

CASE STUDY

Transcriptional Profiling of Pediatric Primary and Metastatic Osteosarcoma with the nCounter® PanCancer IO 360™ Panel

Who

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Product Focus

nCounter® PanCancer IO 360™ Panel
GeoMx® Digital Spatial Profiler

Background and Objective

Osteosarcoma (OS) is the most common bone tumor in pediatric and adolescent/young adult patients. While immune checkpoint blockade has revolutionized the therapeutic landscape of various adult malignancies, the same cannot be said for patients battling OS. In fact, significant improvements in the survival rates for patients with OS have not been made in over 30 years. The objective of this study was to understand the interaction between the malignant OS cells and its surrounding environment to gain therapeutic and mechanistic insight into this disease.

Study Summary and Results

We used formalin fixed paraffin embedded tissue samples (FFPE) from pediatric patients with metastatic and non-metastatic tumors who did not respond to any prior therapies. RNA was extracted from 10-12 serial sections and the nucleic acid was quantified and used for gene expression profiling with the nCounter PanCancer IO 360 Panel. Immunohistochemistry was performed on serial FFPE sections. Data were combined to integrate the analyses at the gene and protein levels.

Gene expression analysis with the IO 360 panel revealed distinct and non-overlapping profiles in the metastatic versus non-metastatic tumor specimens. Immune cells were more abundant in non-metastatic specimens. With the exception of VISTA, PD-L1, and HVEM/TNFRSF14, the non-metastatic specimens exhibited increased expression of various immune checkpoints when compared to the metastatic specimens.

Metastatic specimens demonstrated lymphocyte exclusion, with immunohistochemistry confirming that T cells were confined to the periphery of the lesions. This finding was further supported by data showing evidence of vascular dysfunction and endothelial cell anergy as indicated by increased expression of VEGFA, increased ANGPT2:ANGPT1 gene expression ratio, and decreased expression of the gene encoding E-selectin.

“We plan on utilizing the nCounter PanCancer IO 360 Gene Expression Panel as we move on to Digital Spatial Profiling (DSP) with the GeoMx® system to generate some of our gene signatures and explore new regions of interest. With osteosarcoma specifically, we are interested in applying our discovered gene signatures to generate new data and push the limits of the technology.”

—Dr. McEachron



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Our observations were not attributable to differences in vascularity as we did not observe differences in the abundance of endothelial cells between the metastatic and non-metastatic specimens. Furthermore, correlation analyses showed an inverse relationship between T cell abundance and markers of vascular dysfunction and endothelial cell anergy exclusively in the metastatic specimens.

Together, our data showed that lymphocytes are excluded from infiltrating metastatic specimens. Moreover, non-metastatic OS specimens expressed increased levels of immunotherapeutic targets when compared to metastatic specimens. These data may provide insight into the inefficacy of therapeutic immune checkpoint blockade in metastatic OS and identifies lymphocyte exclusion as important targetable processes for therapeutic intervention in metastatic disease.

Next Steps

The IO 360 panel was an incredibly useful and effective tool to perform comparative targeted gene expression profiling the OS microenvironment using archived acid-decalcified FFPE specimens. Our data highlights the necessity of understanding the spatial context of the tissues to allow for a more accurate interpretation and biological comprehension of the data. That said, we are currently pursuing digital spatial profiling of matched primary and metastatic OS specimens to further unravel the complexity of the OS microenvironment. We anticipate that our integrated multi-platform approach will allow us to identify actionable mechanisms that underlie the resistance to immune checkpoint therapy as well as identify new molecular and cellular targets for therapeutic development.

To learn more about GeoMx DSP at NanoString, visit nanosttring.com/geomx

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OCT 2020 MK2825

