Combination of PI3K and MEK Inhibitor Chemosensitivity in Human Tumor Explants and Cell Lines using the Mosaic Blue Assay and Relationship to Biomarkers by Immunohistochemistry


Mosaic Laboratories, Lake Forest, CA 92630, (949) 472-8080, www.mosaiclabs.com

Abstract

The PI3K and MEK pathways promote tumor cell survival through a variety of downstream signals and are commonly explored in cancer research. Many agents are in clinical development and new evidence suggests that a combination of these inhibitors may be required to achieve optimal anti-tumor activity. Twenty-one viable colorectal and non-small cell lung cancer tissue explants with matched formalin-fixed, paraffin embedded (FFPE) tissue and 8 cell lines (A431, AGS, HCT-116, HT-29, MCF-7, MDA-MB-468, MKN45, and T47D) were used for this study. Fresh tissues were disaggregated into single cells and exposed to GDC-0941, a PI3K inhibitor, and AZD6244, a MEK inhibitor, both as single agents and in combination in the Mosaic Blue assay. Combination results were compared to single agent results. Samples were classified as resistant or sensitive. Matched FFPE tissues and cells were stained with the following biomarkers to identify predictive relationships: pAKT, p4EBP1, pPRAS40, pp70S6 kinase (T421/S424 and T389), pS6K, pS6, pRB (S240/244), pEGFR (Y1086), cMET (cytoplasmic), pHER3 (membrane) and Ki-67. The combination of PI3K and MEK inhibitors demonstrated additive growth inhibition in the explants and cell lines. The average combined activity of both PI3K and MEK was more potent than single agents, with greater additivity demonstrated in colorectal cancer compared to lung cancer. Predictive and pharmacodynamic biomarkers were identified with both single agent and combination treatments, and differences were observed between the two inhibitors. In summary, this study demonstrated that the Mosaic Blue assay is a useful tool for classifying sensitive and resistant tumor explants and cell lines and also confirmed that dual targeting of PI3K and MEK pathways is a valuable approach to achieving higher therapeutic activity in vitro. Evaluation of biomarker profiles revealed predictive and pharmacodynamic relationships in this setting.

Materials and Methods

The Mosaic Blue Assay® is a soft agar ex vivo tumor response assay that tests expanded viable cancer cell explants 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. PI3K inhibitor GDC-0941 and MEK inhibitor AZD6244 were purchased from Selleck Chemicals (Houston, TX). Eight cell lines, A431, AGS, HCT-116, HT-29, MCF-7, MDA-MB-468, MKN45, and T47D, were purchased from ATCC (Manassas, VA). Lung and colon cancer human tissues were provided by Mosaic Laboratories and procured under an IRB-reviewed protocol (MS001), which allows for use of remnant, de-identified, or anonymized human samples for in vitro analysis under the guidelines defining Exemption from Human Subject Research. Cell lines and human explants were exposed to GDC-0941, AZD6244, and GDC-0941 + AZD6244 in an 8-point titration between 0.003 µM and 10 µM using the Mosaic Blue Assay®. A representative adjacent tissue sample from each fresh tumor explant was fixed in formalin, paraffin-embedded and available for IHC staining. The 8 cell lines were exposed to 10 µM of GDC-0941, AZD6244, or GDC-0941 + AZD6244 for 24 hours at 37°C. After exposure, cells were fixed in 10% neutral buffered formalin, processed and embedded into paraffin. FFPE tissue from the colon and lung cancer explants and the cell lines exposed to single and double agent in vitro treatment were sectioned at 4 microns onto positive charged glass slides. FFPE cancer explant tissues were stained by immunohistochemistry (IHC) for the following biomarkers to identify predictive relationships between expression and response in the Mosaic Blue Assay®: pAKT, p4EBP1, pPRAS40, p56 kinase, p56 ribosomal protein, pEGFR, pHER3, pMET, cMET, and Ki-67. Pre and post-treatment cell lines were stained with the following IHC assays to identify pharmacodynamic relationships: pAKT, p4EBP1, pPRAS40, p56 kinase, p56 ribosomal protein, pEGFR, pHER3, pMET, cMET, and Ki-67. Note: All IHC assays are fully validated at Mosaic Laboratories. IHC was evaluated on a semi-quantitative scale, and the percentage of cancer cells stained 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 percent positive expression and H-Score.

Results

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

Pharmacodynamic Biomarker Expression in Cell Lines

Conclusions

- The Mosaic Blue Assay® identified differential response to combination PI3K and MEK inhibitors in colon and lung cancer explants at drug concentrations similar to reported clinical IC50s.
- Combining the Mosaic Blue Assay® and immunohistochemistry on matched FFPE tissues is a useful in vitro surrogate model for combination therapy and identifying biomarker expression patterns.
- Biomarkers that predicted sensitivity to GDC-0941 + AZD6244 included p4EBP1, pPRAS40, and pHER3.
- Biomarkers that predicted resistance to GDC-0941 + AZD6244 included pAKT, pMET, cMET, EGFR, and Ki-67.
- GDC-0941 + AZD6244 in combination caused greater growth inhibition than single agents in cell lines, and demonstrated greater reduction in biomarker expression.

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