April 3, 2017

Digital Spatial Profiling Technology Enables Quantitative Protein Profiling in FFPE Tissue Providing Detailed Molecular Characterization of Immune Response to Tumors

The Yale School of Medicine Presents Validation of NanoString's Novel Digital Spatial Profiling Technology by Quantitative Immunofluorescence Technology at 2017 American Association for Cancer Research

WASHINGTON, April 03, 2017 (GLOBE NEWSWIRE) -- NanoString Technologies, Inc. (NASDAQ:NSTG), a provider of life science tools for translational research and molecular diagnostic products, today highlighted the validation of its novel Digital Spatial Profiling (DSP) technology by the current state-of-the-art AQUA® method for quantitating protein targets in FFPE. The data was generated through a collaboration between NanoString and Dr. David Rimm's lab at the Yale School of Medicine as part of a Technology Access Program for NanoString's DSP technology. The data will be presented in a poster session at the American Association for Cancer Research (AACR) conference, being held April 1-5, 2017 in Washington, D.C.

"We are excited to be working with NanoString on their new Digital Spatial Profiling technology. Our AQUA® quantitative fluorescence approach has been limited to 4-5 fold multiplexing, where DSP can currently multiplex up to 50 protein analytes and has the potential to measure hundreds of RNAs simultaneously on the same tissue section," said Dr. David L. Rimm, Professor of Pathology and of Medicine (Medical Oncology); Director of Pathology Tissue Services; Director of Translational Pathology, Yale University. "This brings many advantages, including the ability to include 'housekeeping' proteins for standardization of assays and normalization of pre-analytic variables. Because of the variable sized sample region, it is a perfect match for the 'field of view' assessment we do with the AQUA technology in our lab. We designed AQUA to measure at the molecular level rather than the cellular level, and the same is true of NanoString's DSP."

AQUA technology, which was developed by Dr. Rimm, has been demonstrated to be both reproducible and quantitative when applied to tissue-based immunoassays. This study demonstrated that both DSP and AQUA are quantitative methods for profiling protein expression, eliminating the subjectivity and variability associated with conventional immunohistochemistry techniques. However, benchmarking against AQUA revealed several key advantages of DSP technology, including the high multiplex capability and wide dynamic range. These capabilities are of particular significance when profiling tumor cell specific protein expression from the complex tumor microenvironment.

"Localized expression of immuno-regulatory proteins shapes the immune response against tumors, and the detection of cell specific proteins can reveal the presence of important immune cell populations," stated Alessandra Cesano, M.D., Ph.D, chief medical officer at NanoString. "Spatial profiling of these key molecules could lead to discovery of novel therapeutic targets and signatures that characterize immunological responses. This could enable predictive biomarkers to guide the development of immunotherapies and companion diagnostics."

Digital Spatial Profiling will be featured in two additional posters and a spotlight presentation during the AACR meeting. Merritt et al., will present recent technical advances of the platform including single cell selection and RNA profiling. Balko et al., applied the technology to characterizing checkpoint inhibitor therapy-induced autoimmune toxicities. The spotlight presentation will feature the inventors of the technology, Dr. Gordon Mills and Dr. Joseph Beechem who will speak about the potential of the platform to enable deep molecular characterization of complex biological phenomenon and provide greater insight into tumor immune cell interactions.

NanoString is accelerating the development of the Digital Spatial Profiling technology with customers via its Technology Access Program. Under the program, customers submit up to 20 FFPE tissue sections and NanoString performs a high-plex protein spatial profiling assay with a panel of 30 pre-validated immuno-oncology targets. An assay report along with raw digital data and processed results are provided back to customers. Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact the company at TAP@nanostring.com.

Key DSP Events at AACR 2017

Spotlight Presentation: Monday, April 3: 10:00am - 11:00am ET Spotlight Theater B - Hall A
Powering Precision Oncology Research with 3D Biology Technology: High Plex Multi-Analyte Profiling on FFPE with Spatial Resolution.
Gordon B. Mills M.D. Ph.D., Professor and Chair of Systems Biology at MD Anderson Cancer Center and Joseph M.

Beechem, PhD, Senior Vice President of Research & Development, NanoString® Technologies.

Poster # 3810 - Tuesday, April 4: 8:00am - 12:00pm ET
Validation of novel high-plex protein spatial profiling quantitation based on NanoString's Digital Spatial Profiling (DSP) technology with quantitative fluorescence (QIF) **[Section #32, Board #6]**
Maria I. Toki, M.D., Ph.D., Yale School of Medicine

Poster # 588 - Sunday, April 2: 1:00 PM-5:00 PM
Advanced molecular characterization of severe autoimmune toxicities associated with checkpoint inhibitor therapies **[Section #25, Board #22]**
Justin M. Balko, Pharm.D., Ph.D., Assistant Professor of Cancer Biology, Vanderbilt University

Poster# 3955 - Tuesday, April 4: 8:00 AM-12:00 PM
Spatially resolved, multiplexed digital characterization of protein and mRNA distribution and abundance in formalin-fixed, paraffin-embedded (FFPE) tissue sections based on NanoString's Digital Spatial Profiling **[Section #41, Board #22]**
Chris Merritt, Ph.D., NanoString Technologies

About NanoString Technologies, Inc.
NanoString Technologies provides life science tools for translational research and molecular diagnostic products. The company's nCounter Analysis System has been employed in life sciences research since it was first introduced in 2008 and has been cited in more than 1,500 peer-reviewed publications. The nCounter Analysis System offers a cost-effective way to easily profile the expression of hundreds of genes, proteins, miRNAs, or copy number variations, simultaneously with high sensitivity and precision, facilitating a wide variety of basic research and translational medicine applications, including biomarker discovery and validation. The company's technology is also being used in diagnostics. The Prosigna® Breast Cancer Prognostic Gene Signature Assay together with the nCounter Dx Analysis System is FDA 510(k) cleared for use as a prognostic indicator for distant recurrence of breast cancer. In addition, the company is collaborating with multiple biopharmaceutical companies in the development of companion diagnostic tests for various cancer therapies, helping to realize the promise of precision oncology. For more information, please visit www.nanostring.com.

Forward-Looking Statements
This news release contains forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934 and the Private Securities Litigation Reform Act of 1995. These forward-looking statements include statements regarding the expected performance of the company's DSP technology and its related technology access program. Such statements are based on current assumptions that involve risks and uncertainties that could cause actual outcomes and results to differ materially. These risks and uncertainties, many of which are beyond our control, include market acceptance of our products; delays or denials of regulatory approvals or clearances for products or applications; delays or denials of reimbursement for diagnostic products; the impact of competition; the impact of expanded sales, marketing, product development and clinical activities on operating expenses; delays or other unforeseen problems with respect to manufacturing, product development or clinical studies; adverse conditions in the general domestic and global economic markets; as well as the other risks set forth in the company's filings with the Securities and Exchange Commission. These forward-looking statements speak only as of the date hereof. NanoString Technologies disclaims any obligation to update these forward-looking statements.

ABSTRACTS

David Rimm (Yale) DSP Abstract for AACR 2017

3810 / 6 - Validation of novel high-plex protein spatial profiling quantitation based on NanoString's Digital Spatial Profiling (DSP) technology with quantitative fluorescence (QIF)

Presenter/Authors
Maria I. Toki1, Chris Merritt2, Giang Ong2, Joseph M. Beechem2, Daniel E. Carvajal-Hausdorf1, Yalai Bai1, David L. Rimm1.
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Disclosures
M.I. Toki: None. C. Merritt: ; NanoString Technologies. G. Ong: ; NanoString Technologies. J.M. Beechem: ; NanoString Technologies. D.E. Carvajal-Hausdorf: None. Y. Bai: None. D.L. Rimm: ; Cepheid. ; Gilead Sciences. ; Genoptix. ; Pierre Fabre. ; Perkin Elmer. ; Astra Zeneca, Agendia, Bethyl Labs, Biocept, BMS, Cell Signaling Technology, Cernostics, ClearSight, FivePrime, Genoptix/Novartis, Merck, OptraScan, Ultivue. ; Metamark Genetics. ; PixelGear.

Abstract
Background: Protein expression in formalin-fixed, paraffin-embedded (FFPE) tissue is routinely measured by Immunohistochemistry (IHC) on only one protein, or with quantitative fluorescence (QIF) on a handful of proteins on a single section. NanoString's Digital Spatial Profiling (DSP)* technology can detect and quantify proteins at significantly higher multiplex (currently 30 plex, potential for up to 800 plex) with spatial resolution using molecular digital color-coded "barcodes", within specific regions of interest on FFPE tissue. Here, we compare Nanostring DSP technology to automated QIF (AQUA), for accurate and reproducible measurement of protein expression on a continuous scale. Additionally, using the multiplexing potential of Nanostring technology, we did a pilot study to assess cold ischemic time as a variable to monitor tissue quality by assessment of epitope degradation in Non-Small Cell Lung Cancer (NSCLC).

Methods
The DSP technology uses a cocktail of primary antibodies conjugated to indexing DNA oligos with a UV Photocleavable linker that can be counted on the nCounter™ platform. Regions of interest (ROI) on the tissue are selected with fluorescently labeled antibodies, and oligos from that region are released via UV mediated linker cleavage. Free oligos are captured via microcapillary fluidics into a microtiter plate and then quantitated on the nCounter. The comparator for this technology was the AQUA method of QIF. We examined a breast cancer tissue microarray with a range of HER2 expression, and a NSCLC time to fixation standardization array with timepoints from 20 min to 48 hrs.

Results
Multiple markers were assessed and a high correlation was found between Nanostring DSP counts and QIF scores ($R^2 > 0.7$), when the measurements were performed in the same region of interest (defined by cytokeratin expression). The dynamic range of DSP exceeded the quantification range of QIF (nearly 4 logs vs about 2 logs). When the 28 protein markers' expression was compared at different fixation timepoints, most were found to be stable over different cold ischemic timepoints. Two markers, including phospho-ERK (Extracellular Signal-Regulated Kinases) and phospho-GSK (Glycogen Synthase Kinase) showed epitope loss as a function of delay to fixation ($R^2=0.0064$ and $R^2=0.05$ respectively).

Conclusion
The Nanostring DSP is a reproducible method with the capability of highly multiplexed measurements of protein expression on a field of view averaged basis. It shows high concordance with the AQUA method of QIF, an extensively validated technique for protein quantification. For the first time, the high-plex capacity of DSP allows inclusion of markers that are sensitive to time to fixation as an intrinsic control for tissue quality.
*FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Justin Balko (Vanderbilt) DSP Abstract for AACR 2017

588 / 22 - Advanced molecular characterization of severe autoimmune toxicities associated with checkpoint inhibitor therapies

Presenter/Authors
Justin M. Balko1, Daniel Y. Wang1, Yu Wang1, Rami Al-Rohil1, Margaret Compton1, Jeffery A. Sosman2, Igor Puzanov3, Bret Mobley1, Robert D. Hoffman1, Yaomin Xu1, Javid J. Moslehi1, Chanjuan Shi1, Douglas B. Johnson1. 1Vanderbilt University Medical Center, Nashville, TN; 2Northwestern University, Chicago, IL; 3Roswell Park Medical Institute, Buffalo, NY

Disclosures
J.M. Balko: ; Incyte. D.Y. Wang: None. Y. Wang: None. R. Al-Rohil: None. M. Compton: None. J.A. Sosman: None. I. Puzanov: ; Amgen. ; Roche. B. Mobley: None. R.D. Hoffman: None. Y. Xu: ; Incyte. ; Astex. J.J. Moslehi: ; Bristol-Myers Squibb. ; Novartis. ; Takeda. ; Pfizer. ; Ariad. ; Acceleron. ; Vertex. ; Rgenix. ; Verastem. C. Shi: None. D.B. Johnson: ; Bristol Myers Squibb. ; Genoptix. ; Incyte.

Abstract
Immune checkpoint inhibitors (ICIs) have made a profound impact on the treatment of a variety of cancers. However, as with any systemic treatment, toxicities are inevitable. With most classes of cancer therapies, toxicities are relatively predictable based on clinical trial safety data and therefore can be handled with prophylactic or supportive care measures. However, ICIs are unique in their ability to cause rare but severe auto-immune toxicities. The molecular underpinnings of these toxicities, as well as unique features of the patient, tumor, or affected tissue, have not been extensively explored. We recently reported a small case series of two patients with myocarditis resulting in death arising following combination ICI therapy (Johnson et al, N Engl J Med, 2016). High lymphocytic infiltration, coupled with PD-L1 expression was present in the affected myocardium and skeletal muscle. Common T cell clones were identified between the affected tissue and tumor, and abnormal expression of muscle-specific transcripts was identified in the associated tumor, suggesting release of peripheral tolerance to tumor-expressed self-antigens.To expand upon our reported study, we collected healthy and afflicted tissue from a series of cancer patients with immune-related colitis, myocarditis (MC), and encephalopathy following ICI treatment. We hypothesize that molecular analysis of these tissues will identify causal factors in the etiology of these toxicities, and how to better predict, prevent, and treat them. Thus, we performed molecular characterization of the immune infiltrate and

diseased tissue microenvironment. A total of 20 affected (colon, cardiac, brain) and non-diseased control specimens were examined by spatial digital profiling (nanoString). This process generates a spatial heatmap of digital counts of 20 selected immunology and cellular markers and proteins across each specimen. Using this technology, the landscape of inflammation in ICI-affected organs can be resolved for insights into the mechanism whereby ICI-mediated auto-immunity occurs. Targeted RNAseq for selected immuno-oncology mRNA targets was also performed. In initial RNA sequencing analyses of MC cases, affected myocardium, skeletal muscle, and patient-matched tumors all demonstrated expression of immune activation markers (e.g. interferon-gamma and granzyme B), expression of PD-L1, and muscle-specific genes. In the expanded population, including colitis, digital spatial profiling analyses and targeted NGS (RNAseq) are underway. Although data analyses are incomplete at the time of this abstract, this work will be the largest and most comprehensive analysis of the molecular underpinnings of ICI-mediated auto-immune toxicity reported to date. These data should offer clarity in the mechanisms and features of these adverse events, how to prevent or predict them with precision medicine, and how to treat them when they do occur.

Internal DSP Poster for AACR

3955 / 22 - Spatially resolved, multiplexed digital characterization of protein and mRNA distribution and abundance in formalin-fixed, paraffin-embedded (FFPE) tissue sections based on NanoString's Digital Spatial Profiling

Presenter/Authors
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Disclosures
C. Merritt: ; NanoString Technologies. J. Jung: ; NanoString Technologies. G. Ong: ; NanoString Technologies. Y. Liang: ; NanoString Technologies. F. Pakiam: ; NanoString Technologies. D. Dunaway: ; NanoString Technologies. I. Sprague: ; NanoString Technologies. S. Warren: ; NanoString Technologies. G.B. Mills: ; MD Anderson Cancer Center. ; NanoString Technologies. J. Beechem: ; NanoString Technologies.

Abstract
Introduction: As intra-tumoral heterogeneity has emerged as a challenge in development of targeted cancer therapeutics, the tissue context of biomarker levels and colocalization of key immunoregulatory proteins has become an increasingly important aspect for understanding tumor immune responses, patient classification, and stratification. Historically, immunohistochemistry and in situ hybridization have been used to assess spatial heterogeneity of targets in clinical samples. These approaches, however, have limited multiplexing capacity and dynamic range. Here, we use DSP technology, a spatially resolved approach for quantifying up to 800 protein or RNA targets with over 5 logs of dynamic range in a single FFPE slide to overcome these limits.
Methods: The technology uses DNA oligo tags for either protein or RNA detection. For protein detection, a cocktail of 30+ primary antibodies (Abs), each with a unique, photocleavable oligo tag, and 1-3 fluorescently (FL) labeled Abs was applied to a slide-mounted FFPE tissue section. Regions of interest (ROI), selected based on a FL imaging scan of the entire tissue, were illuminated sequentially with focused UV light to release the oligos. Following each UV cycle, eluent was collected from the local ROI, moved to a microtiter plate, hybridized to NanoString® barcodes, and then analyzed with an nCounter® Analysis System. The resulting digital counts corresponded to the abundance of each targeted protein in the ROI. For RNA detection, a cocktail of multiple UV-cleavable in situ hybridization probes were used in a similar manner.

Results: We demonstrate multiplexed detection from discrete ROIs within a tumor and adjacent normal tissue, enabling systematic interrogation of a heterogeneous tumor microenvironment. In control samples, we found expected levels of protein and RNA targets. We further demonstrate that this approach enables analysis of target abundance from individually selected cells, both contiguous and non-contiguous with the same phenotype, and enables multiplexed detection of key IO targets. Finally, we demonstrate detection of key IO RNA targets using direct hybridization of oligo-labeled probes.

Conclusions: With further development, our novel DSP approach to capture the spatial context of protein and RNA levels will have many applications in biomarker and translational research. The ability to digitally measure RNA and protein at up to 800-plex from FFPE tissues could facilitate drug mechanism-of-action and response studies within the tumor microenvironment. Quantitative, high-plex data should also greatly accelerate the discovery of IO biomarkers in tumors and the development of companion diagnostics for targeted therapies. P) technology: applications to immuno-oncology (IO) and tumor heterogeneity.

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