Evaluation of PI3K Inhibitor Chemosensitivity in Human Tumor Explants using the Mosaic Blue Assay™ and Relationship to Biomarkers by Immunohistochemistry


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Abstract

Several chemotherapeutic agents that target the phosphotyrosinol 3-kinase (PI3K) pathway are currently used in clinical trials for cancer treatment. The AKT protein family plays an important role in cellular survival by inhibiting apoptotic processes and is one of the major downstream targets of the PI3K pathway. Mammalian target of rapamycin (mTOR) is also a key kinase acting downstream of the activation of PI3K. Chemosensitivity testing on human cancer explants can be valuable in identifying biomarkers that relate to the intrinsic sensitivity of the tumor. The Mosaic Blue Assay is an in vitro tumor response assay developed for non-expanded patient cancer cells.

Viable human tumor and matched formalin-fixed, paraffin embedded (FFPE) tissue were used for this study. Fresh tissues were disaggregated into single cells and exposed to various PI3K inhibitors. Samples were classified as resistant or sensitive based on Mosaic Blue Assay results. The corresponding FFPE tissue block was stained by immunohistochemistry for PTEN, CMET, EGFR, K67, p53 and several phosphorylated proteins: AKT, PRAS40, 4E-BP1, SMAD, and 65 ribosomal protein. Additional fresh cells were also treated with inhibitors, harvested, and phosphorylated proteins were evaluated in the pre- and post-dose tumor cells. Chemosensitivity results identified two relationships. First, biomarker status of the tissue block was evaluated in order to understand the intrinsic chemosensitivity. Second, biomarker status after treatment was evaluated to understand modulation of proteins due to therapy.

In summary, use of a soft agar, ex vivo tumor response assay such as the Mosaic Blue Assay is a useful tool to predict relative sensitivity across tumor types and aid investigation of potential biomarkers.

Introduction and Methods

Three PI3K inhibitors were selected for testing in the Mosaic Blue Assay™: XL147, NVP-BEZ235, and GDC-0941. XL147 is a selective and reversible class I PI3K inhibitor for wild type and mutant p110α with IC50 of 40 nM and 40 nM, NVP-BEZ235 is a dual ATP-competitive PI3K and mTOR inhibitor of p110α, p110γ, p110δ, and p110β with IC50 of 4 nM, 5 nM, 7 nM and 75 nM, respectively. GDC-0941 is a potent inhibitor of p110α, p110β, p110δ and p110γ with IC50 of 3 nM, 33 nM, 33 nM and 75 nM.

Eleven tumor explants (5 colon cancer and 6 squamous cell lung cancer) and 3 cell lines (MDA-MB-468, MKN45, and T47D) were exposed to each compound in a 3 point titration between 0.003 µM and 10 µM. Viable cells from select explants were also exposed to 1 µM of GDC-0941 for 24 hours prior to fixation and paraffin embedding. An adjacent portion of cancer from each fresh specimen was fixed in formalin, paraffin-embedded and used for IHC staining.

The matched fixed cancer tissues were stained with the following biomarkers to identify predictive relationships between expression and response in the Mosaic Blue Assay™: PAKT (Ser473), p4EBP1 (Thr37/46), pPRAS40 (Thr246), ppS6 kinase (Thr421/Ser424 and Thr389), pS6 ribosomal protein (RP) (Ser240/244 and Ser235/236), pEGFR (Tyr1068, Tyr1086, and Tyr1173), pHER3 (Y1068, Y1086, and Y1173), MET (Tyr1234, Tyr1235/1235(d), PTK7, (VTh1235/Tyr204), and K67.

Human explants and cell lines treated with 1 concentration of GDC-0941 were stained with the following biomarkers to identify pharmacodynamic relationships between expression and treatment with a PI3K inhibitor: PAKT (Ser473), p4EBP1 (Thr37/46), pPRAS40 (Tyr426), p53 (Thr242, Ser243 and Thr244), p65 (Ser241/242 and Ser253/256), pEGFR (Tyr1068, Tyr1086, and Tyr1173), pHER3 (Tyr1234, Tyr1235/1235(d), PTK7 (Tyr204/204), and K67. The Mosaic Blue Assay™ is a soft agar ex vivo tumor response assay that tests unexpanded viable cancer explant cells for sensitivity to therapeutics over 5 days. Results are reported as percent growth inhibition at each drug concentration. Soft agar ex vivo tumor response assays have demonstrated >92% accuracy at predicting patients that will not respond to therapy.

Immunohistochemistry was evaluated on a semi-quantitative scale, and the percentage of cancer cells staining at each of the following four levels was recorded: 0 (unstained), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). An H-score was calculated based on the summation of the product of percent of cells stained at each intensity and the staining intensity to yield an H-score from 0-300. Results are provided as H-Scores. All immunohistochemical stains are fully validated at Mosaic Laboratories.

Results

Predictive Biomarker Expression in Human Tumor Explants (Baseline Expression Compared to Mosaic Blue Assay™ Results)

The matched fixed cancer tissues were stained with the following biomarkers to identify pharmacodynamic relationships between expression and treatment with a PI3K inhibitor: PAKT (Ser473), p4EBP1 (Thr37/46), pPRAS40 (Thr246), ppS6 kinase (Thr421/Ser424 and Thr389), pS6 ribosomal protein (RP) (Ser240/244 and Ser235/236), pEGFR (Tyr1068, Tyr1086, and Tyr1173), pHER3 (Tyr1234, Tyr1235/1235(d), PTK7 (Tyr204/204), and K67.

Pharmacodynamic Significance in Human Explants

To the right, average H-Score of immunohistochemical staining in the pre-treatment explants were compared to post-treatment explants. Across all explants, a significant decrease in pp70S6K T389 was observed. Near significant reductions in pPRAS40 and pS6 RP S235/236 were also observed. PI3K may become active again.

Pharmacodynamic Biomarker Expression in Cell Lines

The pharmacodynamic biomarker expression results from the Mosaic Blue Assay™ are presented in the table. The matched fixed cancer tissues were stained with the following biomarkers to identify pharmacodynamic relationships between expression and treatment with a PI3K inhibitor: PAKT (Ser473), p4EBP1 (Thr37/46), pPRAS40 (Tyr426), p53 (Thr242, Ser243 and Thr244), p65 (Ser241/242 and Ser253/256), pEGFR (Tyr1068, Tyr1086, and Tyr1173), pHER3 (Tyr1234, Tyr1235/1235(d), PTK7 (Tyr204/204), and K67.

Pharmacodynamic Biomarker Expression in Cell Lines

Conclusions

The Mosaic Blue Assay™ identified differential response to PI3K inhibitors in colon and lung cancer at drug concentrations similar to reported cell line IC50s. The potency was consistent with expectations. The assay is useful for identifying relationships between chemoresistance and protein expression.

Biomarkers that predicted resistance and sensitivity to GDC-0941 in human explants included p4EBP1, p70S6K T421/424, and p65 ribosomal protein S240/244 (low in resistant; high in sensitive explants). Biomarkers that produced near significant relationships included pPRAS40, p53 ribosomal protein S235/236, and PTEN.

○ cMET staining was not included significantly, which suggests that the expression of cMET is not a significant indicator of sensitivity.

The Mosaic Blue Assay™ is a useful tool to predict relative sensitivity across tumor types and aid investigation of potential biomarkers.