important genes, among them E-cadherin, playing an important role for sensitivity of EGFR TKIs.[11]

We conclude that while both increased EGFR gene copy number, EGFR gene mutations and over expression of EGFR protein all associate with high response rates in NSCLC patients after EGFR TKI therapy, only increased EGFR gene copy number by FISH and EGFR protein expression seems to be independent predictive factors for sensitivity to EGFR inhibitors. Patients without increased EGFR gene copy number and lack of EGFR protein over expression do most likely not have any clinical benefit from these treatments.

REFERENCES

1. Hirsch FR, Scagliotti GV, Langer CJ, Varella-Garcia M, Franklin WA. Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies in lung cancer S29–S42; 2003.
2. Merrick D, Kittelson J, Wintherhalder R, *et al*. Analysis of c-erB1 (EGFR) and c-erB2 (HER2) expression in bronchial dysplasia: evaluation of potential targets for chemoprevention of lung cancer. Clin Cancer Res [in press].
3. Gandara, D. R., West, H., Chansky, K., et al. Bronchioloalveolar carcinoma: a model for investigating the biology of epidermal growth factor inhibition. *Clin Cancer Res, 10*, 4205–4209.
4. Hirsch, F. R., Varella-Garcia, M., Bunn, P. A., et al. Epidermal growth factor receptor in non-small cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol, 21*, 3798–3807.
5. Hirsch, F. R., Varella-Garcia, M., Franklin, W. A., et al. Evaluation of HER2/neu gene amplification and protein expression in non-small cell lung cancer. *Br J Cancer, 86*, 1449–1456.
6. Cappuzzo, F., Hirsch, F. R., Rossi, E., et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. *J Natl Cancer Inst, 97*, 643–655.
7. Hirsch, F. R., McCoy, J., West, H., et al. Increased EGFR gene copy number detected by FISH associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes. *J Clin Oncol, 23*, 6838–6845.
8. Tsao, M.-S., Sakurada, A., Cutz, J.-C., et al. Erlotinib in lung cancer-molecular and clinical predictors of outcome. *N Engl J Med, 353*, 133–144.
9. Hirsch FR, Varella-Garcia M, Bunn PA, *et al*. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small cell lung cancer. J Clin Oncol [in press].
10. Hirsch FR, McCoy J, Cappuzzo F, *et al*. FISH and immunohistochemistry can be used to select NSCLC patients, who will benefit from gefitinib treatment. In: Proceedings of 11th world conference on lung cancer, Barcelona, 2005. Lung Cancer **49**(Suppl. 2): S38; 2005.
11. Witta, S. E., Gemmil, R. M., Hirsch, F. R., et al. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res, 66*(2), 944–950.

doi:10.1016/j.ejcsup.2006.04.029

S29. IMPACT OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MUTATIONS ON RESPONSIVENESS OF NON-SMALL CELL LUNG CANCER (NSCLC) TO TYROSINE KINASE INHIBITORS (TKIS): PROSPECTIVE OBSERVATIONS

Nico van Zandwijk, Alexandre Mathy, Daphne de Jong, Paul Baas, Sjaak Burgers, Petra Nederlof. *Departments of Thoracic Oncology and Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*.

Two years have elapsed since mutations of the tyrosine kinase domain of the EGFR were discovered in patients with NSCLC, who had dramatic clinical responses to treatment with gefitinib.[1–3] Additional laboratory studies have provided further insights into the biological impact of EGFR mutations and several clinical observations have in retrospect confirmed the association between mutations and response to the TKIs gefinitib and erlotinib.[4] At the same time there is the suggestion that K-ras mutations predict unresponsiveness to these TKIs and the fact that K-ras mutations and EGFR mutations seem to be mutually exclusive fits well in this concept.[5] To validate the use of mutation status for therapeutic decisions, we have conducted a study in which the mutation status was determined prospectively, i.e. before the start of treatment with TKIs.

Between June 2004 and December 2005 patients with locally advanced or metastatic NSCLC were asked for their consent to analyze diagnostic specimens for EGFR mutations, if they had two out of three of the following characteristics: female gender, non-smoking status and the diagnosis of BAC or adenocarcinoma. Patients with a mutation in the EGFR TK domain were offered treatment with erlotinib or gefitinib within the framework of the compassionate use programs for these agents.

Baseline assessment included medical history (including prior anticancer therapy), smoking history, physical examination and vital signs, PS, complete blood cell count and blood biochemistry, chest X-ray and tumor assessment (X-rays or computed tomography scans). At follow-up (every 4–6 weeks), interval history, chest X-ray, tumor assessment, complete blood count and biochemistry were collected. Response was determined using the RECIST criteria.

We determined EGFR and K-Ras mutations by isolating DNA from formalin-fixed paraffin embedded tumor biopsies. For EGFR mutation analysis, exons 18–21 were PCR amplified using exon specific primers. Since the samples were often small (biopsies) and formalin fixed, primers were designed located in the flanking introns, such that the size of the PCR fragments was reduced compared to most published primers. K-ras mutation analysis of codon 12 was performed as above.

Forty-one patients were selected for the assessment of mutation status. Thirteen (out of 41 = 32%) biopsies were found to contain an EGFR mutation. None of them had a K-ras mutation. The median age of the mutation positive patients was 55, three of them were ex-smokers and 10 were never smokers. Six of them were male. All but four patients were chemonaive. Nine out of the thirteen patients with mutations in the EGFR gene had an in-frame deletion. In seven cases this was a 15 bp deletion in exon 19. The remaining four patients had a point mutation, in three cases located in exon 21 and in one case there were 2 point mutations in exon 18.

All patients with an exon 19 deletion had a swift response on erlotinib or gefitinib. So did three out of four patients presenting

with a point mutation. Two patients with point mutations eventually experienced progression of their disease. Tumor progression was not observed in the patients with exon 19 deletions. The mean progression free interval was 430 days (CI [294,567]) for the whole series.

***Conclusions*:** The observations add support to the importance of EGFR status as predictors of response to TKIs. Exon 19 deletions seem to predict the best responses to TKIs.

doi:10.1016/j.ejcsup.2006.04.030

S30. ANTI-TUMOR APPLICATIONS OF ACTIVATING TOLL-LIKE RECEPTOR 9 WITH PF-3512676 (FORMERLY CPG 7909)

Arthur M. Krieg. *Coley Pharmaceutical Group.*

Unmethylated CpG dinucleotides are relatively common in viral and bacterial DNA, but are rare in vertebrate DNA. Toll-like receptor 9 (TLR9) detects these "CpG motifs" as a sign of infection, and can be activated for therapeutic purposes by CpG motifs in synthetic oligodeoxynucleotides (CpG ODN), such as PF-3512676 (formerly called CPG 7909, or CpG 2006). PF-3512676 engages TLR9 in B cells and plasmacytoid dendritic cells (pDC), thereby stimulating innate and adaptive immunity, including antigen-specific Th1-like T cell responses. Murine studies showed anti-tumor activity of PF-3512676 as a monotherapy for relatively small tumors. Tumor regression was associated with the induction of a tumor-specific CTL response. PF-3512676 has been administered at various dose levels to more than 1000 humans, and has shown activity as a monotherapy in phase I human clinical trials when administered by intratumoral injection in basal cell carcinoma, by subcutaneous injection in cutaneous T cell lymphoma (CTCL), melanoma, and renal cell carcinoma, and by intravenous injection in non-Hodgkin's lymphoma.

Although the demonstrated activity as a monotherapy provides some proof-of-concept for the use of PF-3512676 in cancer therapy, we considered that combination approaches may provide greater efficacy. We theorized that disruption of the tumor using conventional anti-tumor therapies may reduce the tumors' resistance to immune mediated attack induced through TLR9 activation with PF-3512676. In murine models regression of larger tumors could be induced when PF-3512676 was used in combination with other therapies, including radiotherapy, surgical resection, monoclonal anti-tumor antibodies, and chemotherapy. The combination of PF-3512676 or other CpG ODN with certain chemotherapy regimens, including paclitaxel or gemcitabine, increased the generation of tumor antigen-specific CTL and/or improved tumor regression and survival in metastatic tumor models. These studies also demonstrated the involvement of *T* cells in the synergy between CpG and paclitaxel, consistent with the hypothesis that this combination induces enhanced tumor specific adaptive immune responses. These encouraging results in mouse models have been extended into human therapy in a controlled Phase II trial, where 112 patients with locally advanced or metastatic non-small cell lung cancer were randomized to receive either chemotherapy alone, or in combination with PF-3512676. The combination with chemotherapy provided a statistically significant improvement in objective response rate, and a trend to prolonged survival (1 year survival 33% vs. 50%, $P = 0.08$). The safety and tolerability of these TLR9 agonists has generally been good, with the major adverse events being transient injection site reactions and flu-like symptoms. Phase III trials of PF-3512676 in combination with 2 doublet chemotherapy regimens (paclitaxel plus carboplatin or gemcitabine plus cisplatin) for first-line therapy of locally advanced or metastatic NSCLC in 1600 patients were initiated in late 2005.

doi:10.1016/j.ejcsup.2006.04.031

S31. GENE PROFILING IN MELANOMA – WHAT HAVE WE GAINED?

Keith Hoek. *Department of Dermatology, University Hospital of Zürich, Switzerland.*

In the mid 1990s one of the first targets of DNA microarray analysis was melanoma. For over a decade researchers have made increasing use of this technology in their efforts to understand the molecular biology underlying this disease. Most work has concentrated on class-comparison approaches which assess the transcriptional differences between aggressive and less aggressive variants, or explored the consequences of activating mutations in members of the MAPK pathway. Other studies examined the effects of various in vitro treatments including UV, retinoids, demethylation and hypoxia. The general outcome of these studies have been the generation of ever longer lists of genes nearly all of which are guilty through association. But who are the master criminals in the crowd? Where are the strings which draw these multitudinous factors together and who are the puppetmasters behind them? DNA microarrays may have brought us closer to the facts, but what they all mean is not at all obvious to the majority of researchers. Just where in these details lies the devil of malignancy?

doi:10.1016/j.ejcsup.2006.04.032

S32. TUMOR STROMA-ASSOCIATED ANTIGENS FOR ANTI-CANCER IMMUNOTHERAPY

Jürgen C. Becker, Valeska Hofmeister, David Schrama. *Julius-Maximilians-University of Wuerzburg, Department of Dermatology, Wuerzburg, Germany.*

Immunotherapy has been widely investigated for its potential use in cancer therapy and it becomes more and more apparent that the selection of target antigens is essential for its efficacy. Indeed, limited clinical efficacy is partly due to immune evasion mechanisms of neoplastic cells, e.g. downregulation of expression or presentation of the respective antigens. Consequently, antigens contributing to tumor cell survival seem to be more suitable therapeutic targets. However, even such antigens may be subject to immune evasion due to impaired