#4079 Results of BCIRG 103: A presurgical study to evaluate molecular alterations that occur in human breast cancer tissue after short-term exposure to gefitinib

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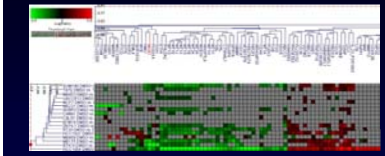
I. Abstract

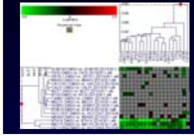
Gefitinib (IRESSA) is a small molecule inhibitor of the epidermal growth factor receptor tyrosine kinase (EGFR-TK), with activity in advanced non-small cell lung cancer (NSCLC) in patients who have previously received chemotherapy. Recently, mutations in the EGFR-TK have been described that may predict for response to gefitinib in NSCLC. These mutations have not been identified in breast cancer to date. Numerous pre-clinical studies have suggested that gefitinib may play a role in the treatment of breast cancer. To determine the potential role of gefitinib in human breast cancer we performed a comprehensive translational science program. We first determined gefitinib sensitivity in vitro in a panel of human breast cancer cell lines. Baseline expression profiles (Agilent microarrays) were generated for all of these cell lines and, potential predictive markers for response and resistance in a subset of cell lines have been identified. 17 cell lines were treated with gefitinib, and pre-and post-treatment expression profiles obtained. In parallel, a presurgical clinical study (BCIRG 103) was conducted at multiple centers in the United States, Canada, Australia, and Europe. BCIRG 103 enrolled 59 women with primary breast cancer. Frozen tumor core biopsies were obtained at baseline and RNA was isolated. Patients then received gefitinib 250 mg orally each day until definitive surgery (minimum 14 days). At the time of surgery, frozen tissue was obtained for RNA isolation. 43 matched pre-and post-treatment tumor samples were evaluable for microarray analysis.

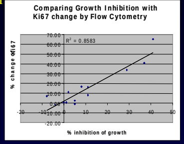
II. Preclinical Data

Change in MKi67 expression in17 Cell Line Treatments



Change in Cell Cycle gene cluster[1] in 17 Cell Line Treatments

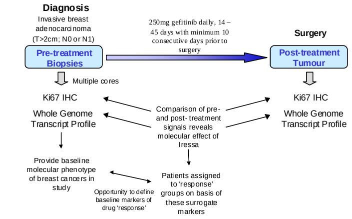




of data		
	1.25	
Cell Line	% Inhibition	IC50
184 A1	91	0.008
MDA-MB-175	90	0.03
BT-474	81	0.13
SK-BR-3	80	0.34
A431	77	0.038
MCF-10A	76	0.00052
184 B5	76	0.29
SUM-190	74	0.38
HCC1954	71	0.235
SUM-225	61	0.19
UACC-812	59	1.25
HCC1419	54	1.1
MDA-MB-468	52	0.78
MDA-MB-361	50	1.2
ZR-75-30	48	1.8
UACC-893	39	2
HCC1187	38	3.6
T-47D	37	1.7
HCC1806	36	2.61
MDA-MB-330	31	7.8
CAL-51	29	3.3
HCC1143	25	29.4
BT-20	23	12.7
ZR-75-1	18	7
HCC1569	17	11.1
MCF-7	15	11.5
EFM-19	13	10.4
MDA-MB-453	10	5.7
MDA-MB-436	9	12.67
HCC1937	7	9.78
CAMA-1	5	15
MDA-MB-157	3	11.5
HCC1500	1	8.5
Hs578T	0	9.75
MDA-MB-231	-1	> 10.0
MDA-MB-435	-2	7
HCC202	47	Flat
HCC70	44	Flat
HCC38	40	Flat

- HER2 amplified cell lines as a group were more sensitive to gefitinib
- Basal (mesenchymal) cell lines were generally more resistant to gefitnib
- Significant decreases in Ki67 and the Cell Cycle Gene Set[1] by microarray were seen in the most sensitive cell lines after treatment with 1µM gefitnib
- These data provide support for using changes in Ki67 and the Cell Cycle Gene Set[1] in the clinical study to define "molecular response" to gefitnib by microarray

III. BCIRG 103: Study Plan


Diagnosis
Invasive breast adenocarcinoma (T>2cm; N0 or N1)
Pre-treatment Biopsies
250mg gefitinib daily, 14 – 45 days with minimum 10 consecutive days prior to surgery
Surgery
Post-treatment Tumour
Multiple cores
Ki67 IHC
Whole Genome Transcript Profile
Comparison of pre- and post- treatment signals reveals molecular effect of Iressa
Ki67 IHC
Whole Genome Transcript Profile
Provide baseline molecular phenotype of breast cancers in study
Opportunity to define baseline markers of drug 'response'
Patients assigned to 'response' groups on basis of these surrogate markers

- Ki67 IHC was done and results were in broad agreement with Ki67 mRNA but the comparison was complicated by low baseline Ki67 levels

IV. Patient Characteristics

	N = 59
Median Age	61(27-79)
Median KPS	100% (80-100)
Pre-menopausal	27%
Post-menopausal	73%

43 Patients Evaluable for molecular Analysis

59 patients registered
- 20 not evaluable for tumor samples
  - 13 inadequate from UCLA lab
  - 7 not evaluable for study treatment

39 patients "clinically" evaluable
- **4 of 7 patients excluded because of dosage had adequate tissue for microarray**
  - 2 received less than minimum dosage
  - 2 stopped drug more than 1 day before surgery

43 patients evaluable for molecular alterations

Tumor Characteristics

	N = 59
Histopathological Grade	
Grade 1 (low)	5 (8%)
Grade 2 (Intermediate)	28 (47%)
Grade 3 (High)	10 (17%)
Not assessable	16 (27%)

	N = 59
Tumor size	
2cm	6 (10%)
>2cm and ≤ 5cm	50 (85%)
> 5cm	3 (5%)
Histopathological Type	
Infiltrating Ductal	38 (64%)
Infiltrating Lobular	8 (14%)
Other infiltrating	13 (22%)

V. Materials & Methods

- Samples were frozen in liquid nitrogen using Gentle Jane device and stored at -80ºC
- Each of 4 cores and portions of the post-treatment tumor were mounted in OCT on dry ice
- 2-3 sections were cut from each block. One slide H&E stained
- H&E slides were evaluated for quality and per cent of tumor cells. One core chosen for RNA processing. Remaining cores saved at -80ºC
- Selected core was removed from the OCT block by partial thawing with the stabilizing reagent RNA Later
- Samples were immediately homogenized and total RNA was extracted using Qiagen Rneasy Minikits (final volume 30-60 ul)
- RNA was quantitated using Nanodrop (2ul)
- RNA integrity was assessed using Agilent Bioanalyzer (1 ul)

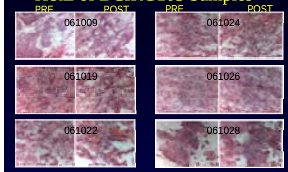
BCIRG 103 Microarray Experiment Set

Baseline Experiments – Comparison of each pre-treatment tumor sample with human breast cancer cell line reference
- subclassify "type"
- identify predictive markers of "response"

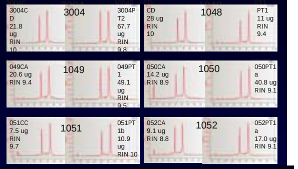
Treatment Experiments – Direct comparison of pre-treatment core with post-treatment surgical specimen
- define growth response
- identify molecular changes with treatment

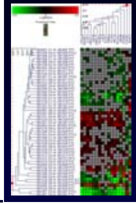
VI. Results

H&E of BCIRG103 Samples



Bioanalyzer and Nanodrop Results





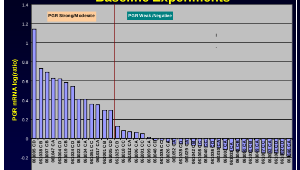
Cell Cycle Marker Genes

Treatment experiments clustered against genes expressed during specific phases of the cell cycle[1]

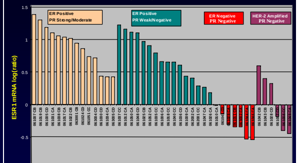
Assigning Experiments to Response Groups

- Review microarray experiment quality
  - Sample quality
  - Labeling quality
  - Correlation between two combined profiles (fluor reversals)
- Cell Cycle Response
- Change in mRNA Ki67 with treatment

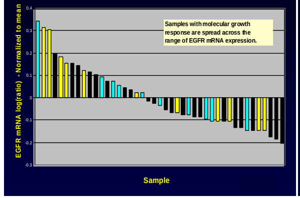
PGR mRNA Expression in BCIRG 103 Baseline Experiments



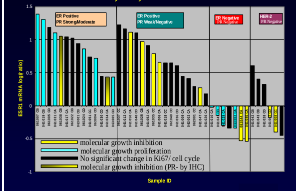
Subgrouping of BCIRG 103 Baseline Experiments based on ER, PR, and HER2



EGFR Expression in Baseline Experiments and Molecular Growth Response



Classification of "Molecular Response" Based on ER, PR, and HER2


ER Positive PR Strong/Moderate
ER Positive PR Weak/Negative
ER Negative PR Negative
HER-2 PR Negative
molecular growth inhibition
molecular growth proliferation
No significant change in Ki67/cell cycle
molecular growth inhibition (PR- by IHC)
ESR1 mRNA log(ratio)
Sample ID

Conclusions:

- Pre-clinical studies with gefitinib, support using changes in expression of Ki67 and a Cell Cycle Gene Set[1] to assign a molecular response
- EGFR expression by mRNA does not predict for molecular growth inhibition to gefitinib
- Molecular growth inhibition or no growth response was seen in ER+/PR- patients and HER2 amplified patients
- Molecular growth proliferation was seen in ER+/PR+ disease. 2 patients with growth inhibition in this group were PR- by IHC
-ER-/PR- disease showed both molecular growth inhibition and proliferation
- Translational studies can help guide the development of targeted therapeutics

Discussion

- Clinically, ER+/PR- breast cancer behaves worse than ER+/PR+ disease
- Several studies suggest that this subtype is EGFR or HER2 *driven*[2,3,4]
- Our data support that ER+/PR- disease is driven by growth factor signaling and therefore may be more likely to benefit from EGFR directed therapies

References

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4.Cui X, Schiff R, Arpino G et al. J Clin Onc 23, 7721-7735 2005