Functional Profiling to Select Chemotherapy in Untreated, Advanced or Metastatic Non-small Cell Lung Cancer*

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Abstract. *Background/Aim: To assess the impact of drug selection upon the treatment of advanced and metastatic non-small cell lung cancer (NSCLC), we applied a functional platform that measures drug-induced cell death in human tumor primary-culture micro-spheroids isolated from surgical specimens. Patients and Methods: At diagnosis, microspheroids isolated by mechanical and enzymatic disaggregation were examined for drug-induced cell-death by morphology and staining characteristics. Drugs were administered using standard protocols. Thirty-one patients, who received at least one cycle of therapy, were evaluable. All patients signed informed consent. Results: Twenty out of 31 patients responded (64.5%), 1 completely and 19 partially, providing a two-fold improvement over historical control of 30% (p=0.00015), a median time-to-progression of 8.5 months and a median overall survival of 21.3 months. Conclusion: This functional platform is feasible and provides a favorable objective response rate, time-to-progression and survival in advanced, metastatic, untreated NSCLC, and warrants further evaluation.*

Bronchogenic carcinoma is the leading cause of cancer-related death in the US with 160,340 estimated deaths in 2012. The majority of patients present with non-small cell lung cancer. While the 1-year survival for advanced lung cancer has improved from 35% to 42% over the past 3 decades (1), the 5-year survival for stage III & IV disease remains only 7% (2), with only 1 out of 6 patients diagnosed with early-stage disease (1).

The American Society of Clinical Oncology (ASCO) clinical practice guidelines recommend that patients with advanced disease and adequate performance status receive chemotherapy consisting of a 2-drug combination, with platinum-based doublets preferred (3). Objective response rates of 20-30% and median overall survival of 9-11 months have been observed (4, 5). More recently, the addition of bevacizumab to carboplatin plus paclitaxel in the Eastern Cooperative Group (ECOG) Study 4599 (E4599) improved the median OS to over 12 months (6), yet presently all patients ultimately suffer disease progression upon or following therapy completion.

There is a growing recognition that enhanced patient selection improves therapeutic outcomes. In recent years, gene arrays have been applied to develop prognostic and predictive molecular signatures (7-9). The BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) study, with a focus upon molecular targets in previously-treated patients (results recently updated (10)) provides support for biomarker-based therapy in this disease. With the expansion of targeted therapies against the epidermal growth factor receptor, ALK-gene re-arrangements and others, the need for predictive signatures will grow. As molecular and proteomic platforms continue to evolve, many cellular signaling pathways remain incompletely understood. The redundancy and crosstalk between cellular pathways in solid tumors pose challenges for analytical methods, despite their proven success in chronic myelogenous leukemia for which the c-Abl oncogene, the target of imatinib, has been fully-characterized (11).

To address the complexity of cancer response to noxious stimuli, we applied a functional platform that examines the effects of cytotoxic drugs, combinations and signal transduction inhibitors at the level of the cellular phenotype. Using human tumor primary culture microspheroids, isolated from surgical specimens and cytologically-positive fluids, the

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Ex Vivo Analysis of Programmed Cell Death (EVA/PCD), examines morphological and metabolic features of drug-induced programmed cell death (both apoptotic and non-apoptotic) to predict response to clinical therapy. Results with the EVA/PCD platform have previously been shown to correlate with response, time-to-progression and survival (12, 13), and with response to tyrosine kinase inhibitors (14).

We applied the EVA/PCD platform to 31 NSCLC patients with previously-untreated, inoperable, advanced disease to select laboratory-directed drug regimens, chosen from among US FDA-approved and compendium listed agents.

Patients and Methods

Patients with histologically-proven non-small cell lung cancer were screened for eligibility. Only patients found inoperable, who were not candidates for definitive radiation or chemo-radiation were eligible. Additional eligibility requirements included performance status of ECOG 0, 1 or 2; adequate pulmonary, cardiac, renal and hepatic function, bi-dimensional measurable disease, a life expectancy ≥3 months, adequate white, red and platelet counts, controlled brain metastases, a successful assay identifying at least one drug with activity and no psychiatric disease that would preclude participation. All patients signed an informed consent. The study was approved by the Memorial Medical Center Human Subjects Committee that serves as the institutional review board for all human studies.

The time sensitivity of *Ex Vivo* Analysis (24-hour window) required that tissues be submitted to the laboratory often prior to formal review of the patient's candidacy for cytotoxic chemotherapy. A total of 98 patients were screened, with 31 qualifying for protocol therapy. The most common reasons for disqualification were lack of measurable disease and performance status ≥ECOG III. The protocol accrual schema is provided in Table I. The reasons for protocol exclusion are provided in Table II. Thirty one patients who received at least one cycle of laboratory-selected chemotherapy are fully evaluable. Patients' characteristics are provided in Table III.

Laboratory analysis. The EVA/PCD laboratory method has previously been described (12). Briefly, surgical specimens obtained at the time of diagnostic biopsy or surgical resections were submitted from the department of pathology in sterile RPMI-1640. Following mechanical dis-aggregation, samples were enzymatically digested in 0.2% (w/v) DNAse and 0.4% (w/v) Collagenase IV. Tumor clusters of desired size (50-100 cell spheroids) were then isolated by density centrifugation over ditriazonate, adjusted to desired density. Fluid specimens were isolated by density centrifugation over ditriazonate, adjusted to desired density. Specimens were washed and re-suspended in modified RPMI-1640, containing fetal bovine serum (FBS) 10%, L-glutamine (2%) and penicillin/streptomycin. Cell counts were adjusted by dilution and cells were gently agitated before distribution into 96-well plates. Serial dilutions of drugs were then added by micropipette. Tumor cell/drug mixtures were incubated for 72 hours at 37˚C in 5% CO_2 in a humidified incubator.

Drugs, selected from among US FDA-approved compendium listed agents, were tested alone and in combination, by class, with cisplatin representing platins, paclitaxel representing taxanes, gefitinib representing EGFr-TKIs, sunitinib representing VEGF inhibitors, and trimetrexate antifols. Table 4 outlines the drugs tested.

At the completion of drug exposure, cell suspensions were examined for evidence of drug-induced cell death. A mixture of Nigrosin B & Fast Green dye with glutaraldehyde-fixed avian erythrocytes, as internal control, was added to each well. Samples were agitated and then cytospin-centrifuged, air dried and counterstained with H&E. Percent viability measured against saline-exposed controls (normalized to 100%) provided 5-point dose-response curves. Best-line plots by least square were used for the calculation of LC_{50} values by interpolation. Comparisons of individual patient LC_{50} values with the NSCLC database allowed the calculation of Z-scores using the formula ($Z=LC_{50}$ sample – LC_{50} Mean/Standard Deviation) with (+) Z-scores reflecting resistance and (–) Z-scores reflecting sensitivity to the drug(s) in question. Samples falling more than ½ STD below the mean were defined as "sensitive". Samples falling more than ½ STD above the mean were defined as "resistant". Those falling between these ranges were defined as "intermediate". Drug combination synergy was assessed using the median-effect analysis of Chou and Talalay (15). Drugs for clinical therapy were selected based upon the following algorithm: 1) Drugs or combinations falling in the "sensitive" range, defined as Z-score more than ½ STD below the mean value, were used. 2) If 2 or more drugs or combinations fell in the "sensitive" range, then the drug or combination with the most favorable Z-score was selected. 3) Drug combinations with similar Z-scores were selected if they revealed synergy. 4) Drug combinations found similar in both activity (Z-score) and synergy were selected based upon the most favorable clinical toxicity profile.

Therapy. Standard treatment protocols, administered in accordance with published results in the thoracic oncology literature, included: carboplatin & paclitaxel; cisplatin & vinorelbine; cisplatin & gemcitabine; carboplatin & gemcitabine; carboplatin & pemetrexed; erlotinib; erlotinib & bevacizumab; carboplatin & paclitaxel & bevacizumab; cisplatin & vinorelbine & bevacizumab; paclitaxel; docetaxel; vinorelbine; docetaxel & gemcitabine; irinotecan and irinotecan & cisplatin. Standard pre-medication, hydration; and anti-emetic therapies were administered in accordance with accepted NCCN clinical guidelines. Growth factor support was administered at the discretion of the treating physician.

Statistical considerations. The trial is a single-arm, non-randomized, historical-controlled design. The primary objective of the trial was to provide a 2-fold increase in objective response rate (CR plus PR) over historical experience of 30%, $p<0.05$, power=0.8. Secondary objectives were progression-free survival and overall survival for patients who received assay-directed therapy.

Response criteria. Patients' responses were measured in accordance with the RECIST criteria (16). Index lesions were identified by pretreatment CT or PET/CT scan conducted not more than two weeks prior to the initiation of therapy. Serial measurements of index lesions were conducted after the second, fourth and sixth cycle of therapy and then every 3 months for the duration of treatment. Time-to-progressive disease was measured from the time of accrual to the time of objective disease progression. Overall survival was measured from the time of accrual to the time of death.

Table I. *Clinical protocol outline for patient accrual.*

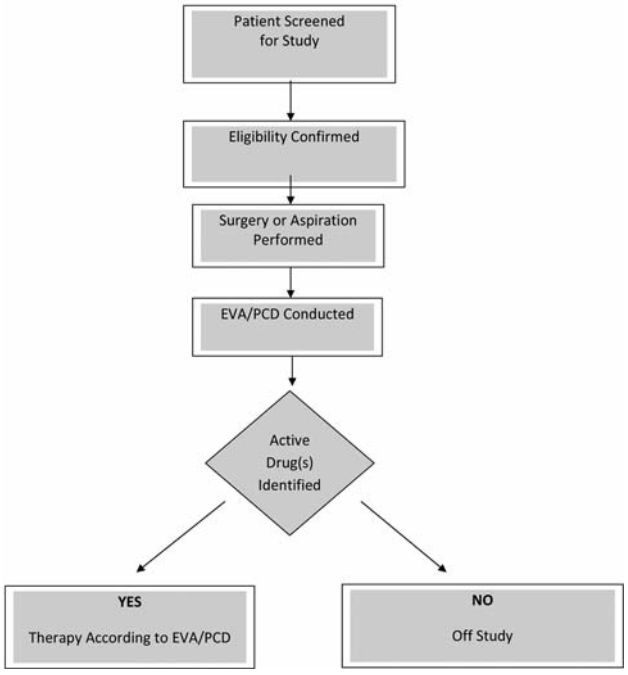
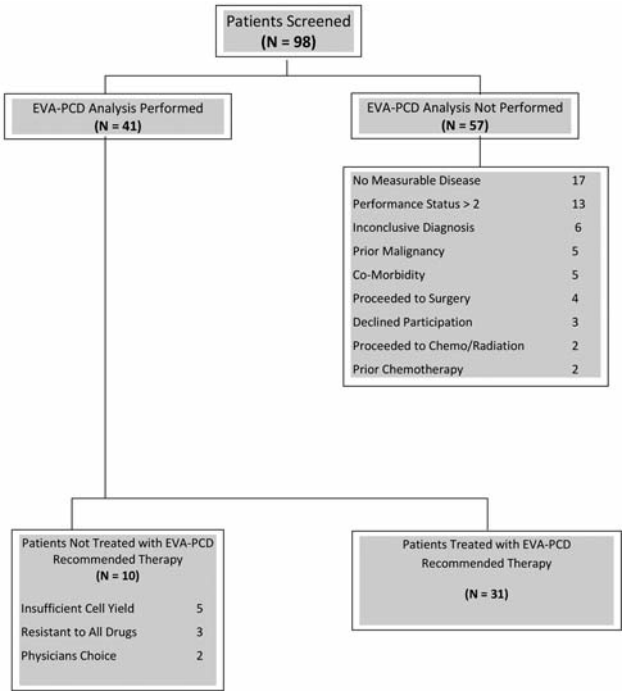


Table II. *Reasons for non-accrual.*



Results

Between December 2003 and July 2010, 98 patients were screened. Thirty-one patients who met inclusionary criteria received assay-directed therapy in accordance with protocol guidelines and are fully evaluable for response, time-to-progressive disease and overall survival.

There were 14 (45.2%) male and 17 (54.8%) female patients, with 23/31 (74.2%) stage IV, 7/31 (22.5%) stage IIIB, and 1/31 (3.2%) bulky stage IIIA. The median age was 59, with a range of 41-83 years. The treatments received under protocol included platinum/taxane 8 (25.8%), platinum/gemcitabine 14 (45.2%), platinum/navelbine 2 (6.5%), platinum/pemetrexed 2 (6.5%), erlotinib 4 (12.9%) and erlotinib/bevacizumab 1 (3.2%).

Statistical analysis. The objective response rate (CR & PR), clinical benefit response (CR & PR & SD) are provided in Table V. The observed Objective Response Rate (ORR) of 64.5%, achieved the study goal of exceeding a two-fold improvement over the historical standard of 30% and it produced a 95% confidence interval (CI) estimate of the true ORR (46.9%, 78.9%) that was substantially above the historical standard. Further support for this finding was provided by a Normal 2-sided Test of the difference between the observed ORR and the historical standard of 30%, which classified this difference as statistically significant ($p<0.0001$) (17).

Survival analyses were performed on the-time-to progression data and the time-to-death data to obtain the median time-to-event and the associated 95% confidence limits for each median: median time-to-progression was 8.5 months (4.5, 10.2) and median time-to-death was 21.3 months (11.1, 27.6). Time-to-disease progression and the overall survival curves for all 31 patients are provided in Figures 1 and 2, respectively. All analyses were performed using the SAS Statistical Software (version 9.2, Cary, NC).

Discussion

NSCLC remains a leading cause of morbidity and mortality. Laboratory platforms that address patient heterogeneity, capable of selecting effective chemotherapies, have the potential to improve response, diminish toxicity and limit futile care. LeChevalier previously calculated that a 2-month improvement in median survival would translate into a 6-month improvement for the 20-30% of responders (18). This strongly supports the use of selective methodologies to identify subsets of responding patients prior to therapy administration.

Following the first published trial in 1954 that used tetrazolium reduction to select chemotherapy (19); metabolic, morphologic, colony formation, H_3-thymidine incorporation, protein synthesis and other methods have been investigated

Table III. *Patients' characteristics*.

Pt ID	Accrual	Age	M/F	Histology	Stage	Sites of disease	Treatment
004	02-04-04	46	M	Adeno CA	IV	Lung, bone	Carboplatin/paclitaxel
006	7-20-04	70	M	Adeno CA	IV	Lung, bone, soft tissue	Cisplatin/gemcitabine
007	09-03-04	82	M	Adeno CA	IV	Lung, soft tissue	Carboplatin/docetaxel
009	09-15-04	46	F	Large cell	IV	Lung, bone	Cisplatin/gemcitabine
010	01-17-05	77	F	Squamous	IV	Lung, adrenal, soft tissue	Carboplatin/paclitaxel
011	03-18-05	59	M	Adeno CA	IV	Lung	Cisplatin/gemcitabine
012	03-21-05	45	F	Adeno CA	IV	Lung, soft tissue	Erlotinib
013	11-15-05	54	M	Large cell	IV	Lung, liver	Cisplatin/gemcitabine
016	02-27-06	59	F	Adeno CA	IV	Lung, soft tissue, bone	Erlotinib
021	06-22-06	60	F	Adeno CA	IV	Lung, bone	Erlotinib
022	11-13-06	67	F	Adeno CA	IV	Lung, bone	Cisplatin/gemcitabine
027	03-02-07	79	F	Adeno CA	IV	Lung, soft tissue	Erlotonib
028	03-06-07	63	M	Adeno CA	IV	Lung, bone, adrenal	Carboplatin/paclitaxel
030	05-10-07	58	M	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/gemcitabine
031	07-06-07	66	M	Large Cell	IV	Lung, soft tissue, adrenal	Cisplatin/gemcitabine
032	08-10-07	41	M	Large cell, undiff	IIIB	Lung, soft tissue	Cisplatin/docetaxel
035	04-17-08	54	M	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/vinorelbine/bevacizumab
036	07-14-08	54	F	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/gemcitabine
037	07-31-08	74	F	Large cell, undiff	IIIA	Lung, soft tissue	Carboplatin/paclitaxel
038	09-10-08	60	M	Adeno Ca	IV	Lung, soft tissue	Cisplatin/gemcitabine
040	10-23-08	52	F	Adeno CA	IV	Lung, brain, soft tissue	Erlotonib/bevacizumab
043	02-10-09	83	F	Adeno CA	IV	Lung, bone, adrenal	Carboplatin/pemetrexed
044	02-17-09	80	M	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/gemcitabine
046	07-06-09	49	F	Adeno CA	IV	Lung, soft tissue, liver	Carboplatin/taxol/bevacizumab
047	08-05-09	52	F	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/gemcitabine
048	11-12-09	41	M	Adeno CA	IV	Lung, soft tissue	Cisplatin/gemcitabine
049	12-22-09	66	F	Adeno CA	IV	Lung, soft tissue	Cis/Vinorelbine/bevacizumab
050	12-31-09	57	M	Adeno CA	IV	Lung, brain	Carboplatin/paclitaxel
051	03-09-10	70	F	Adeno CA	IIIB	Lung, soft tissue	Cisplatin/gemcitabine
052	05-11-10	66	F	Adeno CA	IV	Lung, brain, soft tissue	Cisplatin/gemcitabine
053	07-19-10	69	F	Adeno CA	IV	Lung, soft tissue	Cisplatin/Pemetrexed

Adeno CA=Adenocarcinoma.

Table IV. *Drug panel*.

Single-agents	Combinations
Cisplatin	Cisplatin & gemcitabine
Paclitaxel	Cisplatin & paclitaxel
Vinorelbine	Cisplatin & vinorelbine
Irinotecan	Cisplatin & trimetrexate
Nitrogen Mustard	Cisplatin & irinotecan
Mitomycin-C	Gefitinib & sunitinib
Trimetrexate	
Gemcitabine	
Gefitinib	
Sunitinib	

Table V. *Patients' response*.

Response	Number of patients
Complete response (CR)	1 (3.2%)
Partial response (PR)	19 (61.3%)
Stable disease (SD)	9 (29.0%)
Overall response rate (ORR) (CR & PR)	20 (64.5%)
Clinical benefit response (CR & PR & SD)	29 (93.5%)
Progressive disease	2 (6.5%)

(20). The differential staining method originally reported by Weisenthal *et al*. (21) has been successfully applied in chronic lymphocytic leukemia (22). While the concept of drug selection has remained theoretically attractive, no method has achieved general acceptance. Prior reviews, focused primarily upon the colony formation (human tumor stem cell) assay, did not support their broad application (23, 24), while other reviewers have suggested untreated ovarian cancer and NSCLC (25) as appropriate study models. The latter reviewers conducted a trial in small cell lung cancer that showed a statistically significant improvement in time-to-progression (p=0.035) for assay-directed patients (26).

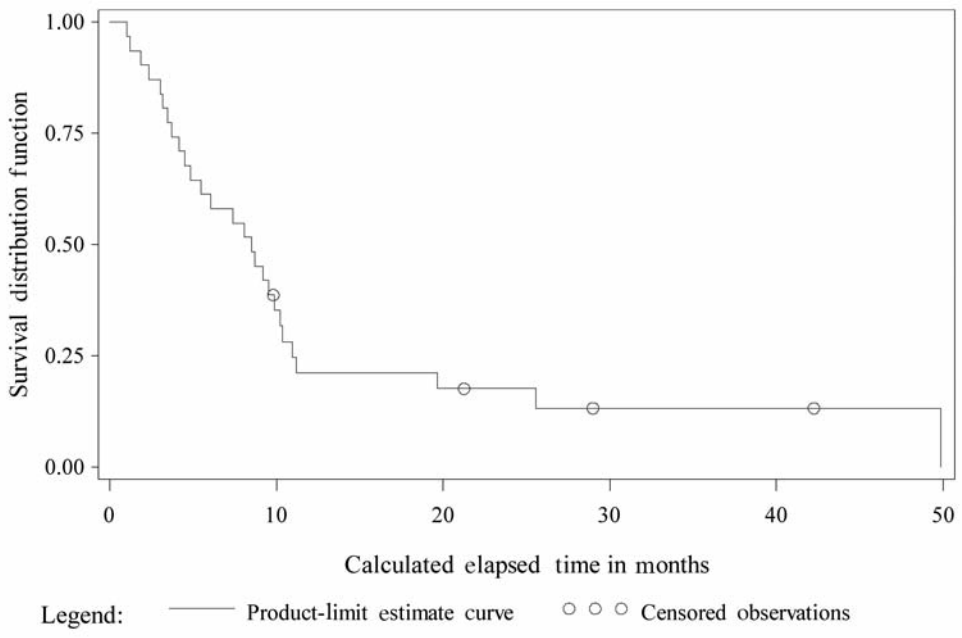


Figure 1. *Time-to-disease progression – time elapsed from accrual to study to objective evidence of progressive disease.*

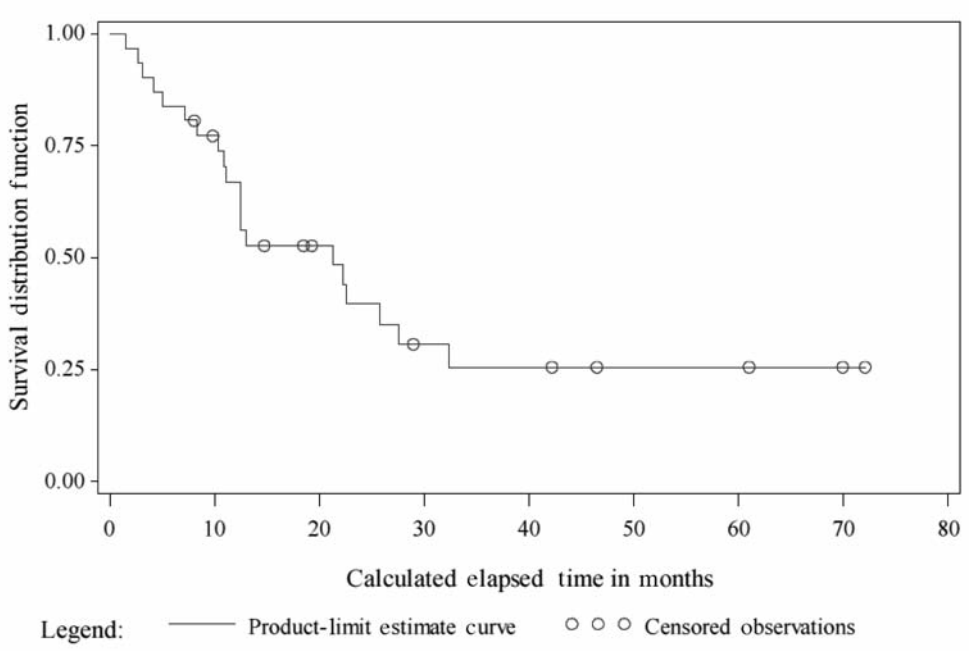


Figure 2. *Overall survival – time elapsed from accrual to study to time of death.*

To address the limitations of earlier methodologies, we incorporated two fundamental changes. First, older cell-proliferation measures (clonogenic, H_3* Thymidine incorporation, *etc*.) were replaced by the measurement of drug-induced cell death, reflecting seminal discoveries in the 1970's (27) that identified perturbations in cell death as the critical drivers of malignant transformation. The primacy of survival signals in human carcinogenesis led us to incorporate the more robust endpoint of programmed cell death for clinical response prediction. Chemotherapy

selection in NSCLC using the related methodology of ATP content to measure drug-induced cell death has been the subject of a prior report (28). The ATP content end-point has also been used as a correlate with molecular markers of response in NSCLC (29).

The second change reflects the use of "native-state" human tumor microspheroids. Using gentle mechanical and enzymatic disaggregation, tumor micro-aggregates are isolated directly from patient biopsies. As these tumor organoids are not sub-cultured or amplified, they retain the human tumor micro-environment, replete with stroma, vasculature, cytokines and inflammatory cells. This maintains the cell-cell, cell-stroma, cell-vasculature, and tumor cell-inflammatory cell interactions, now recognized to be critical for accurate response prediction. A closely related approach has more recently been applied to examine micro-vascular cells following exposure to bevacizumab and small-molecule tyrosine kinase inhibitors (30). Earlier work utilized tissue micro-aggregates propagated in collagen matrices. While this maintained cell-cell interactions, this platform differed somewhat from the one reported in this study, as the histo-culture technique propagated cells *in vitro* (31, 32). The current analysis reflects the incorporation of cell death measures in the native state microenvironment.

This study selected drugs and combinations from among US FDA-approved, compendium listed agents indicated for patients with NSCLC. This enabled us to examine the impact of drug selection upon outcome without the introduction of new drugs or combinations and to compare our results with contemporaneous historical controls. Thus, assay-directed therapy became the principal focus of the Phase II trial.

The 31 of 98 (31.6%) screened patients, who received assay-directed chemotherapy represent patients who met all criteria for inclusion. A large number of patients were disqualified due to lack of measurable disease, performance status, or histology. As a plurality of patients were performance status 2, and several presented with brain metastases at the time of accrual, the study included even the most advanced candidates.

As clinical trial designs continue to refine the concept of personalized medicine, candidates for accrual to treatment protocols are increasingly selected based upon highly specific criteria. The registration trial for Crizotinib screened 1,500 patients to identify 82 candidates for therapy (33). One study that used molecular profiling to select treatment candidates, evaluated 106 patients to identify 66 who received directed treatment (34). A related study, that examined molecular predictors for response to chemotherapy in NSCLC, examined 69 patients' tissues out of the total 170 accrued (9). Similarly, the current trial required a successful laboratory analysis for patients to qualify. Trial designs in the era of biomarker-driven therapeutics are an active area of ongoing investigation (35).

At interim analysis, the objective response rate of 64.5% (20/31), revealed that the trial had achieved its end-point of improving response rates (CR & PR) by two-fold (CI=0.45-0.78, p=0.00015). The median time-to-progression of 8.5 months (CI=4.5-10.2), median overall survival of 21.27 months (CI=11.1-27.5), and percentage of patients surviving at 1-6 (+) years after accrual, also compare favorably with historical controls.

The majority of patients in this trial were stage IV. However, seven stage IIIB and one bulky stage IIIA patient were included. To address the impact of stage upon outcome, we conducted a *post-hoc* analysis limited only to stage IV patients. Out of the 23 stage IV patients, 13 had complete or partial remissions for an objective response rate of 56.5%, similar to the overall objective response rate of 64.5% for the entire study population. For comparison, the ECOG 4599 (36), conducted in stage IV NSCLC, provided an objective response rate of 35% in the positive arm of carboplatin plus paclitaxel plus bevacizumab.

The current trial represents a proof of concept for personalized chemotherapy in advanced NSCLC in the community setting. Harvesting and processing of specimens was quick and reliable with only 8.1% found non-evaluable. Patients were highly motivated to provide tissue and there was no morbidity or mortality associated with tissue procurement.

The trial included conventional cytotoxic chemotherapy and combinations, as well as the targeted agent, erlotinib. Noteworthy, erlotonib, developed as a growth factor inhibitor has proven cytotoxic in our laboratory platform, as we have reported (37), possibly reflecting the phenomenon of oncogene addiction. We first observed this phenomenon with gefitinib and erlotonib, but have now extended these observations to the study of other signal transduction inhibitors (38).

Five out of 31 (16%) patients received erlotinib as first-line therapy. Four out of the 5 had objective responses and the fifth, stable disease. Although high response rates to EGFR-targeted agents are now commonly observed in patients who carry mutations in the EGFR domain (39), the first patients on trial to receive first-line erlotinib, did so before EGFR mutational analyses were available. Several erlotinib-treated patients have enjoyed durable remissions which may reflect the clinical relevance of functional measures, as these were patients who also had the most favorable LC_{50} values for erlotinib, *ex vivo*.

With response rates to EGFR-TKIs characteristically high, we conducted a sub-analysis that excluded the EGFr-TKI treated patients to examine the objective response rate only in patients who received conventional cytotoxic chemotherapy. The response rate of 65.4% (17/26) was essentially the same as the overall response rate of 64.5%, indicating that the tarceva responders did not appear to skew the overall results.

The trial was not designed as a direct comparison of regimens, but instead to examine the impact of patient

selection upon response. This platform has the potential to identify novel combinations and new uses of signal transduction inhibitors. While molecular platforms continue to identify targets, many drugs reveal unexpected activity, while others reveal unanticipated resistance, reflecting the complexity, redundancy and promiscuity of signal pathway networks (40). While the BATTLE study in NSCLC was highly favorable, a trial conducted in a mixed population of recurrent cancers provided an objective response rate of only 10% (33). Functional platforms, by encompassing all of the operative mechanisms of response and resistance acting in concert, could complement genomic and proteomic platforms, particularly with regard to synergy and sequence analyses. The incorporation of functional studies into prospective clinical trials of biomarker-driven therapy like the BATTLE study could offer interesting insights for the comparison of these platforms. The *Ex Vivo* Analysis of Programmed Cell Death (EVA/PCD) warrants further evaluation to assess its capacity to improve response with conventional drugs and to accelerate the introduction of targeted agents into NSCLC management.

References

1 Cancer Facts & Figures, Atlanta: American Cancer Society 2010.

2 Wang T, Nelson RA, Bogardus A and Grannis FW: Five-year lung cancer survival: Which advanced non small cell lung cancer patients attain long term survival? Cancer *116(6)*: 1518-1525, 2010.

3 Azzoli CG, Baker S Jr., Temin S *et al*: American Society of Clinical Oncology Clinical Practice Guideline Update on Chemotherapy for Stage IV Non Small Cell Lung cancer. J Clin Oncol *27(36)*: 6251-6266, 2009.

4 Kelly K, Crowley J, Bunn PA *et al*: Randomized phase III trial of Paclitaxel plus Carboplatin *versus* Vinorelbine plus Cisplatin in the treatment of patients with advanced non-small cell cancer: a Southwest Oncology Group trial. J Clin Oncol *19*: 3210-3218, 2001.

5 Schiller JH, Harrington D, Belani CP *et al*: Comparison of four chemotherapy regimens for advanced non-small cell lung cancer. NEJM *346*: 92-98, 2002.

6 Sandler A, Gray R *et al*: Paclitaxel-Carboplatin alone or with Bevacizumab for non-small cell lung cancer. NEJM *355*: 2542-2550, 2006.

7 Zhu CQ, Ding K and Strumpf D: Prognostic and predictive gene signature for adjuvant chemotherapy in resected non small cell lung cancer. J Clin Oncol *28(29)*: 4417-4424, 2010.

8 Chen HY, Yu SL, Chen CH *et al*: A five-gene signature and clinical outcome in non–small-cell lung cancer. NEJM *356*: 11-20, 2007.

9 Reynolds C, Obasaju C, Schell MJ *et al*: Randomized phase III trial of Gemcitabine-based chemotherapy with in situ RRM1 and ERCC1 protein levels for response prediction in non-small-cell lung cancer. J Clin Oncol *27(34)*: 5808-5815, 2009.

10 Kim ES, Herbst, RS, Wistuba II *et al*: The BATTLE Trial: Personalizing Therapy for Lung Cancer. Cancer Discovery *1(1)*: 42-51, 2011.

11 Deininger MW and Druker BJ: Specific targeted therapy of chronic myelogenous leukemia with Imatinib. Pharmacol Rev *55(3)*: 401-423, 2003.

12 Nagourney RA, Link JS, Blitzer JB *et al*: Gemcitabine plus cisplatin repeating doublet therapy in previously treated, relapsed breast cancer patients. J Clin Oncol *18(11)*: 2245-2249, 2000.

13 Nagourney RA, Brewer CA, Radecki SR *et al*: Phase II trial of gemcitabine plus cisplatin repeating doublet therapy in previously treated, relapsed ovarian cancer patients. Gyn Onc *88*: 35-39, 2003.

14 Nagourney RA, Paulsen, M, McConnell D *et al*: Clinical Responses to EGFR-TKI's identified by drug-induced cell death in human NSCLC primary cultures. Proc ASCO, (abst. 18184), 2007.

15 Chou TC, Talalay P: Applications of the median-effect principle for the assessment of low-dose risk of carcinogens and for the quantitation of synergism and antagonism of chemotherapeutic agents. In New Avenues in Developmental Cancer Chemotherapy, Bristol-Myers Symposium-series, K.R. Harrap and T.A. Connors Eds. New York, Academic Press pp. 37-64, 1987.

16 Therasse P, Arbuck SG, Eisenhauer EA *et al*: New guidelines to evaluate the response to treatment in solid tumors. J Natl Canc Inst *92(3)*: 205-216, 2002.

17 Fleiss, Levin, & Paik. Statistical Methods for Rates & Proportions (3rd Ed.) Wiley, 27, 2003.

18 LeChevalier T: Chemotherapy for advanced NSCLC: Will meta-analysis provide the answer? Chest *109*: 107-109S, 1996.

19 Black MM and Spear FD: Further observations on the effects of cancer chemotherapeutic agents on the *in vitro* dehydrogenase activity of cancer tissue. J Natl Cancer Inst *14*: 1147-1158, 1954.

20 Nagourney RA: *Ex vivo* programmed cell death and the prediction of response to chemotherapy. Curr Treat Options *7*: 103-110, 2006.

21 Weisenthal LM, Marsden JA, Dill PL *et al*: A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. Cancer Res *43(2)*: 749-757, 1983.

22 Bosanquet AG, Burlton AR, Bell PB *et al*: *Ex vivo* cytotoxic drug evaluation by DiSC assay to expedite identification of clinical targets: results with 8-chloro-cAMP. Br J Cancer *76(4)*: 511-518, 1997.

23 Samson DJ, Seidenfeld J, Ziegler K *et al*: Chemotherapy Sensitivity and Resistance Assays: A Systemic Review. J Clin Oncol *22(17)*: 3618-3630, 2004.

24 Schrag D, Garewal H, Burstein H *et al*: American Society of Clinical Oncology Technology Assessment: Chemotherapy Sensitivity and Resistance Assays, J Clin Oncol *22(17)*: 3631-3638, 2004.

25 Cortazar P and Johnson BE: Review of the efficacy of individualized chemotherapy selected by *in vitro* drug sensitivity testing for patients with cancer. J Clin Oncol *17*: 1625-1631, 1999.

26 Cortazar P, Gadzar AF, Wood E *et al*: Survival of patients with limited-stage small cell lung cancer treated with individualized chemotherapy selected by *in vitro* drug sensitivity testing. Clin Canc Res *3*: 741-747, 1997.

27 Kerr JFR, Wyllie AH and Currie AR: Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer *26*: 239-257, 1972.

28 Moon YW, Sohn JH, Kim YT *et al*: Adenosine triphosphate-based chemotherapy response assay (ATP-CRA–guided *versus* empirical chemotherapy in unresectable non-small cell lung cancer. Anticancer Res *29(10)*: 4243-4249, 2009.
29 Glaysher S, Yiannaki D, Gabriel FG *et al*: Resistance gene expression determines the *in vitro* chemosensitivity of non-small cell lung cancer (NSCLC). BMC Cancer *9*: 300, 2009.
30 Weisenthal LM, Patel N and Rueff-Weisenthal C: Cell culture detection of microvascular cell death in clinical specimens of human neoplasms and peripheral blood. J Intern Med *264(3)*: 275-287, 2008.
31 Freeman AE and Hoffman RM: *In vivo*-like growth of human tumors *in vitro*. Proc Natl Acad Sci USA *83(8)*: 2694-2698, 1986.
32 Vascio RA, Redfern CH, Nelson TJ *et al*: *In vivo*-like drug responses of human tumors growing in three-dimensional gel-supported primary culture. Proc Natl Acad Sci USA *84(14)*: 5029-5033, 1987.
33 Kwak EL, Bang YJ, Camidge DR *et al*: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med *364(6)*: 588, 2011.
34 Von Hoff DD, Stephenson JJ Jr., Rosen P *et al*: Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol *28(33)*: 4877-4883, 2010.
35 Mandrekar SJ: Predictive biomarker validation in practice: lessons from real trials. Clin Trials *5*: 567-573, 2010.
36 Sandler A, Gray R, Perry M *et al*: Paclitaxel-Carboplatin alone or with Bevacizumab for non-small cell lung cancer. N Engl J Med *355*: 2542-2550, 2006.
37 Nagourney RA, Chow C, Kollin C *et al*: Activity of ZD1839 (Iressa) alone and combined with cytotoxic drugs in human tumor primary cultures. Proc AACR *43*: 787 (abst. 3902), 2002.
38 Gonsalves FC, Klein K, Ekas LA *et al*: Novel RNAi-based screen identifies three new classes of small molecule inhibitors of the Wnt/wingless signaling pathway. PNAS *108(15)*: 5954-5963, 2011.
39 Lynch TJ, Bell DW, Sordella R *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to Gefitinib. NEJM *350(21)*: 2129, 2004.
40 Nagourney RA, Bernard P, Francisco F *et al*: *Ex vivo* analysis of the mTOR/PI3K inhibitors, BEZ235 & AZD6244, alone and in combination in human tumor primary culture micro-spheroids: Exploration of horizontal pathway targeting. Proc AACR *52*: (abst 650), 2011.

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