Aurora A, pMet, pAKT, and pSTAT3 as Predictive and Pharmacodynamic Biomarkers of EGFR Inhibitor Response in Colon and Lung Cancer Cell Lines

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Abstract

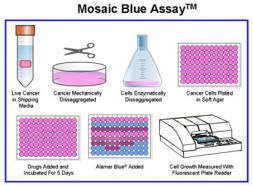
In order to make informed decisions on selecting candidate immunohistochemical assays for clinical trial monitoring, it is helpful to evaluate the sensitivity and pharmacodynamic potential in defined cell lines prior to inclusion in trials. In the current study, 5 colon and 6 lung cancer cell lines were profiled for chemosensitivity to erlotinib and gefitinib using the Mosaic Blue Assay, a soft agar ex vivo tumor response assay. Both drugs were analyzed at 25, 5, 0.5 and 0.05 uM, and the average percent inhibition for all specimens was calculated for gefitinib and erlotinib. At 25 uM, the average effect of erlotinib was greater in lung cancer (60% inhibition) compared to colon cancer (40% inhibition). The average effect of gefitinib was greater in colon cancer (78% inhibition) compared to lung cancer (58% inhibition). In colon cancer, gefitinib demonstrated greater activity than erlotinib; in lung cancer, the two drugs produced similar effects. At 5 uM, the average effect of erlotinib was greater in lung cancer (44% inhibition) compared to colon cancer (17% inhibition). The average effect of gefitinib was similar in colon cancer (38% inhibition) compared to lung cancer (37% inhibition). In colon cancer, gefitinib demonstrated greater activity than erlotinib; in lung cancer, erlotinib demonstrated slightly greater activity than gefitinib.

Cell lines were also treated with erlotinib and gefitinib for 2 hours followed by fixation in 10% NBF and processing and paraffin embedding. Fixed cells were evaluated by immunohistochemistry for the following biomarkers: Aurora A, pAurora A, c-MET, pMET (Y1349 and Y1230/1234/1235), pAKT, pSTAT3, pERK, EGFR, pEGFR, Ki-67, and pHH3. Baseline pMet expression was more prevalent in cell lines that were sensitive to treatment, whereas pSTAT3 was more prevalent in cell lines that were sensitive to erlotinib. Expression of pMET and pSTAT3 was significantly decreased after treatment with erlotinib and gefitinib; however, pAKT was more frequently decreased with gefitinib. Ki-67 and pHH3 expression decreased with erlotinib treatment only. Only a few cell lines demonstrated phosphorylated EGFR, which was ablated after treatment. Aurora A and pAurora A demonstrated differential staining upon treatment with erlotinib and gefitinib that was not consistent across tumor type or drug sensitivity. Neither EGFR nor c-Met demonstrated a predictive association with sensitivity, and the levels were unchanged after short-term drug exposure.

In summary, combining chemosensitivity testing with immunohistochemical characterization of paraffin embedded cells is important to qualify potential predictive and pharmacodynamic biomarkers. In this study, Aurora A, pMet, pAKT, pSTAT3, pEGFR, Ki-67, and pHH3 may be useful for monitoring sensitivity to EGFR therapy.

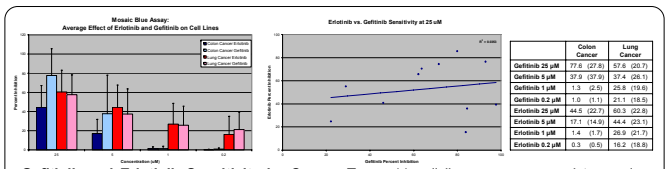
Methods

Five colon and 6 non-small cell lung cancer cell lines (HCT-15, COLO 205, SW480, LOVO, HT-29, A549, H460, Calu-6, H358, NCI-H1975 and H322) were characterized for gefitinib and erlotinib sensitivity using the Mosaic Blue Assay™. The Mosaic Blue Assay™ is a soft agar *ex vivo* tumor response assay that tests unexpanded viable cancer explant cells for sensitivity to therapeutics. Results are reported as percent growth inhibition at each drug concentration and the IC50 is calculated from the dose response. Soft agar *ex vivo* tumor response assays have demonstrated >92% accuracy at predicting patients that will not respond to therapy.



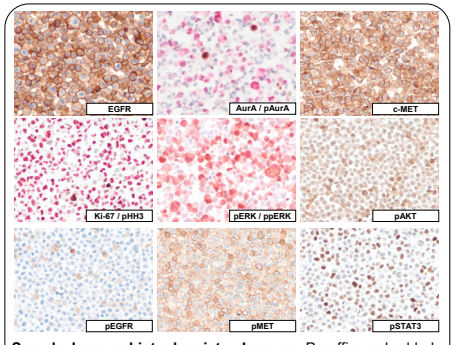
Cell lines treated with 2 concentrations of erlotinib and gefitinib (25 µM and 5 µM) fixed, processed, and paraffin embedded for biomarker testing by IHC. All cell lines were characterized by single stain IHC for the expression of cMET, pMET(Tyr1349), pMET (Tyr1230/1234/1235), EGFR, pEGFR, pAKT, and pSTAT3. Cell lines were also characterized by double stain IHC for the expression of Aurora A + phosphorylated Aurora A, Ki67 + pHH3, and pERK + ppERK. IHC was evaluated using Aperio ImageScope total pixel analysis algorithm.

Results



	Colon Cancer	Lung Cancer
Gefitinib 25 µM	77.6 (27.8)	57.6 (20.7)
Gefitinib 5 µM	37.9 (37.9)	37.4 (26.1)
Gefitinib 1 µM	1.3 (2.5)	25.8 (19.6)
Gefitinib 0.2 µM	1.0 (1.1)	21.1 (18.5)
Erlotinib 25 µM	44.5 (22.7)	60.3 (22.8)
Erlotinib 5 µM	17.1 (14.9)	44.4 (23.1)
Erlotinib 1 µM	1.4 (1.7)	26.9 (21.7)
Erlotinib 0.2 µM	0.3 (0.5)	16.2 (18.8)

Gefitinib and Erlotinib Sensitivity by Cancer Type: 11 cell lines were exposed to varying concentrations of gefitinib and erlotinib and the percent growth inhibition was calculated with the Mosaic Blue assay. Average percent growth inhibition is listed by cancer type. Response to erlotinib and gefitinib trended positively, however, significant differences were observed.



Sample Immunohistochemistry Images: Paraffin embedded samples from untreated and treated cell lines were analyzed using optimized protocols for EGFR, Aurora A/pAurora A, c-MET, Ki-67/Phospho-Histone H3, phospho/diphospho-ERK1, Phospho-AKT, Phospho-EGFR, pMET [Y1349], pMET [Y1230/1234/1235] and pSTAT3.

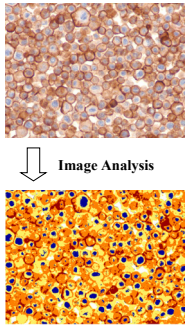


Image Analysis: The percentage of sample stained weak (yellow), moderate (orange) and strong (red) was recorded as well as total %positive.

	Erlotinib PCI @ 25 uM	Erlotinib Resistance Category
SW480	15	Resistant
Calu-6	25	Resistant
Colo205	36	Moderate
HT-29	39	Moderate
A549	41	Moderate
LOVO	55	Sensitive
H460	66	Sensitive
H358	70	Sensitive
H322	74	Sensitive
HCT-15	76	Sensitive
NCI-H1975	86	Sensitive

	Gefitinib PCI @ 25 uM	Gefitinib Resistance Category
Calu-6	22	Resistant
LOVO	29	Resistant
A549	46	Resistant
H460	62	Moderate
H358	64	Moderate
H322	72	Sensitive
NCI-H1975	80	Sensitive
SW480	84	Sensitive
Colo205	84	Sensitive
HCT-15	93	Sensitive
HT-29	98	Sensitive

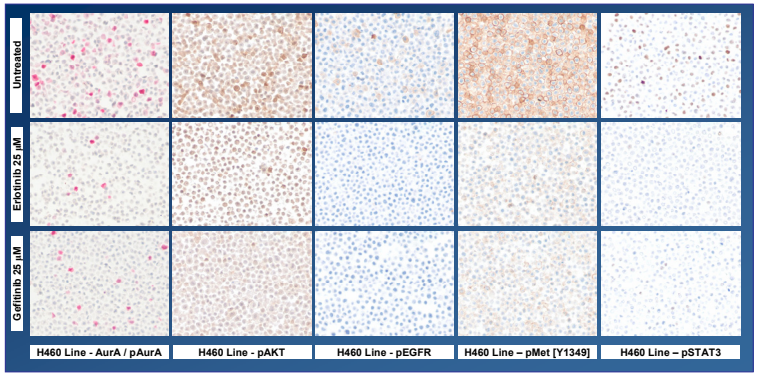
	Aurora A	cMET	cMET>Mod	EGFR	EGFR>Mod	Ki-67	pAKT	pEGFR	pERK	pMET [pYpYpY]	pMET [Y1349]	pSTAT3
Erlotinib Resistant Samples	11.4%	57.3%	28.7%	86.2%	65.0%	31.9%	2.2%	0.1%	85.3%	28.3%	31.8%	0.8%
Erlotinib Sensitive Samples	7.6%	92.6%	44.8%	85.4%	44.2%	29.7%	30.9%	1.3%	34.9%	30.5%	27.5%	14.5%
Difference	-3.8%	35.2%	16.1%	-0.7%	-20.8%	-2.2%	28.7%	1.2%	-50.4%	2.1%	-4.3%	13.7%
T-test	0.29	0.07	0.24	0.44	0.08	0.43	0.14	0.27	0.06	0.46	0.43	0.17

	Aurora A	cMET	cMET>Mod	EGFR	EGFR>Mod	Ki-67	pAKT	pEGFR	pERK	pMET [pYpYpY]	pMET [Y1349]	pSTAT3
Gefitinib Resistant Samples	6.1%	97.9%	51.2%	84.8%	49.8%	24.6%	1.9%	0.0%	68.6%	7.7%	9.3%	0.8%
Gefitinib Sensitive Samples	6.3%	77.5%	28.3%	80.9%	42.5%	26.0%	28.1%	0.6%	32.2%	30.2%	26.4%	5.2%
Difference	0.2%	-20.4%	-22.9%	-3.9%	-7.3%	1.4%	26.3%	0.6%	-36.4%	22.5%	17.1%	4.4%
T-test	0.49	0.21	0.17	0.22	0.31	0.46	0.15	0.16	0.16	0.04	0.10	0.33

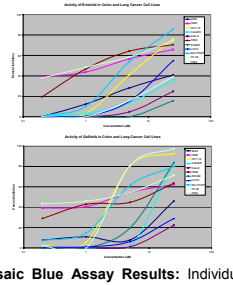
Predictive IHC Biomarkers: Cell lines were categorized as resistant, moderate or sensitive based on the growth inhibition at 25 uM erlotinib or gefitinib. Sensitivity thresholds were based on median and median – 1 SD. The percent staining by immunohistochemistry were compared between sensitive and resistant cell lines. c-MET staining was an average of 35% higher in Erlotinib sensitive samples than resistant samples (p=0.07). pMet was more prevalent in gefitinib sensitive cell lines (p=0.04 for pYpYpY and 0.10 for Y1349). While no correlation between EGFR staining and response to either drug was observed, moderate to strong staining was more prominent in resistant samples. pAKT staining was more prevalent in samples that were sensitive to either drug, while pERK demonstrated the opposite pattern. pSTAT3 levels were higher in cell lines that were sensitive to both drugs. No significant linear correlations were observed.

Biomarker Changes After Erlotinib Exposure	Aurora A	EGFR	EGFR >Mod	Ki-67	pAKT	pEGFR	pERK	pMET [pYpYpY]	pMET [Y1349]	pSTAT3
Average Change	0.4%	-10.5%	-13.8%	-1.8%	-7.0%	-1.6%	-0.5%	-12.4%	-16.0%	-5.7%
Average Change in Resistant Cells	12.6%	-12.5%	-14.8%	25.6%	12.0%	0.0%	-10.9%	-10.2%	-17.5%	-0.3%
Average Change in Sensitive Cells	-3.5%	-11.5%	-15.7%	-12.8%	-6.1%	-2.5%	-11.8%	-19.0%	-20.1%	-9.7%
Number Cell Lines with Increase	4	2	1	5	5	0	6	2	0	1
Number Cell Lines with Decrease	7	9	10	6	6	5	5	9	11	9
Number Cell Lines with Increase >5%	1	1	0	1	2	0	4	0	0	0
Number Cell Lines with Decrease >5%	1	6	8	5	5	1	3	6	7	2

Biomarker Changes After Gefitinib Exposure	Aurora A	EGFR	EGFR >Mod	Ki-67	pAKT	pEGFR	pERK	pMET [pYpYpY]	pMET [Y1349]	pSTAT3
Average Change	0.8%	-2.7%	-6.7%	1.7%	-16.2%	-1.7%	-10.5%	-16.7%	-16.9%	-5.8%
Average Change in Resistant Cells	6.7%	-1.5%	-9.7%	11.9%	-0.7%	NA	-18.3%	-3.0%	-6.1%	-0.8%
Average Change in Sensitive Cells	2.0%	-2.2%	-4.5%	3.5%	-12.5%	-0.6%	-6.7%	-17.5%	-15.6%	-1.9%
Number Cell Lines with Increase	6	6	2	6	3	0	5	2	2	1
Number Cell Lines with Decrease	5	5	9	5	8	5	6	9	9	9
Number Cell Lines with Increase >5%	2	1	1	4	1	0	2	0	0	0
Number Cell Lines with Decrease >5%	1	3	7	3	6	1	4	7	8	3



Pharmacodynamic IHC Biomarkers: The average non-normalized change in percent positive for each IHC marker is listed. Expression of pMET and pSTAT3 was significantly decreased after treatment with erlotinib and gefitinib in the majority of specimens. pAKT was more frequently decreased with gefitinib. Ki-67 demonstrated a greater frequency of specimens with significant reduction (>5% decrease) with erlotinib treatment. When present, phosphorylated EGFR was reduced to 20.8% of untreated levels by erlotinib and to 30.4% of untreated levels by gefitinib . Aurora A demonstrated differential staining upon treatment with erlotinib and gefitinib that was not consistent across tumor type or drug sensitivity.



Mosaic Blue Assay Results: Individual percent growth inhibition curves after treatment with erlotinib or gefitinib in the Mosaic Blue Assay.

Conclusions

- The Mosaic Blue Assay™ identified differential response to EGFR inhibitors in non-small cell lung cancer and colorectal cancer cell lines.
- The construction of cell pellet arrays containing treated and untreated cells processed and embedded in a similar manner to cancer tissues provided a method to evaluate the specificity of IHC assays and whether they demonstrate promise as predictive or pharmacodynamic biomarkers.
- Phosphorylation of MET at both Y1349 and Y1230/1234/1235 demonstrated a predictive relationship with gefitinib and a pharmacodynamic modulation with both erlotinib and gefitinib.
- Phosphorylation of STAT3 demonstrated a positive relationship with both erlotinib and gefitinib. pSTAT3 levels were reduced in most cell lines after treatment with either erlotinib or gefitinib.
- Ki-67, Aurora A and phospho-histone H3 demonstrated differences in staining after drug treatment, but the direction of change varied.