High-plex spatial profiling analysis of multidrug CIVO microdose studies in cancer patients

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ABSTRACT

Introduction: By combining CIVO™ multidrug intratumoral microdosing with digital spatial profiling (DSP) technology, drug specific multitumor parameter responses can be assessed to help drive drug development decisions in the most reliable setting, the cancer patient.

Background: CIVO™ microdosing studies performed in patient tumors in situ allow drug developers to assess localized tumor and microenvironment responses to multiple agents without having to expose patients to high systemic drug levels. By concentrating microdoses of different drugs within a single tumor in situ, it is possible to compare tumor and immune responses in spatially resolved regions of the same tumor. This type of early phase (Phase I) clinical study represents a new path for drug developers to gain insight into drug efficacy, tumor associated immune meal modulation, biomarker discovery and validation, and microenvironment interactions for new drugs earlier in the drug development process.

Traditionally, analysis of FFPE samples from these microdosing studies involve routine immunohistochemistry, immunofluorescence, and in situ assays that reveal changes in protein and RNA expression. These assays offer tissue wide protein and gene expression information but have limited multiplexing capabilities and dynamic range, as well as require precious tissue samples. Novel technologies such as NanoString’s GeoMx™ Digital Spatial Profiler (DSP) enable high-plex spatially resolved analysis of proteins and RNA transcripts in single FFPE tissue sections. In this proof of principle study, we utilized GeoMx™ Digital Spatial Profiler for protein and RNA expression on single FFPE sections from patient sarcoma tumors that were microdosed with multiple FDA approved drugs.

Methods: Single FFPE sections from microdosed patient tumor samples were 4″ stained for DNA and RNA and whole slide imaged. 600um and 100um diameter regions of interest (ROIs) were selected for DSP analysis within drug and control microinjection sites. Imaging and barcode counts were performed using GeoMx™ DSP and nCounter systems.

Results: DSP protein analysis highlighted phosphorylation of ERK, S6, and STAT3 in response to localized doxorubicin exposure compared to vehicle site. Additionally, ROIs sampled along the doxorubicin exposure gradient showed dose-dependent phosphorylation of both ERK and S6 proteins. DSP RNA analysis revealed drug specific transcript regulation of multiple genes in microdosed patient tumors, including upregulation of chemokines CXCL9 and CXCL10 at sites of doxorubicin and aldesleukin injection in a patient tumor but not to other chemotherapy agents.

Conclusion: Early phase CIVO microdosing studies combined with high-plex Digital Spatial Profiling opens the door to generating multi-plex data for multiple microdosed drugs within small patient studies. Analysis of protein and RNA expression using DSP enabled collection of targeted region of interest high density data from a single FFPE section derived from patient samples, conserving precious patient biopsy samples. Through continued expansion of the GeoMx™ DSP analyte panels, collecting an ever-increasing depth of protein and gene expression data is possible in Phase I CIVO microdosing studies.

High-plex investigation in cancer patients with CIVO™ and DSP

- Identifies PD biomarkers
- Reveals immune profiles
- Highlights combination hypotheses
- Enables multidrug comparisons
- Faster, safer, richer translational oncology!

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