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## Summary Report 7th Annual Targeted Therapies of the Treatment of Lung Cancer

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Lung cancer is the leading cause of cancer death worldwide; its 5-year survival rate is only 15%. The therapy for this disease is improving with the advent of new targeted therapies, agents that are potentially more effective and less toxic. This has resulted from a better understanding of the molecular pathogenesis of this disease with the advent of new drugs or combinations for this disease. In February 2007, a 3-day conference in Santa Monica was sponsored by the International Association for the Study of Lung Cancer, which brought together 50 world experts in the treatment of this disease.

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Drs. Bunn Jr. and Johnson acted as meeting chairman and manuscript editors.

## Vascular Endothelial Growth Factor Inhibitors Lawrence Einhorn, MD

Vascular endothelial growth factor (VEGF) is one of the most important growth factors that regulate angiogenesis. Bevacizumab has proven efficacy in colorectal cancer, non-small cell lung cancer (NSCLC), renal cell carcinoma, and other tumor types. It is the only molecularly targeted agent proven to improve survival when combined with chemotherapy as first-line therapy in NSCLC. Molecular markers such as the intercellular adhesion molecule may be useful as a biomarker.

These agents have common toxic effects, especially hypertension, proteinuria, while less frequent toxicities such as thromboses, and hemoptysis are occasionally fatal. Myelosuppression and febrile neutropenic are more frequent when combined with chemotherapy compared with chemotherapy alone. A new toxicity, tracheo-esophageal fistula, has recently been observed with chemotherapy + bevacizumab in a SCLC trial.

This review will cover several new agents that target VEGF and are showing promise in NSCLC.

### AZD 2171

This is a small-molecule tyrosine kinase inhibitor (TKI) selective for all VEGF receptors. A phase I study combined AZD 2171 with carboplatin + paclitaxel (CP) reported 10 objective responses among 20 patients. A 45-mg dose has been chosen for subsequent phase II/III studies in NSCLC. This dose was reduced to 30 mg in a subsequent phase II-III study with carboplatin and paclitaxel being conducted by the Canadian NCI.

### Sorafenib

This agent has been approved for clear cell renal cell carcinoma, and it has promising activity in previously treated NSCLC. Phase III studies are under way, including a 900-patient trial of CP +/- sorafenib (400 mg bid) and a similar phase III trial with cisplatin + gemcitabine. This agent is also being evaluated as third-line or later therapy with randomization to continue or stop therapy after stable disease or objective response. Finally, it will be evaluated with chemoradiotherapy in locally advanced NSCLC.

### Sunitinib

This agent, like sorafenib, is approved for renal cell carcinoma and has activity in NSCLC (with objective responses in 7 of 63 advanced NSCLC patients [11%] objective responses and a 44% stable disease rate with dosage of 50 mg/d for 28 days every 6 weeks). The drug is being studied combined with various chemotherapy regimens or as maintenance therapy in both SCLC and NSCLC.

### ZD 6474 (Vandetanib)

This is a dual VEGF/epidermal growth factor receptor (EGFR) inhibitor. Data previously presented at The American Society of Clinical Oncology Meeting compared this agent with gefitinib and found that progression-free survival favored vandetanib 11 versus 8 weeks. ZD 6474 studied combined with CP in the first line setting as second-line therapy combined with docetaxel and in a separate study, with pemetrexed.

### BIBF 1120

This agent has demonstrated activity in early phase I studies in renal and colorectal cancer and is being studied alone and combined with CP in patients with advanced NSCLC.

Patients with controlled brain metastases and squamous histology are eligible for these studies.

### **VEGF Trap**

VEGF Trap is a fusion protein of key domains from VEGF receptors 1 and 2. Activity has been observed in heavily pretreated patients with NSCLC. This agent is synergistic with several cytolytic drugs and is being studied with Docetaxel in advanced NSCLC.

### **Thalidomide**

This older agent has antiangiogenic activity. An ECOG study evaluated thalidomide combined with chemoradiotherapy in locally advanced NSCLC. There is evidence of benefit in a study from France when used as maintenance therapy in SCLC. A larger study in SCLC randomly allocates patients to receive or not receive maintenance thalidomide after platinum + etoposide in SCLC. Newer agents with better anti-inflammatory and immunomodulatory qualities, such as pomalidomide, are also being studied in lung cancer. Phase III trials are in progress in both NSCLC and SCLC.

### **ABT 869**

This is a multitargeted TKI that has demonstrated activity in previously treated patients with NSCLC.

Other agents discussed in this session included the small molecule VEGFR TKIs AMG 706, pazopanib, AG -013736 (axitinib), and the anti-VEGFR not monoclonal antibody IMC 1121B.

## **EGFR Biomarkers and EGFR Selection – John Minna, David Gandara**

Bruce Johnson, MD, reported that the Cancer and Leukemia Group B (CALGB) is conducting an EGFR biomarker trial to determine the role of EGFR immunohistochemistry (IHC), Fluorescence in-situ hybridization (FISH), and mutation analysis in predicting efficacy of erlotinib treatment, including objective response time to disease progression and development of toxicity. CALGB 30406 randomly allocated patients with IIB/IV chemo-naïve lung adenocarcinoma to erlotinib or erlotinib + CP and adjusted for light smoking or never smoking status with mandatory tissue acquisition. IPASS (centers in East and Southeast Asia) are comparing gefitinib 250 mg/d followed at progression by CP versus CP alone in a randomized trial of patients with III, IIB/IV lung adenocarcinoma with no prior therapy with exploratory biomarker analysis. A prospective Spanish trial randomly allocates patients with untreated NSCLC III and IIB/IV with EGFR mutations to erlotinib 150 mg/d versus cisplatin + taxane or gemcitabine.

Fred Hirsch, MD, PhD, reported that high EGFR gene copy number assessed by FISH in several patient cohorts (SWOG/Italian, ISEL, BR21) predicted beneficial effects of erlotinib, gefitinib, and cetuximab. Using EGFR FISH and IHC, an analysis by TRIBUTE indicated a nonfavorable effect of EGFR TKIs given concomitantly with chemotherapy and EGFR FISH negative IHC negative (30% of patients) resulted in no benefit seen from EGFR TKI therapy. A trial of erlotinib as a single agent or intercalated with chemotherapy in chemo-naïve IIB/IV NSCLC that are EGFR IHC positive or FISH positive is ongoing by Bunn/Hirsch. RADIANT is a erlotinib adjuvant trial of patients with completely resected stage IB-III A EGFR+ NSCLC who then receive (optional) four cycles of standard platinum-based chemotherapy followed by random allocation to placebo or erlotinib treatment (150 mg/p.o. daily for 2 years). This trial will be chaired by Karen Kelly.

Ming-Sound Tsao, MD, presented an update on BR.21. There were 731 patients entered into the study, and 201 had successful EGFR mutation analysis (mutations were found in 24 patients [12%]). Responses to erlotinib therapy were 30% in the mutation-positive tumors and 7.6% in tumors without EGFR mutations ( $p < 0.05$ ). With more sensitive EGFR mutation techniques in 204 patients, mutations were found in 34 (17%) of patients and the response rate 26.7% in mutation-positive tumors and 6.9% in mutation-negative tumors ( $p = 0.035$ ). The survival benefit from erlotinib therapy was slightly but not significantly greater for patients with mutant tumors than for those with nonmutant tumors. An increased gene copy number of EGFR as assessed by FISH showed a greater differential benefit than the mutation status.

David Carbone, MD, reported that serum proteomic biomarkers predict a clinical benefit of EGFR TKIs in studies of pretreatment peripheral blood. These studies had independent analysis at Vanderbilt and the University of Colorado Cancer Center and involved studies of patient serum specimens from Japan and Italy. They were also validated in an ECOG cohort of advanced NSCLC with first-line erlotinib. They provide cross-institution validation and predict survival times from “good” and “poor” prognosis serum proteomic signatures. These lead into the NCI-sponsored Lung Cancer SPECs trial of erlotinib, which is evaluating protein and mRNA expression, and FISH and IHC tumor profiles and proteomics serum profiles.

Adi Gazdar, MD, reported that KRAS mutations are present in NSCLC but never in SCLC and are strongly associated with smoking. The mutational spectrum is limited mainly to G:T transversions in codons 12 and 13. Gender differences in prevalence exist. Independent factors associated with EGFR and KRAS mutations in lung cancer are ever smoking, male sex, ethnicity other than East Asian, poor response to EGFR TKIs, and a high DNA tumor suppressor gene methylation index. KRAS mutations target different subpopulations than EGFR and demonstrate mutual exclusion. KRAS mutation is a potent negative factor for survival indicating different pathways to lung adenocarcinoma in ever and never smokers resulting from activation of these genes.

## Epithelial to Mesenchymal Transition in NSCLC and Derivation of Rationale Targeted Therapy

John Haley, PhD (OSI Pharmaceuticals), presented data on the role of the epithelial to mesenchymal transition (EMT) in determination of sensitivity to EGFR inhibitors. Both erlotinib-sensitive and erlotinib-insensitive NSCLC express EGFR. Erlotinib-sensitive NSCLC also expresses ERBB3 and ECAD, and signaling through the EGFR pathway appears dependent on autocrine production of ligand. Erlotinib-sensitive NSCLC lines express epithelial markers Ecadherin (ECAD, gamma catenin), whereas erlotinib-insensitive NSCLC lines express mesenchymal markers (vimentin, fibronectin, Zeb-1). Epithelial cell EGFR dependence is linked to cell-cell junctions, ECAD expression, and cell polarity. ER3 transcription is frequently attenuated through EMT and acts as a connector of EGFR-Akt Her3 acts as a key mediator of Akt, and erlotinib is capable of inhibiting Akt activity only in sensitive cell lines and this is independent of inhibition of EGFR phosphorylation. In an analysis of TRIBUTE data, ECAD tumor-positive patients had a longer time to progression with erlotinib + chemotherapy than with chemotherapy alone. Lung cancer cells that have undergone EMT are a key source of cancer recurrence, as has been demonstrated in ERBB2 transgenic mouse models of cancer that show tumor regression when ERBB2 expression is removed but recurrence without ERBB2 expression accompanied by tumors with EMT. Thus, mesenchymal cells form a reservoir that promotes cancer recurrence, and EMT regulators are important targets for anticancer therapy. Proteomics analysis has identified signatures of epithelial and mesenchymal pathways in NSCLC lines, and a mesenchymal

phenotype lacking cell polarity defines a loss of cell sensitivity to EGFR TKIs. This has led to measuring EMT in patient samples by differential expression of ECAD and vimentin. Patients with low expression levels of ECAD by fluorescence IHC (N 216) tumors have low survival rates. These data support the use of EGFR in preclinical models, which works in tumor cells with a functional EGFR –ERB3-Akt signaling axis working in epithelial-phenotype tumor cells. The combination of EGFR TKI inhibitor and an mTor inhibitor is additive in the epithelial cell phenotype but synergistic in tumor cells with constitutive activation of Akt in the presence of inhibition of EGFR autophosphorylation. The key is to find new drug targets in NSCLC that are not responsive to EGFR inhibition, particularly if they target what is driving the mesenchymal state.

## Pan Erb and Irreversible Inhibitors

Bruce Johnson, MD, reported that the irreversible pan erbB receptor TKI HKI-272 has activity in Phase I trials in breast cancer. A randomized phase II trial of HKI-272 is being conducted in NSCLC with and without EGFR mutations that have progressed on gefitinib or erlotinib and have also been studied in preclinical lung cancer line models.

Nasser Hanna, MD, reported that the irreversible pan Erb inhibitor BIBW2292 provides dual EGFR and Her2 inhibition. This includes working in NSCLC with EGFR T790M mutations. A phase I monotherapy trial with 145 treated patients (2 partial responses and 56 stable disease) has been conducted along with a BIBW2992 + docetaxel trial with 44 patients (4 objective responses and 13 stable disease).

Tom Lynch, MD, reported that the pan HER TKI (PF-00299804, Pfizer) is a potent, irreversible inhibitor of (Her) ERB1, 2, 3, and 4. It is orally available as a small molecule. Many solid tumors are driven by several proteins in the HER pathways and this has improved pharmacology. The drug is in phase I trials (NKI, Colorado, UCLA, Dana Farber), and phase II trials are planned later in 2007. This drug is effective in HCC827 (EGFR-mutant) NSCLC cells that have been selected to have a T790M resistance mutation in xenograft models.

Heather Wakelee, MD, reported that XL647 inhibits multiple receptor tyrosine kinases (EGFR, HER2, VEGFR2, EPHB4) and has been studied in phase I trials (41 patients, 12 NSCLCs). Fourteen cases of stable disease and 1 partial response were seen, as were a variety of adverse events (particularly diarrhea, rash, nausea, and fatigue). A phase II trial with XL647 (350 mg/p.o qd 5/14 days) as first-line therapy in stage IIB or IV NSCLC is being conducted with patients who have EGFR-activating mutation or at least two of the following features: Asian ethnicity, female sex, minimal smoker status, and adenocarcinoma histology. Also there is an ongoing phase I trial of continuous dosing using plasma for analysis and eyebrow hair bulbs as surrogate tissue of analysis of activity of AKT and ERK by IHC.

## New Trial Designs for Targeted Therapy

J. Jack Lee, PhD, reported that adaptive randomization designs for targeted therapy, which incorporate biomarkers, are particularly suitable for early stage drug development. This was present in the context of the MDACC “BATTLE” Trial (*Biomarker-based Approaches of Targeted Therapy for Lung Cancer Elimination*). This statement is particularly true for identifying high-risk subjects, identifying subpopulations most likely to benefit from the treatment, selecting effective treatment and dropping ineffective treatment early, and thus providing a more ethical design for study. These new trial designs allow the correct conclusion to be reached sooner and allow flexibility in study conduct. The designs aid in

identifying important predictive biomarkers for treatment and provide a large tissue database for future biomarker discovery.

## **Predictive Tests for Chemotherapy Sensitivity – Ramaswamy Govindan, MD**

DNA repair is a critical process that enables cells to identify and repair damaged DNA molecules, thus protecting them from premature death or mutations. Cells exposed continuously to the onslaught of environmental and metabolic insults regularly sustain injury to DNA. Multiple cellular mechanisms have evolved over time to repair the broken, damaged DNA to avoid cell death and, more importantly, disease-causing mutations. It is estimated that approximately 130 genes and their products are involved in the DNA repair processes. It has been known for quite some time that defects in the DNA repair pathway lead to premature ageing, mental retardation, and heightened susceptibility to carcinogenesis—all resulting from the inability of cells to repair DNA damage efficiently.

The ability of cancer cells to survive DNA damage inflicted by platinum compounds depends to a great extent on the efficiency of the DNA repair process. Platinum compounds damage cells by binding to DNA and forming DNA-platinum adducts, resulting in activation of the DNA repair pathways. Cells, both normal and malignant, differ significantly in their ability to carry out this all-important repair process. A brief overview of the cellular response to DNA damage would help us understand how best to exploit the weakness in the system and identify those patients who would benefit from DNA-damaging agents such as platinum compounds.

### **DNA Damage Repair Pathway**

DNA damage alters the configuration of the double helix and is immediately detected by cells. Appropriate DNA repair enzymes are recruited depending on the type of DNA damage. Broadly speaking, there are four types of DNA damage.

1. Covalent modifications of the 4 bases in DNA (A, T, C, G).
2. Mismatches of normal bases because of errors in proofreading during DNA replication.
3. Crosslinks between bases.
4. Single-strand or double-strand breaks.

Damaged bases are repaired by direct chemical reversal or by excision repair. Chemical reversal is a rather inefficient process: multiple different proteins are required for correction of several possible types of chemical alteration of bases. In contrast, excision repair is a very efficient process and is able to repair a wide variety of damages to the bases with a limited set of enzymes. The three modes of excision repair are base excision repair, nucleotide excision repair (NER), and mismatch repair.

Base excision repair involves removal of the damaged base, removal of its deoxyribose phosphate, replacement with the correct nucleotide, and finally ligation of the breaks in the strand. Several genes are involved in this process. NER involves the removal of a nucleotide even if only one base is damaged. NER begins with recognition of the damaged site and follows with unwinding of the DNA using the enzyme system transcription factor IIIH and removal of the damaged portion, which is filled in with a freshly synthesized DNA fragment. DNA polymerases and DNA ligase play a crucial role in accomplishing this process. Xeroderma pigmentosum (XP) is a disease caused by mutation in one of several genes in the NER pathway (XPA, XPB, XPD, XPF and XPG).

The mismatch repair pathway involves correcting the mismatched bases of the normal bases. This process ensures normal base pairing and is accomplished by recruiting enzymes involved in base excision repair and NER and some unique enzymes. These unique enzymes recognize the mismatch (MSH2) and delete the mismatched bases (MLH1). Synthesis of the correct bases involves the use of enzymes in the NER pathway.

This brief overview will focus on two members of the DNA pathway and their ability to predict response to chemotherapeutic agents. Moreover, they may be of importance in predicting outcomes after surgery as well.

## **ERCC1 (Excision Repair Cross-Complementation Group 1)**

Platinum compounds bind covalently to genomic DNA, forming adducts. These adducts alter DNA, resulting in recruitment of the DNA repair pathway enzymes mentioned above. Cisplatin-induced DNA damage is repaired by the NER pathway. ERCC1 is one of 16 genes that are involved in the NER pathway. ERCC1 heterodimerizes with XPF, resulting in 5' excision of the DNA strand. ERCC1 is essential for life. In vitro studies indicate that ERCC1 is perhaps the most important enzyme in the NER pathway. Polymorphisms in the ERCC1 gene and the presence of multiple variants of alternative splicing modify the expression of ERCC1 protein.

## **Regulatory Subunit of Ribonucleotide Reductase (RRM1)**

The RRM1 gene, which codes for the regulatory subunit of ribonucleotide reductase, is located on 11p15.5. Loss of heterozygosity of this region has been well described for lung cancer. Ribonucleotide reductase plays a key role in the synthesis of deoxyribonucleotides from the corresponding ribonucleotides, thus providing the essential building blocks necessary for NER. Preclinical studies suggest that high RRM1 expression is associated with decreased cell proliferation, reduced invasiveness, and lower metastatic potential. Moreover, RRM1 is an important determinant of the efficacy of the nucleoside analog gemcitabine.

## **Predictive Utility of ERCC 1 or RRM1 Expression in Tumor Tissue**

### **Predictive Value of ERCC 1 for Response to Platinum-Based Therapy**

Several studies have correlated ERCC1 expression in NSCLC tumor tissue or ERCC1 germline polymorphisms with response to platinum-based combination chemotherapy. Tumors that are positive for ERCC1 survive platinum-induced cell damage because they have a more efficient DNA repair system than tumors that are negative for ERCC1. Therefore, patients with high ERCC1 expression in their tumors are less likely to benefit from platinum-based therapy than are patients whose tumors have high ERCC1 levels.

The International Adjuvant Lung Cancer Trial (IALT) study reported improved survival for patients with low tumor levels of ERCC 1 when treated with adjuvant chemotherapy. The IALT study randomly allocated 1867 patients with resected stage I-III NSCLC to cisplatin-based chemotherapy or observation. This study demonstrated a 4.1% absolute improvement in 5-year survival after chemotherapy compared with observation alone. Of 761 tumors available, 335 (44%) were positive for ERCC1 by immunohistochemical analysis. Cisplatin-based adjuvant chemotherapy improved survival in patients with ERCC1-negative tumors (adjusted hazards ratio for death 0.65; 95% confidence interval [CI], 0.50-0.86,  $p = 0.002$ ) compared with observation alone. No improvement in survival was observed with adjuvant platinum-based chemotherapy in the ERCC1-positive group (adjusted hazards ratio for death 1.14; 95% CI, 0.84-1.55;  $p = 0.40$ ). Several studies have reported correlation between improved survival with platinum-based chemotherapy and low tumor ERCC1 levels in patients with advanced NSCLC (Table 1). In a retrospective study of 54 patients with

limited-stage SCLC presented at this conference, low levels of ERCC1 were associated with better survival when treated with platinum-based chemoradiation.

### **Predictive Value of RRM1 for Response to Gemcitabine-Based Therapy**

In a small retrospective study of 61 patients with advanced NSCLC treated with cisplatin and gemcitabine, low RRM1 levels were associated with a trend toward improved median survival compared with high RRM1 mRNA levels (13.9 versus 10.9 months;  $p = 0.22$ ). Similar results were observed in the group with concomitant low expression levels of ERCC1 and RRM1. Although the results are intriguing, no definite conclusions could be drawn from this small retrospective study. Until recently, technical limitations precluded immunohistochemical evaluation of RRM1 protein in paraffin-embedded tissue specimens.

### **Prognostic Value of ERCC 1 or RRM1 Expression in Tumor Tissue**

#### **Prognostic Value of ERCC 1**

Among the group of patients who did not receive adjuvant chemotherapy in the IALT study, the survival was better for those with ERCC1-positive tumors than for those with ERCC1-negative tumors (adjusted hazards ratio for death: 0.66; 95% CI: 0.49-0.90;  $p = 0.009$ ).

#### **Prognostic Value of RRM1**

In a retrospective study of patients with resected stage I NSCLC, the median disease-free survival was significantly better in the group with high RRM1 protein expression (more than 10 years) than in those with low RRM1 expression (4.5 years; hazards ratio for disease progression or death, 0.46;  $p = 0.004$ ). It is conceivable that efficient DNA repair (associated with high RRM1 levels) could slow down or prevent molecular events related to progression even in already established tumors and thus contribute to better survival in patients with resected NSCLC. In fact, the combination of high RRM1 and high ERCC1 (a third of patients in one study) predicted better survival than did high expression of either protein alone in that study.

### **Prospective Study Tailoring Chemotherapy Based on ERCC1 and RRM1 Expression**

In a prospective study ("MADe IT") presented at this conference, the investigators from H.L. Moffitt Cancer Center assigned 53 patients with advanced NSCLC to platinum- or nonplatinum-based therapy on the basis of tumor ERCC1 expression: those with low ERCC1 received a platinum (carboplatin)-containing doublet and those with high ERCC1 received a nonplatinum-containing doublet. The choice of the second agent in the platinum-containing doublet group was based on tumor RRM1 expression. Patients with low RRM1 expression received carboplatin and gemcitabine, and those with high RRM1 expression received docetaxel and carboplatin. Similarly, patients assigned to nonplatinum-containing doublet received either gemcitabine and docetaxel (low RRM1 group) or docetaxel and vinorelbine (high RRM1 group). The overall 1-year progression-free survival was 18% (95% CI: 7-33%) and the 1-year overall survival was 62% (95% CI: 43-77%). The median overall survival was 13.4 months, which was better than the traditional 9 to 10 months commonly reported with platinum-based doublet therapy.

### **Future Directions**

The unfolding story of variation in the levels of key enzymes in DNA repair pathway in established malignancies has significant implications in the treatment of cancer. The prognostic and predictive ability of some of the key enzymes of DNA repair pathway would



impact not only the approach to lung cancer but also our approach to a wide variety of other cancer types. Moreover, the preliminary data indicate that expression levels of some of these key enzymes in resected tumors may identify patients who are less likely to relapse. Of course, further studies in larger samples are mandatory before these tests could be used in the clinic. These innovative studies have opened a new avenue for further research to examine more closely the DNA repair pathway in established cancer. Targeting chemotherapy to the right patient is no longer a dream but a reality.

## Novel Drugs – Jack West

Epothilones are a new class of anticancer drugs that bind to and stabilize microtubules in a manner similar but not identical to that of the taxanes, and several epothilones have been developed and are in clinical trials. BMS 247550 is an epothilone B analog that has been studied in two different schedules in phase II trials of previously treated patients with advanced NSCLC, as both a single q21 day dose of 32 mg/m<sup>2</sup> (*N* = 77) and a day 1-5 dose of 6 mg/m<sup>2</sup> q21 days (*N* = 69). Each of these regimens has demonstrated a response rate in the 10 to 15% range and a median survival of 7-8 months. Epothilone D, or KOS-862, has also been evaluated in a phase II trial of 100 mg/m<sup>2</sup> q21 days in previously treated patients with advanced NSCLC (*N* = 55), in which a single response was observed. Other epothilones are still in phase I studies. Despite hints of activity across a range of tumor types and challenging toxicities, epothilones have all demonstrated problematic myelotoxicity and neuropathy. They continue to have no clear role despite years of development, leaving questions about whether these agents will ever demonstrate an adequate therapeutic index for clinical use.

BMS-275183 is an oral taxane similar in structure to paclitaxel that has undergone phase I investigation in a range of solid tumors on a weekly schedule and then on a twice weekly schedule. These studies have demonstrated that the dose-limiting toxicities (DLTs) are neurotoxicity and, to a lesser extent, diarrhea and fatigue on a weekly schedule, while the twice weekly schedule had DLTs of neutropenia and diarrhea and comparably less neuropathy. With 14 responses among 73 patients between the 2 trials, including 7 partial responses among 26 patients with NSCLC and evaluable disease, this agent was felt to be quite encouraging, and the twice weekly schedule was selected for phase II study due to the lower incidence and severity of neurotoxicity. Nevertheless, in subsequent phase II work with BMS-275183 in patients with previously treated advanced NSCLC, excessive and unpredictable toxicity from febrile neutropenia was seen. The development of this agent is currently on hold.

In its IV form, the topoisomerase I inhibitor topotecan is an FDA-approved drug in the setting of recurrent SCLC, but oral topotecan has also been the subject of clinical trials in this setting. In an important phase III trial comparing oral topotecan 2.3 mg/m<sup>2</sup> days 1-5 of a 21-day cycle with best supportive care to best supportive care alone in the second-line setting for 141 SCLC patients, a significant survival benefit was observed (hazard ratio: 0.64). This oral regimen has also been compared in a phase III trial with IV topotecan at the approved dose and schedule of 1.5 mg/m<sup>2</sup> on days 1-5 of a 21-day schedule and was found to have equivalent efficacy in terms of response and survival in the second-line SCLC setting. Finally, oral topotecan combined with IV cisplatin has been compared in a phase III trial to standard cisplatin and etoposide, both administered every 3 weeks for up to 4 cycles or 2 cycles beyond best response in the setting of first-line extensive disease SCLC. This study, with nearly 800 enrolled patients, demonstrated a nearly identical median survival and overall efficacy. Oral topotecan is not yet commercially available but is awaiting FDA review.

Nanoparticle albumin bound (nab) paclitaxel, the novel formulation that does not require Cremaphor or steroid premedication, can be administered as a 20-minute infusion without the risk of solvent-based hypersensitivities. Approved by the FDA in 2005 for the treatment of recurrent or metastatic breast cancer, nab paclitaxel has also been studied in first-line treatment of advanced NSCLC. As a single agent administered at 260 mg/m<sup>2</sup> IV every 3 weeks in 43 patients, it has been shown to produce a response rate of 16%, 33% of patients with stable disease for 4 months or longer, a median progression-free survival and a median overall survival of 11.6 months, and toxicity limited to grade 4 or lower. It has also been studied at a weekly dose of 125 mg/m<sup>2</sup> weekly for 3 of 4 weeks, with a response rate of 30%, 23% with stable disease, and a median survival of 11 months among 40 previously untreated patients. It is the weekly approach that is being studied in combination with carboplatin, with a response rate of 50% and 36% demonstrating stable disease. Among the 50 enrolled patients, 44% had grade 3 or 4 neutropenia, but there was serious neuropathy. A phase III trial is planned in which a combination of weekly nab paclitaxel and q3week carboplatin will be compared with standard CP in 100 or more previously untreated patients with advanced NSCLC for patients who cannot receive bevacizumab due to ineligibility of unavailability.

Paclitaxel polyglumex (PPX) is another novel formulation of paclitaxel in which the taxane is bound to a biodegradable polymer using a polyglutamate drug delivery system, allowing administration without solvents over a recommended infusion time of 10 to 20 minutes and potentially allowing for improved delivery of the agent to the tumor target with relative sparing of normal tissues. PPX has already been studied in several phase III randomized trials in the performance status 2 (PS2) patient population. In the STELLAR 3 trial, the combination of carboplatin/PPX was compared with CP q3weeks, while the STELLAR 4 trial compared single-agent PPX to gemcitabine or vinorelbine as a single agent. Each of these first-line trials demonstrated no significant overall differences in survival or other efficacy end points between the two trials, but an exploratory analysis of the female patients in both of these trials revealed a markedly superior survival in recipients of PPX. Subsequent careful assessment for a potential explanation for this gender-based difference may find that the release of the paclitaxel molecule from the polymer backbone of PPX requires intracellular cleavage by lysosomal proteases, particularly cathepsin B, which is up-regulated by estrogen. On the basis of these results, a follow-up randomized phase III trial of women with PS2, known as PIONEER, was initiated to compare PPX with solvent-bound paclitaxel. This trial was discontinued recently. A follow-up trial is being initiated that will compare carboplatin + PPX versus CP in premenopausal women with PS 2.

## Novel Inhibitors of Mitosis

Aurora kinases are involved in the normal mitotic process, and mutations of these proteins are associated with disruption of cell division. Because these proteins are involved with the polar regions of the cell during mitosis, it was named Aurora after the aurora borealis, or northern lights. Aurora kinases are strongly overexpressed in more than 40 NSCLC tumors tested, and overexpression in lung tumors has been associated with worse survival. There are several aurora kinase inhibitors (all IV drugs,) that have been developed and are now in or about to enter clinical trials. Those latest in development include MK-0457 (Merck), AZD1152 (Astra-Zeneca), and PHA-739385 (Nerviano). Pololike kinase inhibitors are just entering clinical trials and appears to have neutropenia as the most frequent DLT. Thus far, early clinical research indicates that neutropenia is the DLT for all of these. Clinical development of several of these agents in lung cancer trials is ongoing.

Bortezomib is a proteasome inhibitor that stabilizes cell cycle regulatory proteins and can have a wide range of anticancer mechanisms, including antiangiogenic activity and

induction of apoptosis. Davies and colleagues presented the results of a phase II SWOG trial of the combination of bortezomib 1.0 mg/m<sup>2</sup> IV days 1, 4, 8, and 11 with carboplatin + gemcitabine on a q21 days schedule in the first-line treatment of NSCLC, which led to an 11-month survival and 1-year survival of 47%. Based on emerging work out of UC Davis suggesting that sequencing bortezomib after docetaxel can enhance the apoptosis of cancer cells, a subsequent randomized phase II trial will randomly allocate patients with advanced NSCLC to second-line docetaxel at 75 mg/m<sup>2</sup> IV along with bortezomib administered concurrently at 1.6 mg/m<sup>2</sup> on days 1 and 8, or sequentially on days 2 and 9, every 3 weeks. Another randomized phase II trial of erlotinib alone versus erlotinib + bortezomib on the days 1 and 8 q21 day scheduled has finished accrual and awaits further follow-up and presentation. Another phase II trial that has also completed accrual but has not yet been presented randomly allocated patients in the second-line setting to bortezomib, pemetrexed, or a combination of the two. Finally, bortezomib is also being studied in advanced NSCLC.

Pralatrexate (PDX), a novel antifolate, is a chemically modified derivative of methotrexate rationally designed to optimize intracellular concentrations and accumulation. Preclinical studies have demonstrated dramatic antitumor activity in lung and breast cancer xenograft models. A phase I study in patients with extensively pretreated advanced NSCLC (*N* = 33) evaluated a weekly as well as a q2 week dosing schedule and identified an optimal phase II dose and schedule of 150 mg/m<sup>2</sup> q 2weeks in the absence of B12 and folate supplementation. The DLT was stomatitis, and there was no significant myelosuppression. Two partial responses were seen among patients with NSCLC. Subsequent development of PDX with B12 and folate supplementation have allowed for dose escalation as a single agent to doses twice that possible without supplementation. PDX is being studied further as a single agent and in combination with docetaxel or paclitaxel. There is potential for development of phase III trials comparing PDX to one or more currently available second- and third-line treatment options in advanced NSCLC.

Vinflunine is a rationally designed microtubule inhibitor that has demonstrated antitumor activity against a wide range of human tumor xenografts and additivity or synergy with multiple currently used chemotherapeutic agents. Vinflunine is freely soluble in water, allowing for IV administration without Cremaphor or polysorbate 80 as solvents, no need for steroid premedication, and a short infusion time of 10-20 minutes. Clinical studies that have focused on a dose and schedule of 320 mg/m<sup>2</sup> IV q21 days have thus far demonstrated myelosuppression, fatigue, and constipation as the leading adverse effects. A phase II trial at this dose in the second-line setting for NSCLC demonstrated an 8% RR and a 50% SD rate among 60 patients, with a median duration of response of 5.8 months and a median OS of 7 months. These results have been considered promising enough to pursue a randomized phase III trial of vinflunine 320 mg/m<sup>2</sup> IV q21 days versus docetaxel 75 mg/m<sup>2</sup> IV q21days as second-line therapy, which accrued 551 patients before enrollment was closed in 2005. In addition, vinflunine is being studied in trials combining it with multiple agents in the first-line and second-line settings as well as concurrently with radiation.

## Novel Targets

John Minna, Giorgio V. Scagliotti

The growing knowledge about altered molecular mechanisms in cancer cells is driving the discovery of potential novel agents against new cellular targets which can act alone or in combination with cytotoxic chemotherapy or other targeted agents to ultimately improve cancer control. Several of these novel targets have implications for treating lung cancer stem cells or the niche they reside in (these include Notch pathway inhibitors, semaphorin replacement, Hedgehog pathway targets, and agents targeting telomerase). Other novel

agents target the metabolism of oncogene products, key signaling pathways for growth factors, and related pathways.

**Heat Shock Protein Inhibitors.** Heat shock protein-90 (Hsp-90) is a chaperone protein responsible for the proper folding and function of several “client” oncogenic proteins, including bcr-abl, c-kit, EGFR, Her-2, Flt-3, VEGFR/HIF-1 $\alpha$ , and p-Akt. Consequently, the inhibition of Hsp-90 represents an alternative possibility to inhibit in cancer cells the activity of these proteins. IPI-504 is a potent and selective inhibitor of Hsp-90. It is administered in a 30-minute IV infusion and is currently in phase I-II clinical testing. IPI-504 could represent a therapeutic alternative for those NSCLC cells harboring a secondary EGFR mutation (T790M mutation) but which are still dependent on Hsp-90 function.

**Notch Pathway Inhibitors.** Notch was discovered more than 80 years ago in *Drosophila* when haploid insufficiency resulted in “notches” at wing margins. In mammals, Notch is important for the proper development in many organs (lymphogenesis, neurogenesis, and hair/sensory development). Notch is also crucial for vascular development and in normal adults, Notch expression is reduced to vascular systems. High expression levels of Notch1-3, Jagged1, and Hes1 can be detected in resected lung cancers or lung cancer cell lines, and activated Notch3 in developing lung inhibits terminal differentiation and induces hyperplasia of immature pneumocytes. Notch3 cooperates with the EGF pathway in maintaining the malignant phenotype, while inhibition of Notch3 activation in vitro induces apoptosis and results in loss of malignant phenotype. Preliminary data indicate that inhibiting Notch by using the  $\gamma$ -secretase inhibitor MRK003 reduces tumor growth in vivo. The cross-talk between Notch3 and the EGF pathway also suggests that inhibiting Notch3 may enhance tumor sensitivity to an EGFR TKI.

**Semaphorins as Novel Therapeutics.** SEMA3F is one of 2 secreted semaphorins (SEMA3B and SEMA3F) residing in the 3p21.3 tumor suppressor gene region. Their expression is inactivated in most lung cancers and in other common human tumors, such as breast cancer. SEMA3F binds to neuropilin receptors and thus can antagonize the function of VEGF, which also binds to these receptors. SEMA3F expression is lost in lung cancer, and replacing SEMA3F results in reversal of much of the invasive malignant phenotype. Thus, SEMA3F and SEMA3B as soluble molecules (or small molecule mimics) are candidates for development as new targeted therapeutic agents to replace this function lost in lung cancer cells. Because of their role in counteracting VEGF, they may play a special role in VEGF-targeted therapy as well.

**Insulin-Like Growth Factor (IGF) Signaling Pathway Inhibitors.** Increased IGF levels and higher expression of IGF-1R have been found in cancer patients. IGF-1R is involved in cellular transformation and mediates tumor survival and growth. In addition, human tumors produce IGF-II as an autocrine growth factor. BIIB22 is a high-affinity, fully human IgG4 nonglycosylated antibody that blocks the interaction of IGF-I and IGF-II with IGF-1R, inhibits downstream signaling (pIGF-1R, pAkt, pErk), and down-regulates IGF-1R cell surface expression. BIIB22 inhibits IGF-I and IGF-II-mediated growth of tumor cell lines and in vivo tumor growth. IMCA12 is a fully human IgG1 monoclonal antibody with high-affinity binding to human IGF-1R and no affinity for the human insulin receptor. It inhibits IGF-I and IGF-II binding to IGF-1R, inhibits IGF-1R phosphorylation, and promotes IGF-1R degradation. Antitumor activity has been shown in xenograft and orthotopic models when administered alone, in combination with chemotherapeutic agents, and in combination with cetuximab. In phase I studies it has been administered in weekly, every 2 weeks, and every 3 weeks schedules. CP-751,871 is a fully human IgG2 monoclonal antibody against IGF-1R and, similar to IMCA12, does not bind to the insulin receptor and induces IGF-1R internalization and degradation. Currently, no DLT has been identified for IGF alone or with

chemotherapy when given up to 10 mg/kg. An ongoing phase II randomized study (2:1 randomization) of CP +/- CP 751,871 has response rate as a primary end point.

**Hedgehog Pathway Inhibitors.** Like the Notch and Wnt pathways, the Hedgehog signaling pathway is important in embryonic development, including lung development. The Hedgehog pathway is especially important in neuroendocrine lung development and in SCLC. These tumors depend on ligand dependent activation. Cyclopamine, an antagonist of one component of the Hedgehog pathway, smoothened, has preclinical activity against SCLC. Other smoothened antagonists can be developed. Likewise, antibodies against the ligand Hedgehog are candidate novel therapeutics directly analogous to monoclonal antibodies directed against VEGF, which are known to be successful in the treatment of lung cancer. The Hedgehog pathway will probably be important in the maintenance of lung cancer stem cells. Thus, therapy directed against this pathway will likely target tumor stem cells.

**Telomerase Inhibitors.** Telomerase activity is activated in perhaps all lung cancers of all histologic types. This enzyme and its RNA cofactor are absolutely required for the immortal growth of tumor cells and the maintenance of telomere ends of chromosomes. While normal stem cells in the body also express telomerase, preclinical studies have shown that there is a large therapeutic window for telomerase to be inhibited in tumor cells without harming the organism as a whole. The telomerase RNA component inhibitor GRN153L has been developed and is effective in telomerase inhibition. Extensive preclinical studies have demonstrated its significant activity against lung cancer xenografts. It has entered phase I clinical trials, and phase II trials in lung cancer are being designed. The key for its development is to integrate it with standard chemotherapy. Preclinical lung cancer xenograft models show that this is possible.

**Src Inhibitors.** C-src is a cytoplasmic nonreceptor tyrosine kinase family that links signaling from growth factor, integrin, and cytokine receptors on the surface of cells to their downstream effector signaling cascades, such as PI3K/PTEN/Akt, Stats, Ras/Raf/ERK, and focal adhesion kinase. Regulation of these key pathways allows SRC to control cellular growth and proliferation, survival, invasion and metastasis, and angiogenesis. In lung cancer, c-Src can cooperate with EGFR and src activity can be required for transformation by EGFR. Currently C-Src TKIs entering in clinical programs include dasatinib (which inhibits also abl, EPHA2, PDGF-R and c-kit), AZD-0530, and SKI-606. The effect of dasatinib on NSCLC cells is cell line dependent and includes cell cycle arrest, apoptosis, and reduced cellular invasion. In cell lines EGFR status or dependency is predictive: EGFR mutant cells undergo apoptosis, while EGFR wild-type cells are dependent on EGFR for proliferation undergo G1 arrest. The association of dasatinib and erlotinib results in synergistic inhibition of cellular proliferation in cell lines responsive to EGFR TKI. In Phase I studies with dasatinib, main toxicities included gastrointestinal toxicity, fatigue, and rash.

A phase I/II study testing the safety and tolerability of erlotinib + dasatinib in previously treated NSCLC in patients with good performance status and without previous exposure to EGFR TKI is already planned. AZD 0530 has been tested in a phase I study in solid tumors with doses ranging from 60 to 250 mg/d p.o. for 14 days. DLTs included febrile neutropenia and fatigue, and the maximum tolerated dose was 175 mg/d. Phase II studies have been planned or initiated for NSCLC, prostate cancer, melanoma, colorectal cancer, and pancreatic cancer.

## Novel Targets 2

Steven Dubinett

Histone deacetylase inhibitors increase histone acetylation, resulting in DNA with a more open chromatin, thus favoring transcription. HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) have been found to inhibit proliferation of NSCLC cells in preclinical studies. Thus, reversible histone acetylation regulates gene expression, and epigenetic modification has become an important target for anticancer therapy. Previous preclinical studies provided rationales for combining HDAC inhibitors with CP. These include the finding that SAHA can enhance the antitumor efficacy of platinum compounds. The rationale for combination with the taxanes includes the fact that HDAC6 interacts with beta-tubulin and alters microtubule dynamics; thus, inhibition of HDAC6 leads to mitotic arrest. SAHA has also been shown to lower the apoptotic threshold to other agents by causing inhibition of Akt phosphorylation. Dr. Chandra Belani presented new findings regarding a phase I study with the HDAC inhibitor vorinostat (SAHA) in combination with CP for patients with advanced solid malignancies. Of 19 patients with advanced NSCLC in this trial there were 10 PR and 4 patients with SD. On the basis of this study, SAHA 400 mg/d for 2 out of 3 weeks in combination with full doses of CP was the recommended phase II dose. On the basis of these results a randomized phase II study will enroll 80 stage IIIb/IV patients with PS 0/1 and no prior chemotherapy. The primary end point will be the response rate. The discussion period also included mention of additional ongoing studies in which SAHA is being combined with erlotinib. Here the rationale includes the HDAC inhibitor-mediated up-regulation of tumor E-cadherin expression, which may enhance the efficacy of the EGFR TKI.

In related studies, Dr. Charles Rudin described a combination therapy targeting epigenetic silencing by using therapy with both HDAC inhibitors and demethylating agents. The combination therapy is intended to target multiple epigenetic levels by concurrent targeting of promoter hypermethylation (using 5-azacytidine, a DNMT inhibitor) and histone deacetylation (with MS-275, an HDAC inhibitor). The overall goal of this approach is to induce a durable reversal of gene silencing.

The mammalian target of rapamycin (mTOR) has emerged as an important therapeutic target for cancer. Rapamycin and its derivatives that specifically inhibit mTOR are undergoing evaluation in clinical trials. Preclinical studies indicate the mTOR inhibitors are active in lung cancer models, and increased efficacy has been demonstrated for the combination of mTOR inhibitors with chemotherapy or EGFR inhibitors. Early reports have indicated clinical responses to monotherapy with mTOR inhibitors. Dr. Fadlo Khuri reviewed the rationale for the use of rapamycin in combination therapies. He discussed the trial conducted by Vince Miller in which the mTOR inhibitor RAD001 was used with gefitinib. Preliminary results in the phase I trial indicated 2 PR out of 10 patients treated. These patients with PR were male, were smokers, and had no EGFR mutations.

The rationale for combination therapies with arachidonic acid (AA) pathway inhibitors was discussed in two presentations. The first presentation encompassed the work of Dr. Martin Edelman, who found that COX-2 expression is a positive predictive factor for celecoxib plus chemotherapy in advanced NSCLC (CALGB 30203). The study was based on the hypothesis that COX-2 or 5-LOX inhibition will enhance the efficacy of platinum-based chemotherapy in advanced NSCLC. The investigators sought to inhibit both pathways because AA metabolites have been shown to regulate angiogenesis, apoptosis resistance, and invasion and metastasis. Patients with IIIb (pleural effusion) or IV NSCLC PS0-2 received (1) carboplatin (C) AUC = 5.5, gemcitabine (G) 1000 mg/m<sup>2</sup> and zileutin (Z) 600 mg po qid; (2) C + G + celecoxib 400 mg po bid; or (3) C + G + Z + celecoxib. End points included PFS (9 months) and OS. The study failed to achieve its predefined goal of a 9-month PFS >50%. In correlative studies, IHC indicated that COX-2 is a negative prognostic factor for survival but a positive predictive factor for survival if patients received celecoxib.

Multivariate analysis confirmed the interaction of COX-2 expression and response to celecoxib. A phase III randomized trial testing the hypothesis that COX-2 inhibition is beneficial in patients with COX-2-overexpressing tumors is under discussion.

In a second presentation regarding combination therapy targeting the AA pathway, Dr. Karen Reckamp described a phase I trial combining erlotinib with celecoxib. Overexpression of COX-2 activates extracellular signal-regulated kinase/mitogen-activated protein kinase signaling in an EGFR TKI-resistant manner. Overexpression of tumor COX-2 also suppresses E-cadherin expression in a prostaglandin E<sub>2</sub>-dependent manner. This is important because low E-cadherin expression has been associated with resistance to EGFR TKI. Because preclinical data indicated that tumor COX-2 expression caused resistance to EGFR TKI, a phase I trial to establish the optimal biologic dose (OBD), defined as the maximal decrease in urinary prostaglandin E-M (PGE-M), and the toxicity profile of the combination of celecoxib and erlotinib in advanced NSCLC was performed. Twenty-two subjects with stage IIIB/IV NSCLC received increasing doses of celecoxib from 200 to 800 mg twice daily (bid) and a fixed dose of erlotinib. Primary end points included evaluation of toxicity and determination of the OBD of celecoxib combined with erlotinib. Secondary end points investigated exploratory biologic markers and clinical response. Twenty-two subjects were enrolled, and 21 were evaluable for the determination of OBD, toxicity, and response. Rash and skin-related effects were the most commonly reported toxicities and occurred in 86% of patients. There were no DLTs and no cardiovascular toxicities related to study treatment. All subjects were evaluated on intent to treat. Seven patients showed partial responses (33%), and five patients developed stable disease (24%). Responses were seen in patients both with and without EGFR-activating mutations. A significant decline in urinary PGE-M was shown after 8 weeks of treatment, and the OBD of celecoxib of 600 mg bid. This study defines the OBD of celecoxib with a fixed dose of EGFR TKI. These results show objective responses with an acceptable toxicity profile. Future trials using COX-2 inhibition strategies should use the OBD of celecoxib at 600 mg bid. A randomized phase II trial in patients with advanced NSCLC comparing erlotinib plus celecoxib to erlotinib plus placebo will begin this year.

## Combinations of Radiation and Targeted Agents

Laurie Gaspar, Hak Choy

There are emerging data regarding the combination of targeted agents and radiation therapy. Studies have been performed, or are ongoing, of the combination of radiation with EGFR inhibitors, antiangiogenic agents, and multitargeted agents such as pemetrexed and ZD6474.

Interest in EGFR inhibitors in combination with radiation therapy has increased following the positive results of a randomized phase III study of cetuximab and radiation versus radiation alone in head and neck cancer (Bonner JA, et al. *NEJM* 2006). Patients randomly allocated to receive cetuximab, a monoclonal antibody to the EGFR receptor, received a loading dose in week 1 and then a weekly dose through radiation. There was no chemotherapy in this study. The median survival was 54 months for patients who received cetuximab and 28 months in the control arm ( $p = 0.02$ ).

RTOG 0324 was a phase II study of cetuximab with chemoradiation in patients with stage III NSCLC. A week after a loading dose of cetuximab, patients went on to receive concurrent radiation with weekly cetuximab, paclitaxel, and carboplatin. After completion of concurrent chemoradiation, patients received 6 more weeks of weekly cetuximab with 2 cycles of CP given on a 3 weekly schedule. The study closed in May 2005 having accrued 93 patients. Treatment was reasonably tolerated: 9% of patients experiencing grade 4 or 5 toxicity, and 3 patients developed grade 4 or 5 pneumonitis. Preliminary survival data

indicate that the 1-year survival rate is 68%. RTOG is now planning a phase III study of chemoradiation alone versus chemoradiation and concurrent cetuximab.

Other studies have looked at EGFR inhibition as maintenance therapy following standard chemoradiation for locally advanced NSCLC. SWOG 0023 was a randomized phase III study for patients with unresectable stage III NSCLC. Following registration, all patients received concurrent chemoradiation (cisplatin/vincristine/61 Gy) and then 3 cycles of docetaxel as consolidation. Patients were then randomly allocated to maintenance therapy with gefitinib (an EGFR TKI) or placebo. Patients were stratified by stage IIIA versus IIIB, measurable versus nonmeasurable disease, and squamous versus nonsquamous histology. This study was closed early due to an interim analysis that showed that the gefitinib arm could not have a median survival superior to that of the placebo arm. The median survival was 19 months for all patients entered on the study. There was no statistically significant difference in the incidence of grade 3 or higher pneumonitis observed between the two treatment arms.

There is also considerable interest in combining anti-angiogenic agents with standard chemoradiation for stage III NSCLC. Preclinical work has demonstrated increasing tumor cell apoptosis with increasing doses of antiangiogenic agents given with radiation therapy. SWOG 0533 is an ongoing phase I trial integrating bevacizumab, an antiangiogenic agent, into the treatment of locally advanced stage III NSCLC. All patients receive concurrent cisplatin, etoposide, and 64.8 Gy thoracic radiation therapy followed by 3 cycles of consolidation docetaxel. With each new patient cohort, the bevacizumab is moved earlier and earlier into the treatment regimen. Initially bevacizumab is only given to patients after completion of concurrent chemoradiation. Following successful enrollment to this cohort, bevacizumab will begin day 15 of chemoradiation. The final cohort will have bevacizumab starting day 1 of chemoradiation. Patients are placed into high- and low-risk categories. High risk is defined as squamous cell histology or tumor with cavitation or proximity to a major vessel, or a history of hemoptysis greater than 1/2 tsp within 28 days of registration. The study looks at these 2 risk groups separately. SWOG 0533 has an extensive correlative component looking at serum and circulating endothelial cell and tumor markers associated with hypoxia and angiogenesis.

Paracrine versus Perfusion Effects of Angiogenesis. Drugs such as ZD6474 that have both antiepidermal growth factor and antiangiogenic properties have been combined in animal models and have shown to have more activity than radiation alone or radiation plus paclitaxel.

Pemetrexed is a multitargeted antimetabolite that has shown activity in mesothelioma. Several studies are evaluating pemetrexed in NSCLC. Preclinical work has demonstrated that it has additive or supra-additive effects when combined with radiation therapy. A phase I study of escalating doses of pemetrexed and carboplatin have shown that the 2 drugs can be given in full dose (pemetrexed 500 mg/m<sup>2</sup> and carboplatin AUC 5 on a q 3 week schedule) with concurrent thoracic radiation with a reasonable toxicity profile. There did not appear to be any major enhancement of radiation pneumonitis or esophagitis. CALGB 30407 is a randomized phase II study designed to test this combination of pemetrexed and carboplatin with 70-Gy concurrent thoracic radiation in the phase II setting. Patients receive either pemetrexed or carboplatin with thoracic radiation or the same regimen with the addition of cetuximab. The regimen will be considered of further interest if the median survival is 20.9 months or more. Preliminary toxicity data has shown an increase in grade 3-4 thrombocytopenia and neutropenia in the cetuximab arm but no increase in esophagitis or pneumonitis.



Another industry-sponsored phase I study is combining pemetrexed with escalating doses of cisplatin, given concurrently with 74-Gy thoracic radiation. This will then be compared in a randomized phase II design with the same regimen but substituting carboplatin for cisplatin. Both arms receive 3 cycles pemetrexed. Numerous other phase I/II studies are ongoing to look at pemetrexed in the combined modality setting.

## Apoptosis

D. Ross Camidge, Roy S. Herbst

Two main molecular pathways lead to effector caspase activation and programmed cell death (apoptosis). The intrinsic pathway depends on the release of mitochondrial-derived cytochrome C. The extrinsic pathway depends on the activation of cell-surface death receptors (Fas, TNF-receptor, or TRAIL [TNF-related apoptosis inducing ligand] receptors). Cytotoxic chemotherapy and radiotherapy are known to trigger cell death predominantly through p53-dependent induction of the intrinsic apoptotic pathway, although considerable cross-talk between the pathways exists. The down-regulation of apoptosis in many cancer cells, through a variety of different means, may underlie their resistance to standard treatments, thus offering several targets for potential therapeutic intervention.

**TRAIL Agonists (Ligand).** TRAIL, or Apo2L, is a naturally occurring 281-amino-acid secreted protein that binds to both TRAIL-R1 and TRAIL-R2 (also called death receptors 4 and 5 [DR4 and DR5], respectively) and triggers apoptosis through the extrinsic pathway. Recombinant Apo2L, which is a soluble zinc-coordinated homotrimer, has been codeveloped by Genentech and Amgen and preclinically appears to selectively induce apoptosis in cancer cells while sparing the majority of normal cells (with the notable exception of hepatocytes). Surface expression of 3 other “decoy” receptors (TRAIL-R3, TRAIL-R4, and TRAIL-R5 [Osteoprotegerin]), which also bind TRAIL, may be one protection mechanism used by the normal cells. Preclinically, the greatest effects in Colo205 colon cancer xenografts were seen when Apo2L was combined with a traditional cytotoxic in the form of CPT-11. A phase I all-comers dose-escalation study of Apo2L given IV daily on D1-5 on a 21 day schedule to patients with and without liver metastases is ongoing. Apo2L was well-tolerated to up to 15 mg/kg/d. The plasma half-life was approximately 2 to 3 hours. The linear pharmacokinetics over the dose range was tested, and there was no suggestion that the presence of hepatic metastases or mild hepatic dysfunction affected exposures. One confirmed partial response, in a patient with metastatic synovial chondrosarcoma, has been noted to date.

**TRAIL Agonists (Monoclonal Antibodies).** Although Apo2L targets both TRAIL-R1 and TRAIL-R2, its relatively short plasma half-life, leading to an intensive regimen of administration, and novel manufacture have helped to drive the exploration of other, better-established drug formats for targeting death receptors. Several companies have developed agonistic monoclonal antibodies (mAb) directed against TRAIL-R1 or TRAIL-R2. Mapatumumab (HGS-ETR1) is a fully human agonistic mAb directed against TRAIL-R1. It has single-agent activity in H460 NSCLC xenograft models, and there is evidence of synergy in combination with both carboplatin and docetaxel. Two separate phase I all-comers single-agent dose-escalation studies have been completed. HGS-ETR1 was well-tolerated up to 20 mg/kg administered on day 1 of a 28 day schedule or 10 mg/kg on a 14 day schedule. The plasma half-life was 16-17 days, which is consistent with other mAbs, and no human antihuman antibody responses were detected. The best response in the single-agent phase I studies was stable disease. Three separate single-agent phase II studies have been completed at 10 mg/kg every 14 or 21 days (NSCLC, colorectal cancer, and NHL). No responses were seen in either the NSCLC or colorectal cancer studies, but one complete

response and two partial responses were noted in the 15 patients with follicular lymphoma in the NHL study. Phase Ib combination studies with full-dose CP and with full-dose gemcitabine and cisplatin in patients in whom these drugs would be considered “appropriate treatment” are complete or near completion. No evidence of PK interaction has been noted. Responses to date in the NSCLC patients have been in the expected range for the use of cytotoxic agents alone. Although these TRAIL agonist approaches appear well-tolerated and there are early hints of activity in certain rare tumors, the next challenge is to determine what these tumors have in common to facilitate preidentification of those most likely to respond to death receptor stimulation alone or in combination with standard therapies.

**Survivin Antisense.** Survivin is an intracellular protein that acts as a negative regulator of effector caspase activity and has an as yet incompletely understood role in the physiology of many normal cells. By both immunohistochemical and transcriptional analysis, survivin is overexpressed in a wide range of malignancies but appears absent from adjacent normal tissues. Its expression in tumors correlates with poorer prognosis, increased recurrence rates, and increased resistance to therapy in NSCLC and head and neck cancer. LY2181308 is an 18-mer antisense oligonucleotide that is nuclease resistant and highly potent preclinically in down-regulating surviving expression. It shows single-agent pre-clinical activity against xenografts. A single-agent all-comers phase I study of IV LY2181308 is ongoing. Toxicities to date include transient clotting abnormalities, reversible complement activation, and mild flu-like symptoms during the infusion. No activity signals have yet been detected, but phase II studies are planned. There is interest from preclinical data in combinations of LY2181308 with radiotherapy in NSCLC.

**Peroxisome Proliferator-Activated Receptor- $\gamma$  (PPAR- $\gamma$ ) Agonists.** PPAR- $\gamma$  is a nuclear receptor that dimerizes with the retinoid X receptor to influence transcription that promotes differentiation (E-cadherin expression) and inhibit the growth of NSCLC cell lines and xenografts. PPAR- $\gamma$  is intimately associated with the Wnt7-a/Fzd9 pathway. Wnt7-a expression is lost in some lung adenocarcinomas, and preclinically, restoration of Wnt7-a expression reduces the soft-agar growth of cell lines lacking Wnt7-a. Nonsteroidal anti-inflammatory drugs that reduce the development of new tumors in experimental models of lung tumorigenesis act through PGI<sub>2</sub>-mediated PPAR- $\gamma$  stimulation. This stimulation, which is reproducible via the PGI<sub>2</sub> agonist Iloprost, requires Fzd-9 expression. Randomized chemo-prevention studies with Iloprost or placebo in smokers with established bronchial dysplasia are ongoing.

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TABLE 1

ERCC1 Expression-Predicting Response to Chemotherapy

Author	N	Stage	Type of Chemotherapy	ERCC1 Level	Median Survival	p	Response CR&PR	p
Olaussen et al.	389	I-III	Cis-Vinorelbine	Low High	56 (mo) 50 (mo)	0.34	NA	NA
Lord et al.	56	IIIB-IV	Cis-Gencitabine	Low High	62 (wk) 20 (wk)	0.009	52% 36.4%	0.38
Ceppi et al.	61	III-IV	Cis-Gencitabine	Low High	17.3 (mo) 10.9 (mo)	0.0032	NA	NA
Rosell et al.	160	IV	Cis-Gencitabine	Low High	18.3 (mo) NR		56.6% 37.7%	0.02