treatment is compromised.

According to Dr. Root, the new reimbursement environment looks promising for the new MS-based assays. If a laboratory discovers or implements a new biomarker for patient care, there is an avenue now to get reimbursed at least for a while until more labs are adopting the test or a single lab provides data on non-CMS and third party reimbursement of that test. This segment of the panel discussion was well received and audience participation was excellent.

In summary, critical updates were presented to the attendees regarding regulatory and reimbursement issues affecting adaptation of novel diagnostics into clinical practice. It was recently announced that the adaptation of the new regulatory requirements for the LDTs which had been expected to take effect by the end of 2016, are now on-hold indefinitely pending the decision by the President Trump's administration.

The next session, co-chaired by Dr. Victoria Zhang (University of Rochester) and Steven Harris, M.D. (University of Illinois Chicago), focused on Managing Advances in Clinical and Translational MS. 2016 AACC President Dr. Patricia M. Jones from Children's Medical Center (Dallas, TX) started the session by describing the history and current status of testing for Inborn Errors of Metabolism (IEM) using MS. Testing for IEM has progressed dramatically since its inception, driven largely by advances in technology, with both GC/MS and LC-MS/MS playing an important role in screening and confirmation. From an assay performed to detect one condition, IEM testing has grown to a suite of tests screening for a large and growing number of conditions. Case studies illustrated the importance of this testing and the role MS plays in IEM testing. Additional diagnosable IEM conditions exist; the development of LC-MS/MS based laboratory developed tests (LDTs) for these conditions is needed.

The topic switched from a well-established set of tests to a research-based talk when status of *MALDI-TOF MS and Its Applications in Laboratory Medicine* was presented by Dr. Yusheng Zhu from the Medical University of South Carolina. MALDI-TOF is a developing technique that shows promise for targeted molecule analysis and molecular profiling, as well as applications in microbiology, molecular pathology, flow cytometry and tissue imaging. The fundamentals of the technique were covered, along with a discussion of quantification of targeted molecules, emphasizing the analysis of molecules larger than conventional small molecule drugs and metabolites. MALDI-TOF molecular profiling, used to distinguish between, for example, cancerous tumors and non-cancerous, is still in development and needs large-scale validation before being implemented as a clinical test.

A different, but very important topic was covered by Dr. Charles B. Root of CodeMap, LLC. His presentation, *Present and Future Reimbursement for Mass Spec Testing,* explored the complex world of current and future regulations that govern reimbursement for both lab tests and

MS based tests. The roles of Current Procedural Terminology (CPT) codes and Medicare G-codes along with the need for expansion of coding to reflect current and ever-changing tests were discussed using current CPT coding for mass spec drug tests as examples. The impact and timeline of the impending Protecting Access to Medicare Act (PAMA) rules covering reimbursement and data reporting were explained, along with Advanced Laboratory Test (ADLT) requirements. Finally, the importance of correct coding for new mass spec assays was discussed.

Doug Jeffery, PhD, from the U.S. Food and Drug Administration concluded the session with a glimpse of the future state of another aspect of clinical MS with his overview of the recent FDA workshop – *Using MS for Proteomic and Peptide Analysis*. Key topics discussed included validation and when to re-validate, standards and controls, method comparison studies, data management and sample collection / handling. The presentation went on to discuss FDA views on peptide and protein analysis by LC-MS/MS as an extension of current practice, needing validation to existing (CLSI) guidelines for precision and reproducibility, linearity, carryover, detection limits and specificity. Dr. Jeffery concluded by emphasizing that input from the clinical LC-MS community is desired as the FDA moves forward.

The Key Note Lecture of the Session on Translational and High Resolution Mass Spec for Pathology and Beyond, co-chaired by AACC President Jones and CAP President Friedberg, was delivered by John D. Pfeifer, M.D., Ph.D., Professor of Pathology, and Vice Chair for Clinical Affairs, Department of Pathology, Washington University School of Medicine. Dr. Pfeifer's authoritative presentation on Clinical Tissue Imaging by MS - The Future is Now, was based on a collaboration with Dr. Richard Caprioli and Dr. Jeremy Norris of the National Research Resource for Imaging at Vanderbilt University Medical Center. He demonstrated the use of Imaging MS for lipid in a rat brain using High Mass Resolution MALDI Fourier Transform-Ion Cyclotron Resonance MS (MALDI FTICR MS), using positive ion mode MS/MS, and phosphopeptide enrichment on TiO2 surface. Diagnostic applications included using IMS on FFPE tissue sections for differentiating melanoma and breast lesions at proteomic level, renal tumors, and pathogen identification. As an adjunct for Precision Medicine, IMS peptide measurement might be used to evaluate pathway activation for immunomodulatory therapy involving PD-1 and PD-L1 inhibitors. Dr. Pfeifer state that CAP has already provided checklist for MS applications, and will develop future ones for IMS hardware and software. Dr. Victoria Zhang of University of Rochester established the current need and future plans for MS education. AACC offers MS certificate programs, and well attended workshops during annual meetings as well as hands-on workshops at the University of Pennsylvania (Co-Chairs, Les Shaw, Ph.D. of UPenn and Doug Stickle, Ph.D. of Thomas Jefferson University) , University of Chicago (Chair, Edward Leung, Ph.D.) and Northwestern/Lurie Children's Hospital (Chair, Shannon Haymond, Ph.D.). Further, several sessions were offered as part of the annual meetings of the Chinese Association of Laboratory Medicine. Clinical Laboratory News of AACC has offered regular practical articles on MS. She was positive that these combined efforts would provide ample opportunities for MS at various levels. Ms. Nidia Lauzon, a Ph.D. student from the Department of Chemistry at Universite de Montreal described another novel about forensic analysis of latent fingermarks by silver-assisted Laser Desorption Ionization imaging MS (LDI IMS) on non-conductive surfaces. Since the initial detection of cocaine in fingermark in 2008 by Graham Cook at Purdue University, her laboratory in Montreal had developed a fast, simple and highly sensitive methodology by silver sputtering onto collected finger mark surface. LDI- TOF IMS of the finger mark surface produced, in positive mode, Ag+-fatty acids, cholesterol and

lipids. Finger marks might be detected in surfaces such as paper, cigarette cardboard, and garbage bag. It can also be used for the detection of illicit drugs such as cocaine, THC and heroin. Dr. Edward Leung of University of Chicago demonstrated a high sensitivity Micro-LC-MS/MS clinical method for estradiol in human serum without derivatization. Negative ionization was used along with alkaline pH mobile phase. MicroLC clogging was minimized by cleaning in sample preparation and assay performance. Detection limit of estradiol was about ~3 pg/mL.

Industry Viewpoint session was co-Chaired by Brent Dixon, PhD (currently with South Carolina Dept. of Health & Environmental Control**)** and Ming Jin, PhD (University of Illinois at Chicago). Crystal Holt, MS of Sciex presented "A Focus on Education". The aim of Sciex is to provide world-class technologies to help answer scientific challenges to improve our world. Sciex offers a network of resources to support instrument management, support/service calls, knowledge articles and training access. The Sciex University module offers training programs covering full workflows, maintenance, LC-MS fundamentals with 24 hour/ 7 day access and certification tracking. Sciex University is fully customizable per laboratory site to their instrumentation and staff. Carrie Adler, MS, MT of Agilent Technologies presented an "Agilent Perspective: Accurate Mass as a Powerful Screening Tool". She highlighted the importance of choosing the best MS platform to address the specific assay's requirements of sensitivity, selectivity and compound identification. These mass spectrometers may be applied to targeted quantitation, non-targeted screening or unknown profiling. There are software packages in the MassHunter suite that are used to reduce the data to meaningful results. Edward Goucher, PhD of Thermo Fisher Scientific discussed the opportunities in the clinical MS and their future directions. This technology has gained adopters and higher demand is anticipated as a function of aging global populations and the Precision Medicine effort. There are broad applications including translational and clinical research, In-Vitro Diagnostic MS, forensic toxicology and anti-doping for sports medicine. ThermoFisher Scientific has a wide portfolio spanning benchtop Orbitrap (high resolution), triple quadrupole, and ion trap mass spectrometers. They are actively engaged in the next frontier of "clinical omics".

The session on Emerging and Translational MS Applications, co-chaired by Dr. Yusheng Zhu (formerly at Medical University of South Carolina, currently at Penn State University Hershey Medical Center) and Dr. Shannon Haymond (Lurie Children's Hospital and Northwestern University), started with a keynote presentation by Ronald R. Flegel from Substance Abuse and Mental Health Services Administration (Rockville, MD). The title of the talk was "Oral Fluid and Alternate Samples for Workplace Drug Testing". Urine is a common specimen for Drug Free Workplace Programs, but it has some limitations. In the Proposed Mandatory Guidelines for Federal Workplace Drug Testing Programs, oral fluid is added as an alternative specimen and the Guideline is in the approval process. In addition, the testing of Delta-9-tetrahydrocannabinol (THC) to detect cannabis use or testing of Delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA) within the federal drug testing process is being reviewed. The proposed guideline allows federal executive branch agencies to test four additional Schedule II narcotic prescription medications (i.e., oxycodone, oxymorphone, hydrocodone, and hydromorphone). In addition, the Hair Mandatory Guidelines are being drafted by the Division of Workplace Programs.

This regulatory update was followed by a scientific talk entitled "Renal Metabolomics for Personalized/Precision Medicine" presented by the AACC Past President, Dr. Steven H. Wong from Wake Forest University School of Medicine (Winston-Salem, NC) . Dr. Wong first gave an introduction to MS based metabolomics in translational and clinical laboratories including

experimental design, work flow, data analysis, and quality control. Then Dr. Wong shared his experience in renal metabolomics and pain management drug metabolomics studies. Finally, he discussed future metabolomic applications for personalized medicine.

Use of another matrix, umbilical cord tissue for neonatal drug testing was presented by Dr. Steven W. Cotten from Ohio State University Wexner Medical Center (Columbus, OH), whose presentation was "Umbilical Cord Tissue Analysis via LC/MS". Umbilical Cord Tissue has a similar detection window for neonatal drug testing compared to amniotic fluid and meconium. Dr. Cotten described the process of using umbilical cord tissue for neonatal drug testing including specimen processing and analysis and the quality matrix such as the times from collection to receiving and from receiving to report. The performance study showed that higher sensitivity and negative predictive value made the cord assay a better rule out (screening) test for neonatal abstinence syndrome, not a rule in (confirmation) test.

Finally, a technology topic was presented by Dr. R. Brent Dixon from PCLS (Rock Hill, SC), entitled "Columnless Direct Sample Analysis by MS". The limitations of columnless MS are lower signal to noise for low level concentration analytes, higher background signal level due to suppression, ionization competition and co-eluted components, and challenge for isobaric compounds etc. However, this method has its own advantages such as providing a fast methodology to get quick results, minimal sample preparation, ability to retest within turnaround time constraints, lower sample volume requirements, less consumable waste, lower solvent consumption, and shorter run times. Then, he used RapidFire/MS (RF-MS) as an example to demonstrate this technology and the applications of columnless MS in drug testing, tissue imaging, and molecular profiling.

The session on Next Generation Clinical MS: Enabling Personalized/Precision Medicine, co-chaired by Dr. Steven Cotten (Ohio State University) and Dr. Ed Leung (University of Chicago), began with a keynote presentation featuring Dr. Valentina Pirro from the University of Turin and Purdue University who discussed ambient ionization MS for brain cancer diagnosis. In her talk Dr. Pirro detailed the role that MS can play in the surgical resection of brain tumors. The two main challenges for brain cancer are accurate diagnosis and efficient treatment. The diagnosis involves a combination of histology and molecular information which can be laborious and time intensive and only occurs post operatively. Efficient treatment requires maximal tumor resection that minimizes neurological damages. Currently there is no assessment of surgical margins intraoperatively. Using a specialized surgical handpiece, the sample can be introduced in the MS using ambient ionization in real time to provide in vivo, intrasurgical analysis of tissue. The full scan mass spectral patterns acquired can be used to classify tumor types or distinguish normal from cancerous tissue. This approach can also be used to map tumor heterogeneity and tumor boundaries using contrast agents. Dr. Pirro finished by highlighting how ambient ionization can be integrated online and offline into traditional pathology and molecular diagnostics.

The second speaker, Dr. Angella Charnot-Katsikas from the University of Chicago, provided an update on MS for Microbial Speciation and Drug Resistance in the Clinical Microbiology Lab. Dr. Charnot-Katsikas began with a review of the two current commercially available MALDI-TOF systems for clinical microbiology speciation followed by advantages of MALDI-TOF. These includes a faster turnaround time to ID, not vulnerable to gain or loss of function, no need to pre-differentiate and doesn't require primer or probe designed. She then discussed the clinical impact that MALDI has had on treating sepsis and infection by reducing time to antibiotic optimization, length of stay and mortality. Dr. Charnot-Katsikas concluded with a discussion of

MALDI to detect antibiotic resistance and the future application of susceptibility, stain typing and biothreat agents.

The final speaker of the session was Dr. Henry Rodriguez from NCI who spoke on Proteogenomics and Precision Medicine that detailed areas of Accuracy and Performance for AACC Consideration. Dr. Rodriguez discussed the White House Cancer Moonshot whose goals is to make 10 years of progress in cancer in 5 years. He described the creation of the APOLLO (Applied Proteogenomics Organizational Learning and Outcomes) Clinical Network where all veterans seen for lung cancer will be molecular and proteomic profiling that will shared across. Discussion of the moonshot initiative revealed many opportunities for contribution from the MS laboratory medicine community and AACC.