Unlocking Predictive Potential for Response to Immunotherapy
Digital Spatial Profiling Technology

MK1351 | March 2019
NanoString Technologies®, Inc.

Authors: Katie Buchanan and Sarah Church
Unlocking Predictive Potential for Response to Immunotherapy: Digital Spatial Profiling Technology

Introduction
Stage III melanoma is characterized by the spread of the cancer cell from the skin into the lymph nodes. Surgery is the first line of treatment to remove the tumor(s), cancerous lymph nodes, and healthy tissue adjacent to the tumors. Often adjuvant therapy is administered post-surgery. This can include weeks of radiation, chemotherapy, targeted drug therapy, or immunotherapy. These therapies can have mixed results and side effects that vary by patient. Researchers are actively investigating these differences in outcome to identify combinations of biomarkers that may predict a patient’s response to treatment. These expression signatures may help guide the physician to administer more effective treatments in a deliberate, evidence-based manner.

The challenge today is in identifying these combinations of biomarkers at play in the tumor microenvironment. Quantitating the levels of proteins and RNAs in a tumor sample often requires destroying the tissue and sacrificing spatial information. Alternatively, while fluorescence and brightfield imaging provide a visual map of expression they are limited by the number of fluorophores that can be captured in one experiment. While it is possible to perform multiple rounds of immunostaining and imaging on the same sample, the sample will degrade over time and errors in image registration can lead to misinterpretation of results.

The GeoMx™ Digital Spatial Profiler (DSP) is a new platform that leverages the nCounter® barcoding technology to both spatially resolve and digitally quantify protein and mRNA expression. The assay uses either antibody or RNA probes coupled to photocleavable oligonucleotide tags. After binding of oligo-conjugated probes and up to four morphology markers (fluorescent probes) to slide-mounted formalin fixed paraffin-embedded (FFPE) tissue sections, the oligonucleotide tags are released from selected regions of the tissue by UV exposure. Released tags are quantitated in a standard nCounter assay, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance (FIGURE 1).

**FIGURE 1:** GeoMx DSP Overview: Samples are stained with oligo-conjugated antibodies, imaged and ROIs selected. Oligos are then cleaved from the targeted ROIs, collected, and analyzed.
GeoMx DSP Profiling Modalities

Five main GeoMx DSP profiling modalities have been identified to define regions of interest (ROI): Geometric, Segment, Contour, Gridded, and Rare Cell (FIGURE 2). Geometric profiling uses geometric shapes to quantify expression within the chosen boundaries. The same shape can be reused, ensuring that the specific area (in pixels) is the same between ROIs. Segment profiling recognizes the differences between high and low signals from morphology markers (fluorescent targets) to identify and profile distinct biological areas within a ROI, for instance CD45-positive versus S100B-positive tissue (FIGURE 3). Contour profiling can illustrate how proximity affects biological response by examining the local microenvironment around a central structure using radiating ROI. Central structures can be compact, such as clusters of immune cells, or complex, like a neuron or blood vessel. Gridded profiling creates a tunable gridding pattern that is overlaid on the image to drive deep spatial mapping of a sample. Finally, Rare Cell identifies distinct cell populations based on cell type specific morphology markers, shining a spotlight on rare events in an otherwise crowded field.

FIGURE 2: Five profiling modalities of GeoMx DSP

FIGURE 3:
A. Tumor sample stained with S100B (tumor cells) and CD45 (immune cells). Segments are generated based on S100B and CD45 cellular morphology.

B. Enlarged view of segmentation: Green = S100B-positive tumor cells, red = CD45-positive immune cells, and blue = DNA. Each segment is collected and quantified separately within the ROI.
**Highlighted Results in Melanoma**

GeoMx DSP is being used to gain insight into the tumor microenvironment and the real-time effect of checkpoint inhibitors on tumors from patients with melanoma. The use of immune checkpoint inhibitors has been shown to be effective in decreasing relapse rates post-surgery. Programmed cell death ligand (PD-L1) is expressed on the surface of healthy cells to protect tissues from autoimmune attack. PD-L1 can also be expressed on the surface of melanoma cells, enabling them to escape detection by activated T cells that express the receptor PD-1. Nivolumab is a human monoclonal antibody against PD-1 and has been administered post-surgery in patients with melanoma. It blocks the PD-1:PD-L1 interaction, allowing continued immune surveillance by activated T cells. Ipilimumab is another human monoclonal antibody used post-surgery to disrupt the inhibition of T cells, thereby increasing the number of active T cells present peripherally. As neither of these treatments is specific to the cancer itself, but rather the immune system, there are often adverse side effects associated with their use. Nonetheless, using a combination of both drugs was approved by the FDA for use in treatment of melanoma in 2015.

Neoadjuvant therapy, or the administration of therapeutic agents before surgery, shows promise as a method to decrease the size or extent of the cancer, as well as release tumor-associated antigens prior to surgery. Two recent papers published in Nature Medicine investigate the use of nivolumab and nivolumab plus ipilimumab neoadjuvant therapy for patients with resectable stage III melanoma. FFPE tumor sections were examined by standard H&E and IHC staining before and after treatment. In addition to standard IHC analysis, these two groups used NanoString’s novel GeoMx DSP to characterize the tumor microenvironment prior to and during treatment.

In “Neoadjuvant Immune Checkpoint Blockade in High-Risk Resectable Melanoma” Jennifer Wargo and her team at MD Anderson Cancer Center compared neoadjuvant monotherapy using nivolumab only (N) to combination therapy using ipilimumab plus nivolumab (I+N). They demonstrated that combination therapy was associated with improved overall survival. However, for both treatment regimens it was noted that some patients responded to treatment better than others within the same group; these patients were designated as “responders” and “non-responders”, respectively. The team used GeoMx DSP to characterize the tumor microenvironment of responders and nonresponders using tumor biopsies taken prior to and during treatment. ROIs were selected via Segment Profiling for intratumor immune dense regions, identified by high CD45 signal concurrent with high concentrations of DNA staining. When tumors were examined from patients during treatment, responders had higher levels of CD45+ expression, CD8+ infiltrate, increased PD-L1, CD4, granzyme B, FoxP3, CD20 and PD-1 expression over non-responders (FIGURE 4). These differences were observed not only in on-treatment samples but in baseline samples, suggesting that these markers might be used to predict the success of a treatment prior to administration; potentially reshaping the type of therapy selected for the patient.

A separate study from Christian Blank and the team at the Netherlands Cancer Institute, “Neoadjuvant versus Adjuvant Ipilimumab Plus Nivolumab in Macroscopic Stage III Melanoma” compared the effects of using I+N as either an adjuvant or a neoadjuvant treatment. Neoadjuvant treatment was successful in decreasing the tumor size, resulting in less extensive surgical intervention. One key finding that explained this result was that neoadjuvant I+N expands more resident T cell clones than adjuvant I+N as demonstrated by TCR sequencing before and after treatment. A second key finding was that levels of interferon-γ (IFN-γ) RNA within pretreatment tumor biopsies correlated to clinical outcome and relapse rates after treatment. FFPE biopsies taken prior to treatment with I+N were stained with 29 targets of interest, and S100B an antigen expressed on melanocytes, to identify tumor rich ROI. Six ROIs per tumor were chosen via GeoMx DSP profiling of pretreatment and on treatment biopsy identifies multiple markers associated with response.Amaria, RN et al. Neoadjuvant Immune Checkpoint Blockade in High-risk Resectable Melanoma. Nat Med. 2018; 24(11): 1649-1654.

**FIGURE 4:** NR=No Response, R=Response. GeoMX DSP profiling of pretreatment and on treatment biopsy identifies multiple markers associated with response.
FIGURE 5:

A. Geometric ROI selection strategy

B. Volcano plot measuring differential expression of proteins between patients with melanoma that relapsed or did not relapse after neoadjuvant therapy analyzed by GeoMx DSP. Note the increased levels of β2M, CD3, and PD-L1 in patients without relapse. None of the other proteins are shown in the figure and CD3 is associated with the adaptive immune response (figure reproduced from Nature Medicine).

Geometric Profiling. CD45 staining was also used to establish three ROIs with high immune infiltrate and three ROIs with low immune infiltrate. The group quantified levels of CD3, β-2 microglobulin, and PD-L1 protein with GeoMx DSP and also stratified IFN-γ RNA levels as low, intermediate, and high. Patients with decreased levels of CD3, β-2 microglobulin, and PD-L1 and low levels of IFN-γ RNA relapsed. Patients with intermediate to high levels of IFN-γ RNA did not relapse (at time of publication), indicating that this biosignature has the potential to be used to predict the patient’s response to treatment (FIGURE 5).

Recent work from David Rimm and colleagues at Yale University looked at specific cell types within the tumor microenvironment to identify prognostic biomarkers. Compartments were elucidated with the Rare Cell Profiling using serial masks, focusing on macrophage (CD68+), melanocyte (S100B+), and non-macrophage immune cells (CD45+CD68-). The team was particularly interested in differentiating between the tumor and the stromal areas. CD3, CD8, β-2 microglobulin, PD-L1, and HLA-DR all demonstrated cell type-specific predictive power both in overall survival rates and progression free survival. PD-L1 showed strongest association with overall survival in the macrophage compartment (FIGURE 6). β-2 microglobulin in the immune, non-macrophage compartment was associated with both overall survival and progression free survival. With this information, the group has identified compartment specific markers associated with potential prognostic biomarkers of survival.

Conclusion
Using GeoMx DSP technology, potential predictive biomarkers have been identified for clinical responses to both ipilimumab and nivolumab. Whether used as neoadjuvant or adjuvant therapies, there is an inherent toxicity associated with these checkpoint inhibitors. Side effects of nivolumab and ipilimumab can range depending on the patient and can be severe enough to discontinue use. While at this time the side effects one might experience cannot necessarily be predicted, we are gaining a better understanding of whether a therapy will be beneficial prior to beginning treatment.

Moving beyond ipilimumab and nivolumab, there are many other single agent and combination immunotherapies that still need to be characterized for predictive biomarkers. GeoMx DSP has shown results that strongly track with established immunohistochemistry and pathology methods and offers the potential opportunity to profile and establish more biomarkers in a single experiment than these techniques. Moreover, its utility to perform high-plex analysis of RNA and protein for discovery of prognostic and predictive markers holds great potential for application and advancement in clinical practice and medical technology.
FIGURE 6: PD-L1 expression specifically in macrophages potentially predicts outcome. 44 proteins were profiled across 3 unique compartments from 59 immuno-therapy treated melanoma patients using rare cell profiling. Five compartment specific biomarkers were identified with PD-L1 expression in macrophages showing the strongest predictive power.

High PD-L1 expression in macrophages is associated with prolonged survival

PD-L1 in macrophage (CD68+) ROI

- Low PD-L1 in CD68 + ROI (n = 38)
- High PD-L1 in CD68 + ROI (n = 19)

p = 0.0089
HR: 4.3, 95% CI: 1.86 - 10.22

PD-L1 in melanocyte (mel.) ROI

- Low PD-L1 in melanocytes ROI (n = 40)
- High PD-L1 in melanocytes ROI (n = 19)

p = 0.02
HR: 2.32, 95% CI: 1.21 - 4.46
REFERENCES


For more information, please visit nanostring.com/products/geomx-digital-spatial-profiler/geomx-dsp

NanoString Technologies, Inc.
530 Fairview Avenue North Seattle, Washington 98109
T (888) 358-6266 F (206) 378-6288 nanostring.com

Sales Contacts
United States us.sales@nanostring.com
EMEA: europe.sales@nanostring.com
Asia Pacific & Japan apac.sales@nanostring.com
Other Regions info@nanostring.com

MAR 2019 MK1351

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.
© 2019 NanoString Technologies, Inc. All rights reserved. NanoString, NanoString Technologies, the NanoString logo, GeoMx DSP and nCounter are trademarks or registered trademarks of NanoString Technologies, Inc., in the United States and/or other countries. All other trademarks and/or service marks not owned by NanoString that appear in this document are the property of their respective owners.