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

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Abstract

The MEK pathway is activated in most cancers. Several chemotherapeutic agents target this pathway through inhibition of MEK, ERK or upstream receptor tyrosine kinases and are in clinical trials to treat patients with solid tumors. The use of chemosensitivity testing using human cancer explants is very useful in identifying biomarkers that relate to the intrinsic sensitivity of the tumor to a chemotherapeutic agent. The Mosaic Blue assay is a soft agar, ex vivo tumor response assay that utilizes non-expanded patient cancer cells.

Viable colorectal and non-small cell lung cancer tissues with matched formalin-fixed, paraffin embedded (FFPE) tissue were used for this study. Fresh tissues were disaggregated into single cells and exposed to various inhibitors of the MEK pathway, specifically AZD6244 and GSK1120212. Samples were classified as resistant or sensitive. The inhibitors demonstrated different resistance and sensitivity profiles in the human tumors, and samples demonstrated a higher frequency of resistance to AZD6244 than to GSK1120212 at similar concentrations. The corresponding FFPE tissue block was stained by immunohistochemistry for phosphorylated AKT, PRAS40, 4EBP1, S6 kinase, S6 ribosomal protein as well as PTEN, EGFR, cMET, HER3, and Ki-67 in accordance with validated protocols. In addition to analysis of primary cancer samples, the MDA-MB-468, MKN45, and T47D cell lines were also treated with AZD6244 and GSK1120212, harvested, and phosphorylated proteins were evaluated in the pre- and post-dose tumor cells. Immunohistochemistry results were compared to chemosensitivity results and 2 types of relationships were identified. First, biomarker status of the archival tissue was evaluated in order to understand the intrinsic chemosensitivity. Resistant tissues demonstrated higher expression of pAKT, PTEN, cMET, EGFR, HER3, and Ki-67 and lower expression of phosphorylated S6 ribosomal protein (S240/244 and S235/236) and phosphorylated ERK compared to sensitive tissues. Second, biomarker status upon treatment in cell lines was evaluated to understand modulation of proteins due to therapy.

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

Introduction and Methods

Two MEK inhibitors were selected for testing in the Mosaic Blue Assay™: AZD6244 and GSK1120212. Eleven tumor explants (5 colon cancer and 6 squamous cell lung cancer) and 3 cell lines (MKN45, T47D, and MDA-MB-468) were exposed to each compound in an 8-point titration between 0.003 µM and 10 µM. A representative adjacent tissue sample from each fresh tumor explant was fixed in formalin, paraffin-embedded and available for IHC staining (considered the archival block). The 3 cell lines were also exposed to 10 µM of AZD6244 for 24 hours prior to fixation and paraffin embedding.

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.

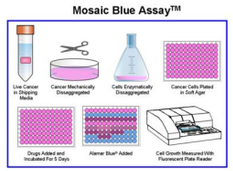
The matched fixed explant tissues were stained with the following biomarkers to identify predictive relationships between expression and response in the Mosaic Blue Assay™: pAKT, p4EBP1, pPRAS40, pp70S6 kinase (T421/S424 and T389), pS6 ribosomal protein (S240/244 and S235/236), pERK, PTEN, cMET, EGFR, HER3, Ki-67, and p53.

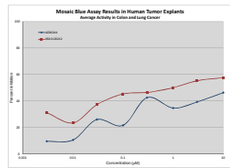
Pre and post AZD6244 treatment cell lines were stained with the following biomarkers to identify a pharmacodynamic relationship: pAKT, p4EBP1, pPRAS40, pp70S6 kinase (T421/S424 and T389), pS6 ribosomal protein (S240/244 and S235/236), pMET, pERK, pEGFR (T1173, T1086, and T1068), pHER3, and Ki-67.

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. All immunohistochemical stains are fully validated at Mosaic Laboratories. The MKN45 cell line was found to be MET amplified and the T47D cell line was found to be PIK3CA amplified using validated FISH assays at Mosaic Labs.

Results

Mosaic Blue Assay™ Results in Tumor Explants and Cell Lines



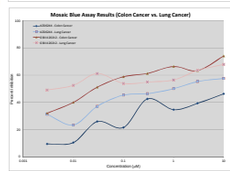


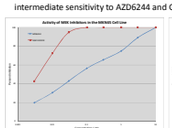
Mosaic Blue Assay™ Results.

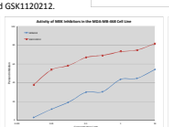
Activity of 2 MEK inhibitors in 6 colon cancer and 5 lung cancer explants (right) and 3 cell lines (below) using the Mosaic Blue Assay. Results are graphed as percent inhibition.

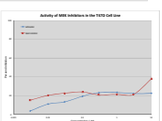
In human tumors, activity was highest with GSK1120212 followed by AZD6244. The average IC50 for GSK1120212 was between 0.01 and 0.03 µM and the IC50 for AZD6244 was between 3 and 10 µM. Within tumor types, activity was similar between colon and lung cancer with slightly higher activity of AZD6244 in lung cancer.

In cell lines, the MKN45 cell line was sensitive, the T47D cell line was resistant, and the MDA-MB-468 cell line was intermediate sensitivity to AZD6244 and GSK1120212.





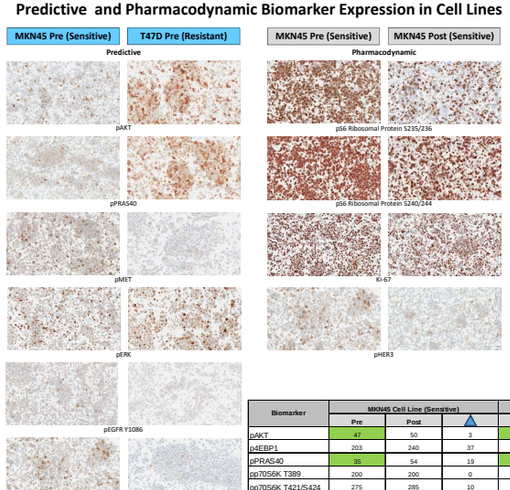




Percent Inhibition in the Mosaic Blue Assay

Concentration	MKN45 Cell Line (MET Amplified)		T47D Cell Line (PIK3CA Amplified)		MDA-MB-468 Cell Line	
(uM)	AZD6244	GSK1120212	AZD6244	GSK1120212	AZD6244	GSK1120212
10	100	100	22	38	54	82
3	89	100	22	21	45	75
1	75	100	24	21	44	73
0.3	65	100	23	21	30	69
0.1	56	100	19	24	30	67
0.03	43	95	13	23	19	58
0.01	30	72	11	20	12	54
0.003	20	43	4	15	3	38
	Sensitive	Sensitive	Resistant	Resistant	Intermediate	Sensitive

Predictive and Pharmacodynamic Biomarker Expression in Cell Lines



Biomarker Relationships in Cell Lines.

The pre and post-treatment cell pellets were stained for various biomarkers and compared to the Mosaic Blue Assay™ results. The MKN45 cell line was sensitive and the T47D cell line was resistant to AZD6244. Pharmacodynamic relationships in yellow demonstrated the greatest change from pre to post treatment. Predictive relationships in green demonstrate the greatest change in pre treatment between the sensitive cell line (MKN45) and resistant cell line (T47D). Select images (20x) are illustrated.

Biomarker	MKN45 Cell Line (Sensitive)			T47D Cell Line (Resistant)			MDA-MB-468 Cell Line (Intermediate / Sensitive)		
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ
pAKT	47	50	3	155	150	-5	164	285	121
p4EBP1	203	240	37	214	215	1	220	230	10
pPRAS40	35	54	19	139	152	13	171	205	34
pp70S6K T389	200	200	0	200	200	0	180	180	0
pp70S6K T421/S424	275	285	10	290	295	5	235	265	30
pS6 RP S235/236	274	150	-124	268	250	-18	280	295	15
pS6 RP S240/244	288	240	-48	290	291	1	300	282	-18
pMET	133	112	-21	28	35	7	70	107	37
pERK	75	82	7	111	132	21	183	127	-56
pEGFR T1068	2	20	18	0	2	2	270	283	13
pEGFR T1086	57	51	-6	2	15	13	125	180	55
pEGFR T1173	3	0	-3	0	0	0	175	190	15
pHER3	39	7	-32	0	2	2	4	34	30
Ki-67	279	203	-76	271	190	-81	287	285	-2

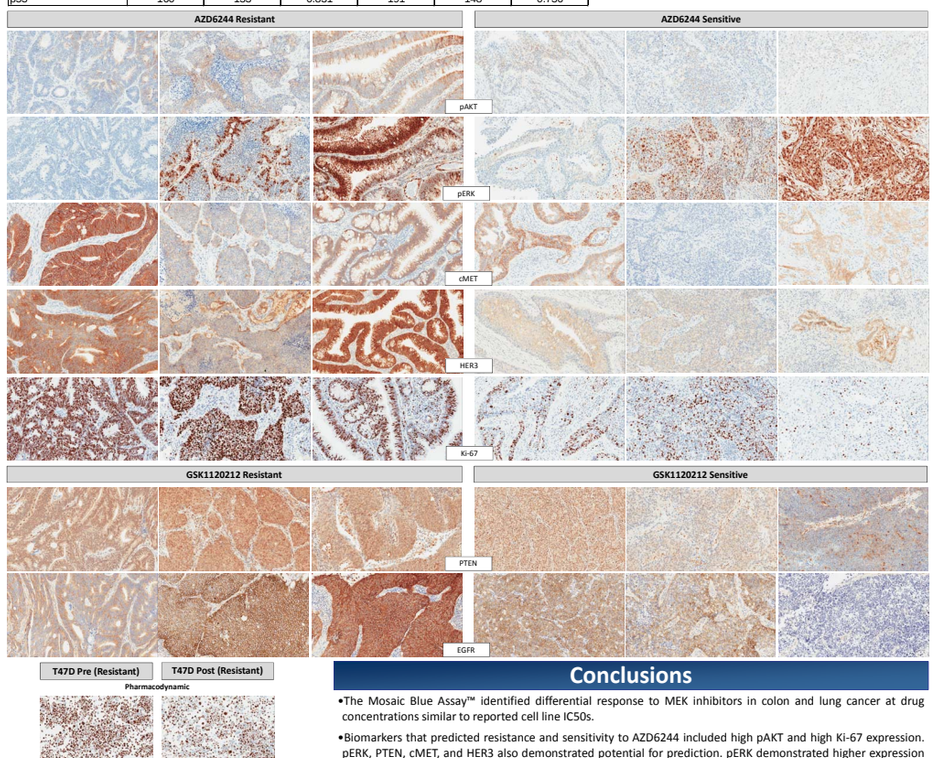
Predictive Biomarker Expression in Human Tumor Explants (Baseline Expression Compared to Mosaic Blue Assay Results)

Biomarker	AZD6244 Resistant	AZD6244 Sensitive	AZD6244 Significance	GSK1120212 Resistant	GSK1120212 Sensitive	GSK1120212 Significance
pAKT	100	17	0.037	70	49	0.619
p4EBP1	119	103	0.617	121	116	0.936
pPRAS40	84	65	0.744	54	56	0.972
pp70S6 T389	167	130	0.413	146	132	0.768
pp70S6 T421/S424	211	192	0.199	200	200	0.985
pS6 S235/236	53	63	0.791	30	75	0.258
pS6 S240/244	137	165	0.681	65	166	0.123
pERK	91	140	0.650	35	82	0.417
PTEN	130	62	0.391	194	94	0.002
cMET	159	85	0.497	137	52	0.396
EGFR	250	235	0.851	250	103	0.116
HER3	204	23	0.103	144	17	0.154
Ki-67	175	56	0.011	178	156	0.683
p53	160	133	0.831	191	148	0.736

Predictive Biomarker Relationships.

Immunohistochemical H-Scores were compared to activity of 2 MEK inhibitors in the Mosaic Blue Assay™. Resistant and sensitive explants were selected based on activity at the approximate IC50 concentration. Three explants were selected as resistant and 3 explants were selected as sensitive for each inhibitor. For AZD6244, 2 colon and 1 lung cancer explants were resistant and 3 lung cancer explants were sensitive. For GSK1120212, 1 colon and 2 lung cancer explants were resistant and 2 colon and 1 lung cancer explants were sensitive. Only 2 explants were common between the 2 MEK inhibitors.

Data is reported as average H-Score values. Significant relationships are highlighted in yellow. Increased expression is highlighted in green. pAKT and Ki-67 were predictive for AZD6244 (higher expression in resistant; lower expression in sensitive). PTEN was predictive for GSK1120212 (higher expression in resistant; lower expression in sensitive). Resistant tissues demonstrated higher expression of pAKT, PTEN, cMET, EGFR, HER3 (membrane localized), and Ki-67, and lower expression of pS6 ribosomal protein (S240/244 and S235/236) and pERK compared to sensitive tissues. Select images of IHC staining are presented.



Conclusions

- The Mosaic Blue Assay™ identified differential response to MEK inhibitors in colon and lung cancer at drug concentrations similar to reported cell line IC50s.
- Biomarkers that predicted resistance and sensitivity to AZD6244 included high pAKT and high Ki-67 expression. pERK, PTEN, cMET, and HER3 also demonstrated potential for prediction. pERK demonstrated higher expression in sensitive explants and PTEN, cMET, and HER3 demonstrated higher expression in resistant explants.
- Biomarkers that predicted resistance and sensitivity to GSK1120212 included PTEN, which was high in resistant explants and low in sensitive explants. pS6 ribosomal protein (S235/236 and S240/244), pERK, PTEN, cMET, EGFR, and HER3 also demonstrated potential for prediction. pS6 ribosomal protein (S235/236 and S240/244) and pERK demonstrated higher expression in sensitive explants and PTEN, cMET, EGFR, and HER3 demonstrated higher expression in resistant explants.
- In the MKN45 cell line (MEK sensitive), pharmacodynamic changes were observed with pS6 ribosomal protein (S235/236 and S240/244) (higher in predose) and Ki-67 (higher in post-dose). Comparing biomarker expression between MKN45 cell line (MEK sensitive) and T47D cell line (MEK resistant), pAKT, pPRAS40, and pERK were higher in the resistant cell line while pMET, pEGFR T1086, and pHER3 were higher in the sensitive cell line.
- Testing of additional explants are being performed to further characterize the staining trends observed in this study.

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