changing PCR conditions (n≥16-32 per conditon) 2) Inter-assay variation within replicate amplifications of a single genome (n≥750 LOHs for each of the 20 markers) 3) Inter-assay variation arising from amplifications of different genotypes (n=940 LOHs, 20 genotypes). Coefficients of determination (r2) from regression lines fitted to allele amplification product scatter graphs were used to quantify variation and rank markers for reproducibility. Histograms of LOHs were used to construct various marker-specific probability distributions for confidence intervals.

Results: 1) All PCR conditions affected either the relative extent or quality of allele amplification. 2) Most markers at the chosen loci could be reproducibly amplified (0.996>r2>0.885) with exceptions reflecting natural-variation in allele sizes (eg r2=0.635), which caused an increase in LOHs variation. 3) LOHs variation correlated with increasing difference in allele sizes. Finally, population homozygosity at some loci rendered the assay less informative.

Conclusion: Many parameters were identified that contribute to variation in LOHs measurement. For microsatellite technology (and LOHs) to be predictive of bladder cancer, optimised amplification conditions need to be strictly adhered to. The number of markers involved in the current protocol provide considerable challenges for sample handling and data interpretation. Results from ongoing trials of clinical samples will determine if the natural variation in observed LOHs is sufficiently low to achieve high sensitivity and specificity in cancer detection.

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p53 overexpression predicts the resistance to chemotherapy in advanced non small cell lung cancer (NSCLC)

V. Gregorc[1], V. Ludovini[1], L. Pistola[1], S. Darwish[1], V. De Angelis[1], V. Minotti[1], G. Bellezza[2], A. Sidoni[2], E. Bucciarelli[2], M. Tonato[1]. *[1] Division of Medical Oncology, Policlinico Hospital, Perugia, Italy; [2] Department of Pathology, University, Perugia, Italy*

Purpose: Tumors with p53 overexpression have been associated with enhanced resistance to cisplatin-based chemotherapy in limited studies involving NSCLC. Ours is the largest study on p53 and response to chemotherapy in patients (pts) with locally advanced and advanced NSCLC. The relationships and interactions between p53, Rb and bcl-2 immunostaining, clinical parameters and response and overall survival were considered in analysis.

Methods and population: Paraffin-embedded bronchial biopsies and transthoracic agobiopsy specimens of 102 pts who underwent cisplatin based-chemotherapy between 1991 and 1999 were evaluated using an immunostaining method. Pts were 88 males and 14 females, with a mean age of 61.5±9.9 yrs; 47 were stage III and 55 were stage IV.

Results: Median survival was 12.2 months and the 5 year survival was 6.1%. Only 15 specimens (14.7%) were normal for all three markers. Fifty-six tumor samples (54.9%) had normal p53, 41 (40.2%) had normal Rb and 94 (92.2%) had normal bcl2 expression. The univariate analysis indicated that the pts with normal p53 and association of normal p53 and deleted Rb had better response to chemotherapy. The response rate of the p53 positive group was 26% versus 57% of p53 negative group ($p<0.002$). We also compared the different associations of Rb and p53 protein expression: group I (p53-Rb+), group II (p53-Rb-), group III (p53+Rb+), group IV (p53+Rb-) including 17, 39, 24 and 22 patients, respectively. Group II was found to have the best response rate (62%, $P<0.003$). Moreover, bcl-2 overexpression, TNM (III vs IV) and normal LDH serum levels were associated with better overall survival ($p<0.03$, $p<0.02$ and $p<0.001$). In multivariate analyses, p53 overexpression for resistance to chemotherapy and TNM for overall survival were identified as independent predictive factors ($p<0.02$, $p<0.001$,respectively).

Conclusion: Our study demonstrates a predictive role of p53 for response rate and confirmed TNM as the only predictive factor for survival. It may be important to consider these data for stratification in prospective randomized clinical trials because the difference in the response rate due to p53 expression is too high to be ignored.

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Relationship between a clinical drug resistance and immunohistochemical expression of p-glycoprotein and p53 in small cell lung cancer

R. Ishibashi, Y. Nakanishi, M. Izumi, T. Harada, N. Inoshima, K. Inoue, H. Wataya, T. Minami, Y. Horiuchi, N. Hara. *Graduate School of Medical Sciences, Kyushu Univ., Research Institute for Diseases of the Chest, Fukuoka, Japan*

Background: Although small cell lung cancer (SCLC) is in general chemosensitive, nearly 20% of SCLC is refractory to chemotherapy. While resistance to chemotherapy is a major problem in treatment of SCLC, there is no useful predictor of treatment response. One reason of drug resistance is the overexpression of the drug efflux pump, P-glycoprotein and other multidrug resistance protein. On the other hand, inactivation of the p53 tumor suppressor gene is reported to result in chemoresistance due to deletion of p53-mediated apoptosis. Several markers of drug resistance have been described in preclinical models, but the mechanism of drug resistance in lung cancer patients remains unknown.

Purpose: We designed this study to determine the utility of p53, P-glycoprotein and multidrug resistance protein expression in predicting the response to chemotherapy in patients with SCLC, retrospectively.

Material and Methods: We evaluated transbronchial biopsy (TBB) specimens from 62 patients with previously untreated SCLC. Formalin- fixed, paraffin-embedded TBB specimens were immunostained using anti-p53 antibody (DO-1, Oncogene Science, New York, U.S.A.), anti-P- glycoprotein antibody (JSB-1, Nichirei Co., Japan).

Results: The positive rate of P-glycoprotein was 25.8%, and that of p53 was 76.7%. No correlation was observed between P-glycoprotein and p53 immunostaining. The expression of P-glycoprotein and p53 were not correlated with clinical backgrounds including age, sex, disease extension, tumor markers, or chemotherapy regimen. However, the expression of P-glycoprotein was correlated with clinical chemotherapy resistance in SCLC ($P < 0.001$), while that of p53 was not ($P = 0.999$).

Conclusion: These results suggest that immunostaining of P- glycoprotein for TBB specimens may help to predict clinical drug resistance in SCLC. Stainning results of other ABC superfamily such as MRP1 and MRP2 are also to be presented.

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Predictive value of p16ink4a and p53 alterations for the prognosis of resected early stage non-small cell lung cancer

J. Niklinski[1], W. Niklinska[2], L. Chyczewski[3], G. Lapuc[1], J. Laudanski[1]. *[1] Medical Academy of Bialystok, Departmemnt of Thoracic Surgery, Bialystok, Poland; [2] Medical Academy of Bialystok, Department of Histology, Bialystok, Poland; [3] Medical Academy of Bialystok, Department of Clinical Molecular Biology, Bialystok, Poland*

Purpose and Methods: The association of p16 and p53 gene abnormalities with the prognosis of patients with non-small cell lung cancer (NSCLC) has been widely investigated to date, however, this association is still controversial.

Therefore, we investigated the prognostic significance of p16 abnormalities (mutations by sequencing and hypermethylation by methylation-specific PCR), and p53 alterations (by immunohistochemistry and serum p53 antibody assessment, and by direct sequencing through exons 4 to 8) in 72 radically resected stage I-II NSCLCs. Kaplan-Meier estimates of survival were calculated for clinical variables and molecular markers using the Cox model for multivariate analysis.

Results: Sequencing analysis of the p53 gene showed mutations in 32% of stage I-II NSCLC; serum p53 antibodies were detected in 14%, and p53 protein expression in 40%. p16 abnormalities were found in 33% of patients (point mutations in 2 (3%) and promoter hypermethylation in 22 (30%) cases). No correlation was found between p53 and p16 abnormalities and various clinicopathologic factors, including age, sex, histological type of tumour and TNM (I vs. II) stage. The overall survival rate of NSCLC revealed that both the patients with p53 and 16 abnormalities tended to have a poorer prognosis than the patients without p53 ($p< 0.02$) and p16 (< 0.01) abnormalities. In the multivariate analysis, however, when the types of p53 and p16 alterations were analyzed, only p53 gene mutations and p16 hypermethylation were associated with poor prognosis.

Conclusion: These results indicated that p53 point mutation and p16 hypermethylation could be a useful molecular markers for the prognosis of patients with resected early stage NSCLC.