Establishing Robust Pharmacodynamic Immunofluorescence Assays of Clinical Biopsies at the National Cancer Institute

Optimized Quality Control Procedures for the Evaluation of DNA Damage Response and Epithelial Mesenchymal Transition (EMT) Biomarkers

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Introduction

Robust PD assay results are valuable for informing decisions about the development of new agents and combinations. The National Cancer Institute’s Division of Cancer Treatment and Diagnosis (DCTD) develops and validates PD assays to obtain information about drug effect on intended molecular targets in first-in-human trials, which will enable clinical decisions. Our group utilizes quantitative immunofluorescence assays (qIFAs) of PD biomarkers in FFPE slides prepared from pre- and post-dose tumor biopsies collected from patients on early-phase clinical trials. Stringent methods are employed during fixation, blocking, and microtomy to maximize the success rate of generating sufficient slides with optimal areas for analysis and to ensure proper preservation of tissue morphologies. The qIFAs and slides are utilized to assess the quality of the tumor biopsies. Pathology-guided analysis, as appropriate for the intended molecular measurements, allows the assay operator to focus on tumor regions of interest and avoid normal tissue or other confounding regions compromised by sample-handling artifacts and helps to ensure a non-subjective analysis.

Figures and Discussion

Optimized procedures for FFPE blocking and microscopy in preparation for quantitative pharmacodynamic IFA analyses of DNA damage-induced biomarkers: DCTD SOP340522

- Pre- and post-dose biopsies are fixed and paraffin blocked in parallel with a positive biomarker control tissue.
- Positive control acts as a fixation and staining control for evaluation of positive biomarker staining on each slide.
- Methods are utilized to maintain and preserve full-face longitudinal presentation of the biopsy during sectioning.
- Sections from two blocks containing the pre- and post-dose are sectioned onto a series of slides in parallel.

H&E slide quality control review by a pathologist

- The following H&E review criteria are customized to the intended biomarker analysis:
  - Evaluation of each tissue section.
  - Morphology of each section should indicate sufficient nuclear and cellular definition.
  - Sufficient cellularity should be present in each section so that at least one 20x field (0.5 mm²) can be analyzed per slide.
  - Minimum number of regions of interest for analysis are presented by the quantitative biomarker analysis protocol (providing HS).

H&E Slide Annotation

- In these cases, the specimens do not advance to the assay operator for analysis, which eliminates the analysis and possible reporting of inappropriate biomarker data.
- The clinical sample data report for these patient samples will provide an explanation to the clinical investigator regarding the insufficiency of the tumor materials for analysis.

Quantitative H&E Analysis of a Pre-dose and Post-dose Biomarker: SOP340523, 340533, 340534

- Panel A: Positive biomarker control tissue, mouse tissue is processed in parallel with each pair of clinical biopsies. This tissue shows consistent strong staining for H&E, serving as a fixation and staining control for each clinical slide.
- Panel B shows a pre-dose and C panel shows a post-dose biopsy from a patient on clinical trial. The drug treatment led to an increased level of HSAX in this patient’s tumor, which is analyzed and reported using clinically validated methods.
- Panel D: Drug responsive tissue shows loss, mid, and high level of HSAX in mouse xenograft tumor quadrants derived from vehicle- and drug-treated animals. These calibrators serve as a reference standard for drug effect on target and are included in each run of clinical slide.

Common Findings during Modified QC Review: Minimal Tumor Content

- Representative H&E slide annotated by a pathologist is shown in the top panel, and a slide from the same patient started for a multiplex assay to assess EMT is shown in the bottom panel.
- In this case the pathologist annotated areas to exclude from the analysis, such as normal tissue, stromal areas, and processing artifacts (biopsy artifacts), and all tumor areas (green).
- The quantitative IFA assay tissue architecture is performed from a whole slide scan and involves analysis of the entire tumor biopsy, excluding only the areas inappropriate for analysis as described by the pathology review.

Summary and Conclusions

- Our group supports the development, validation, and implementation of robust PD assays to evaluate drug mechanisms of action during clinical trial evaluations of new drug regimens. Quantitative IFA measurements in needle biopsies is one such assay type.
- Integral quality control procedures are applied during each phase of these quantitative qIFA methods to help ensure quality from biopsy collection, tissue preservation via FFPE, microtomy, and H&E review through qIFA imaging, analysis, and reporting.
- Using pathology guidance to assist the assessment of the biopsy tissues, both to eliminate the analysis of inappropriate specimens and to guide the analysis of sufficient samples via annotation of a whole slide image is a critical tool in our qIFA workflow.

This process helps to streamline the analysis process and helps to ensure a more accurate and non-subjective analysis of tumor areas for pharmacodynamic biomarker modulation, both as applied to the evaluation of DNA damage response biomarkers, such as HSAX, and as applied to whole tissue assessments, such as EMT.

References

1. DCTD Standard Operating Procedures SOP340507, 340522, 340523, 340524, 340533, 340534.
3. Quantitative immunofluorescence assay of MET and epithelial-mesenchymal transition (EMT) biomarker modulation by antiangiogenic inhibitors in xenograft tumor tissues; Navas T et al., 2014, Cancer Res., 74; Abstract 3049.

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