The application of robust pharmacodynamic (PD) assays as part of clinical trial design is valuable for confirming putative drug mechanisms in patients. Our group supports the development, validation, and clinical implementation of PD assays, including quantitative immunofluorescence (qIFA) measurements in formalin-fixed, paraffin-embedded (FFPE) human tumor biopsies. One such assay is a multiplex qIFA method, referred to as RxI-IFA4, for evaluating biomarker modulation in response to DNA-damaging agents.

**Materials and Methods**

- Human breast cancer cell line MDA-MB-231 was used to produce xenograft tumors in athymic nude mice. Animals were treated with vehicle or a single dose of a CIAP inhibitor at 3 or 20 mg/kg and tumors were collected, quartered, and flash frozen 7e post-dose.
- Tumor quadrants and control tumors were FFPE treated, and slides were prepared as described.  
  - Slides were stained using a Bond-Max Autostainer (Leica Microsystems) with biotinylated-\(v\)H2AX mAb (Millipore, 315U-15) plus Alexa-660 streptavidin (Invitrogen, #S12777), anti-\(\alpha\)-FR (Abcam, clone SP6), anti-\(\alpha\)-CPT (R&D Systems, #AR4855) plus Alexa-546 anti-FITC (Invitrogen, #IA-1035), and DAPI.  
  - Images were captured using a Nikon Eclipse B200 (Nikon) microscope and Q-image, Metafluor-2000 fast 13904 mono-cooled camera (QImaging), and analyzed using a custom macro developed with Image-Pro (Media Cybernetics) to quantify % nuclear area positive (NAP) for \(v\)H2AX and K67, and % cyttoplasmic area positive (CAP) for CPT3.

**References**


**Results**

Figure 4: RxI-IFA data showing comparable performance for \(v\)H2AX, K67, and CPT3 across two lots of slide blocks. Individual calibrator blocks were prepared from different tumor quadrants derived from the same drug-treated animal. Lot 10090120 (S1 analysis) and Lot 1009070 (S3 analysis) results, and the mean value for each group are shown.

Figure 5: RxI-IFA data showing comparable performance for \(v\)H2AX, K67, and CPT3 analyzed over a period of 120 days. Based on a drop in SCAP signal for CPT3 at Day 60, the stability of cut calibrator/control slides was set at 6 weeks.

**Discussion**

- Our group supports the development, validation, and clinical implementation of robust PD assays to evaluate drug mechanism of action during clinical trial evaluations of new drug compounds and combination therapies. Multiplex qIFA measurements in needle biopsies of tumors collected pre- and post-dose is one such assay type.
- Integral quality control tools developed and validated to support clinical implementation of the multiplex qIFA RxI-IFA4 include xenograft tumor calibrators derived from mouse treated with compounds known to modulate the PD markers of interest and animal model-derived control tissues, which serve to verify the expected analytical performance for each biomarker on each calibrator/control and clinical slide.
- Methods developed to reproducibly generate, qualify, and store the xenograft calibrator/control slides for the multiplex qIFA RxI-IFA4 were developed.
- Use of tumor quadrants allows for a large number of slides to be produced from a qualified calibrator/control block (~150 per block).
- Advantages over cell pellet slides derived from control or drug-treated cells include biomarker expression levels in cells rarely correlates with expression in vivo and accurate quantitative image analysis with Image Pro requires tissue context.

**Abstract #1173**

**Title:** Development of Calibrators and Controls as Quality Control Tools for Clinical Implementation of Quantitative Immunofluorescence Assays for Pharmacodynamic Biomarkers of Molecular Targeted Agents in Tumor Biopsies

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