So far, 168 patients with non-small cell lung cancer (NSCLC) or prostate cancer have been treated with L-BLP25 vaccine in phase I or II clinical trials. A randomized phase IIb study was performed that compared the safety and efficacy of L-BLP25 in patients with stage IIIB or IV NSCLC against best supportive care (BSC). Data from this study revealed an overall median survival of 17.4 months for patients on the vaccine arm versus 13 months for the patients in the BSC arm. Two-year survival was 43% for the L-BLP25 vaccine arm versus 29% for the BSC arm. The median survival for patients with locoregional Stage IIIB disease (without pleural effusion) was 30.6 months in the L-BLP25 arm and 13.3 months for patients in the control arm. Two-year survival was 60% for the L-BLP25 vaccine arm versus 37% for the BSC arm. Safety data from the phase IIb study showed an incidence of adverse events similar to those previously reported in L-BLP25 clinical trials, i.e. mild to moderate flu-like symptoms and injection site reactions. Although the differences in survival were statistically not significant, these results suggest a survival advantage in locoregional stage IIIB patients treated with L-BLP25 vaccine. A phase III trial in unresectable stage III NSCLC patients will be launched in 2006.

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S27. SURVIVING – PRECLINICAL RATIONAL AND PRELIMINARY RESULTS

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Prognosis of most disseminated solid tumors remains gloomy as neither chemotherapeutic nor unspecific immune modulatory approaches were able to improve the overall survival of these patients. Hence, specific immunotherapy has received increasing attention. Disappointing clinical results, however, indicate that the choice of suitable antigens is of special importance. To this end, the inhibitor of apoptosis protein survivin, which is over-expressed in several tumours but is largely undetectable in adult tissues, appears to be a promising target for vaccination purposes, since downregulation or loss of expression is associated with impaired tumour progression. Consequently, heavily pretreated patients suffering from advanced, therapy-refractory melanoma, pancreatic, cervical or colorectal cancer were vaccinated with affinity-improved HLA-A1, A2 or B35-restricted survivin-derived peptide epitope together with Monatamide ISA-51 in a compassionate use setting. Preliminary results from an interim analysis of this ongoing clinical trial demonstrated that patients mounted strong survivin specific T cell responses as measured by ELISPOT assay and tetramer-staining. Furthermore, in situ peptide/HLA-A2 multimer staining confirmed that these survivin reactive cells infiltrated both visceral and soft tissue metastases. Most importantly, clinical activity was suggested by both disease stabilisation as well as objective responses.

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S28. THE ROLE OF EGFR FAMILY IN PRENEOPLASIA AND LUNG CANCER; PERSPECTIVES FOR TARGETED THERAPIES AND SELECTION OF PATIENTS

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The Erb-B family of receptors seems to play an important role in the lung carcinogenesis. Several studies have demonstrated over expression of epidermal growth factor receptor (EGFR) in bronchial dysplasias.[1] We have studied 268 bronchial biopsies from high-risk individuals participating in a high-risk sputum cohort (>30 pack-years of smoking history and COPD defined as FEV1 < 70% of expected), and EGFR protein was strongly expressed in about 60% of patients with normal bronchial histologies, but increased with increased level of dysplasia. Also HER2 was highly expressed in both normal bronchial epithelium as well as in preneoplasias.[2]

The expression of EGFR/HER2 in malignant lung tumors was studied in several studies. Squamous cell carcinomas has most often high expression of EGFR, but less HER2, while adenocarcinomas has more often high expression of HER2 and less EGFR. Of interest is that bronchioloalveolar carcinomas has high expression of both EGFR and HER2, which can contribute to explain why this subtype seems to be more sensitive to EGFR inhibitors than the other non-small cell subtypes.[3]

The *prognostic role* of EGFR and HER2 has been addressed.[4,5] In our study 183 patients with resected NSCLC were studied, and no significant prognostic association was demonstrated in this study. However, other studies have reported that EGFR overexpression is associated with poorer prognosis.[5] In the UCCC study also the EGFR gene copy number was studied by FISH, and a tendency to a shorter survival was seen for increased gene copy number.[4]

The *predictive role* of EGFR and HER2 for sensitivity to EGFR TKIs has been studied at UCCC in two separate cohorts of NSCLC patients. In an Italian cohort of 108 NSCLC patients we found that EGFR protein expression, increased gene copy number detected by FISH and EGFR gene mutations were all associated to treatment outcome after EGFR TKI therapy.[6] Patients with high EGFR gene copy number (high polysomy/amplification) had a high objective response rate (36%), disease control rate (67%) and median survival of 19 months, which was significantly better than the patients with no or low gene gain. The same was found in another study cohort (SWOG 0126) for patients with BAC subtypes.[7] The association between increased EGFR gene copy number and survival has been demonstrated in two randomized placebo controlled studies, one with erlotinib with hazard ratio (HR) = 0.44 in the FISH positive group and in the ISEL study with gefitinib (HR = 0.61), while no differences was seen in the FISH negative groups.[8,9]

In order to identify a panel of markers, which can predict sensitivity to EGFR TKIs we studied the predictive value of *combined markers*. While a combination of positive IHC and positive FISH were associated with very high response rates (41%) and prolonged survival (median 21 months), negative IHC and FISH assessment was associated with no response and very short survival (median 6months) indicating no clinical benefit in this group of patients.[10] We have also performed in vitro NSCLC cell line studies with EGFR TKIs and characterized sensitive and resistant cell lines by Affymetrix gene chips. We have identified several

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.

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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

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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