Mosaic Blue Assay: Erlotinib and Gefitinib Chemosensitivity in Non-small Cell Lung Cancer Explants and the Relationship to EGFR and Other Biomarkers

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Abstract

Erlotinib and gefitinib are epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) used to treat patients with non-small cell lung cancer. The use of cancer explant testing is very useful in identifying biomarkers that relate to the intrinsic sensitivity of cancers to therapeutic agents, since it allows single-agent testing on non-expanded patient cancer cells and has demonstrated excellent predictive accuracy for clinical results.

In the current study, non-small cell lung cancer specimens were profiled for chemosensitivity to erlotinib and gefitinib using the Mosaic Blue soft agar *ex vivo* tumor response assay. Specimens were histologically classified as adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma and other. Both drugs were analyzed at 50, 5, 0.5 and 0.05 μM; and the average percent inhibition for all specimens was calculated for gefitinib and erlotinib.

Specimens were evaluated for EGFR expression by immunohistochemistry using Zymed clone 31G7. EGFR expression was identified in the majority of specimens, and varied from weak to strong in intensity. The average percentage of cells staining for EGFR was highest in squamous cell carcinoma, followed by adenocarcinoma and adenosquamous cell carcinoma. Specimens were also profiled for phospho-EGFR, phospho-STAT3, and HER3 to evaluate biomarkers related to intrinsic chemosensitivity.

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

Erlotinib (Tarceva®) and Gefitinib (Iressa®) are selective inhibitors of EGFR with IC50's of 20 nM and 33 nM, respectively, that are used for the treatment of non-small cell lung cancer (NSCLC). Response rates in unselected NSCLC patients are between 4.1% and 27%. An overall survival benefit has only been demonstrated with erlotinib. Numerous biomarker studies have been performed in order to identify tests to predict patient response. EGFR protein, EGFR gene copy number, EGFR mutation status, K-RAS mutation status, smoking status, gender, ethnicity and histological subtype of cancer have demonstrated varying degrees of relationship with response.

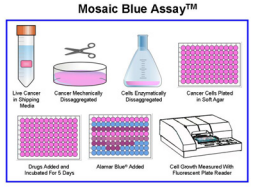
Twenty-six non-small cell lung cancers were characterized for gefitinib and erlotinib sensitivity using the Mosaic Blue Assay™. The Mosaic Blue Assay™ is a soft agar *ex vivo* tumor response assay that tests unexpanded viable cancer explant cells for sensitivity to therapeutics. Results are reported 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. Representative adjacent cancer from fresh specimens was fixed in formalin, paraffin-embedded and tested for biomarkers. All 26 samples were characterized for EGFR by immunohistochemistry using Zymed clone 31G7, and for mutations in EGFR and K-RAS. Fifteen samples were also analyzed by immunohistochemistry for phospho-EGFR, phospho-STAT3 and HER3.

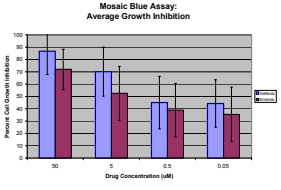
Immunohistochemistry was evaluated on a semi-quantitative scale, and the percentage of cancer cells staining at each of the following four levels was recorded: 0 (unstained), 1+ (weak staining), 2+ (moderate staining) and 3+ (strong staining). An H-score was calculated based on the summation of the product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

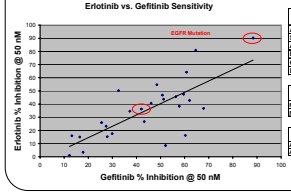
Results

Specimen ID	Cancer Type	Histology	Gender	Age	EGFR Mutation	KRAS Mutation
001	Non-small Cell Lung Cancer	Adenosquamous cell carcinoma	M	57	None	G12V, Exon 2
002	Non-small Cell Lung Cancer	Adenosquamous cell carcinoma	M	64	None	None
003	Non-small Cell Lung Cancer	Adenosquamous cell carcinoma	M	54	L858R, Exon 21	None
004	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	58	None	None
005	Non-small Cell Lung Cancer	Squamous cell carcinoma	F	69	None	None
006	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	67	None	None
007	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	63	None	None
008	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	57	None	None
009	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	66	None	None
010	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	59	None	G12C, Exon 2
011	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	60	None	None
012	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	77	None	None
013	Non-small Cell Lung Cancer	Squamous cell carcinoma	M	61	None	None
014	Non-small Cell Lung Cancer	Adenocarcinoma	F	53	None	None
015	Non-small Cell Lung Cancer	Adenocarcinoma	F	55	L858R, Exon 21	None
016	Non-small Cell Lung Cancer	Adenocarcinoma	F	51	None	None
017	Non-small Cell Lung Cancer	Adenocarcinoma	M	47	None	None
018	Non-small Cell Lung Cancer	Adenocarcinoma	M	66	None	None
019	Non-small Cell Lung Cancer	Adenocarcinoma	M	70	None	G12D, Exon 2
020	Non-small Cell Lung Cancer	Adenocarcinoma	M	60	None	None
021	Non-small Cell Lung Cancer	Adenocarcinoma	NA	NA	None	G12V, Exon 2
022	Non-small Cell Lung Cancer	Bronchioalveolar carcinoma	F	72	None	None
023	Non-small Cell Lung Cancer	Large cell carcinoma	M	48	None	None
024	Non-small Cell Lung Cancer	Large cell carcinoma	M	58	None	None
025	Non-small Cell Lung Cancer	Large cell carcinoma	M	58	None	None
026	Non-small Cell Lung Cancer	Clear cell carcinoma	M	66	None	None

Mosaic Blue Assay Results, Relationship to Histology, Gender, Age





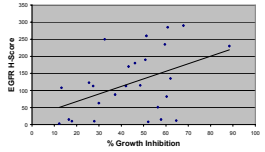


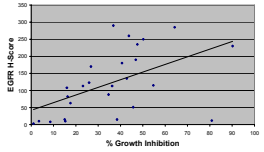
	Gefitinib Inhibition (%)		Erlotinib Inhibition (%)	
	5 uM	50 nM	5 uM	50 nM
Adenosquamous (n=3)	60	39	24	26
Squamous Cell Carcinoma (n=10)	78	43	62	42
Adenocarcinoma (n=8)	60	47	52	37
Bronchioalveolar (n=1)	77	66	64	46
Clear Cell (n=1)	58	12	24	1
Large Cell (n=3)	84	56	59	29

	Gefitinib Inhibition (%)		Erlotinib Inhibition (%)		EGFR	pEGFR	pSTAT3	HER3
	5 uM	50 nM	5 uM	50 nM	H-Score	H-Score	H-Score	H-Score
Male	69	42	51	32	141	37	60	36
Female	77	59	62	53	182	29	138	74

	Gefitinib Inhibition (%)		Erlotinib Inhibition (%)		EGFR	pEGFR	pSTAT3	HER3
	5 uM	50 nM	5 uM	50 nM	H-Score	H-Score	H-Score	H-Score
Age<55	62	47	46	33	204	22	110	115
Age>55	73	45	65	37	135	33	77	26

Gefitinib and Erlotinib Response vs. EGFR IHC





	EGFR % Positive	EGFR H-Score
Adenosquamous (n=3)	39	100
Squamous (n=10)	83	194
Adenocarcinoma (n=8)	65	123
Other (n=5)	56	102

	Gefitinib Inhibition (%)		Erlotinib Inhibition (%)	
	5 uM	50 nM	5 uM	50 nM
EGFR H-Score <100	63	39	45	25
EGFR H-Score >100	76	48	58	43
T-Test, p-value	0.049	0.134	0.077	0.019

There was a positive trend between EGFR H-score and response to both gefitinib and erlotinib. Squamous cell carcinomas had a higher average H-score than adenocarcinomas, adenosquamous carcinomas and other (large cell, clear cell and bronchioalveolar) carcinomas.

Gefitinib and Erlotinib Response vs. Mutation Status

	Gefitinib Inhibition (%)		Erlotinib Inhibition (%)		EGFR
	5 uM	50 nM	5 uM	50 nM	H-Score
EGFR Mutant (n=2)	71	65	61	63	255
EGFR WT (n=24)	70	43	52	33	134
KRAS Mutant (n=4)	55	27	31	16	62
KRAS WT (n=22)	73	47	56	39	158

Cancers with EGFR mutations are more sensitive to both gefitinib and erlotinib than EGFR wild type cancers. Cancers with K-Ras mutations are more resistant to both erlotinib and gefitinib

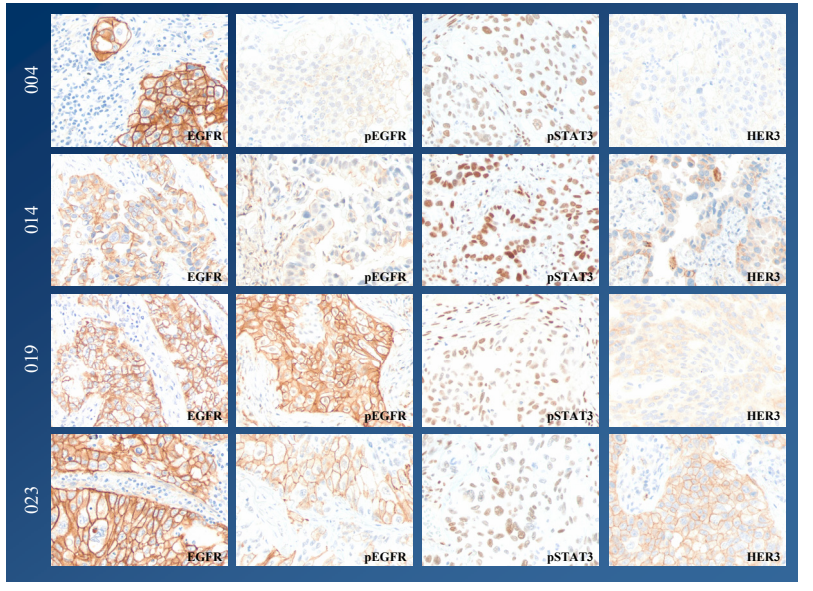
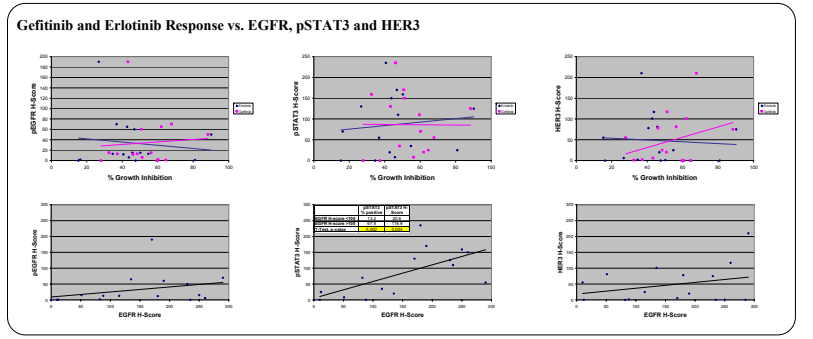


Figure 7: Immunohistochemistry for EGFR, pEGFR, pSTAT3 and HER3

Gefitinib and Erlotinib Response vs. EGFR, pSTAT3 and HER3



Conclusions

- The Mosaic Blue Assay™ identifies differential response to EGFR inhibitors in non-small cell lung cancer at drug concentrations similar to reported cell line IC50s. Response to erlotinib and gefitinib trend together.
- Cancers that contain EGFR mutations were significantly more sensitive to low concentrations of both gefitinib and erlotinib, while cancers with K-Ras mutations were significantly less sensitive.
- A positive trend exists between EGFR immunohistochemical staining using clone 31G7 and growth inhibition by gefitinib and erlotinib. Both erlotinib (50 nM) and gefitinib (5 µM) demonstrated a statistically significant higher activity in non-small cell lung cancers with an EGFR H-score of at least 100 units vs. those scored under 100 units.
- A statistically significant positive relationship was identified between EGFR H-Score and phospho-STAT3 H-score.