Background: Caspase-3 (CASP-3) is a primary effector CASP that executes programmed cell death, and it plays an important role in the development and progression of cancer. Polymorphisms in the CASP-3 gene may influence CASP-3 production and/or activity, thereby modulating the susceptibility to lung cancer. To test this hypothesis, we investigated the association between CASP-3 polymorphisms and the risk of lung cancer in a Korean population.

Methods: We first screened single nucleotide polymorphisms (SNPs) in the CASP-3 gene by direct sequencing of genomic DNA samples taken from 27 healthy Korean individuals. We selected identified SNPs based on their frequency, linkage disequilibrium (LD) status and haplo-type tagging status, and then genotyped the selected SNPs in 582 lung cancer patients and 582 healthy controls who were frequency matched for age and gender.

Results: We identified eight SNPs: six known SNPs (-928A>G, 246C>T, 829C>A, 1143G>C, 17532A>C and 20541C>T); and two novel SNPs (77G>A and 163G>T). Individuals with at least one variant allele of the -928A>G, 77G>A and 17532A>C polymorphisms were at a significantly decreased risk for lung cancer in comparison to the carriers with each homozygous wild-type allele [adjusted odds ratio (OR) = 0.79, 95% confidence interval (CI) = 0.62-1.00, P = 0.05; adjusted OR = 0.78, 95% CI = 0.61-0.99, P = 0.04; and adjusted OR = 0.74, 95% CI = 0.58-0.95, P = 0.02, respectively). Consistent with the results of genotyping analysis, the GAGC haplotype carrying the variant allele at all of the -928A>G, 77G>A, and 17532A>C loci was associated with a significantly decreased risk of lung cancer compared to the AGGA haplotype carrying no variant alleles at the three loci (adjusted OR = 0.66, 95% CI = 0.51-0.86, P = 0.002 and Bonferroni corrected P = 0.008).

Conclusions: These results suggest that the CASP-3 polymorphisms and their haplotypes contribute to the genetic susceptibility to lung cancer.

A7-03   **Prevention & Early Detection, Mon, 13:45 - 15:30**

Sputum cytometry to detect lung cancer

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Background: Improved early detection remains the most promising method of improving patient survival from lung cancer, and is a realistic goal in the short term. This study reports the findings of a large scale trial evaluating a novel, fully automated method of DNA cytometry on sputum to detect lung cancer.

Methods: Over a period of 18 months, 1235 patients clinically suspicious of having lung cancer were recruited into a multinational validation trial of the LungSign™ sputum test. Induced sputum was collected at the time of initial presentation, fixed and treated with dithiothreitol (DTT). Papanicolaou stained smears were prepared and analysed by conventional cytology. Monolayer cytospin slides were prepared and stained using the Feulgen-thionin method. Cytospin slides were scored using LungSign, a test that uses a fully automated computerised DNA cytometry system to analyze thousands of epithelial cell nuclei to generate a measure associated with malignancy.

Results: Of the 1123 patients analysed, 370 proved to have lung cancer (prevalence 33%). The LungSign test provided an ROC "area under the curve" value of 0.692, and for a specificity of 91% it detected 40% of all lung cancers (Figure 1). Results were similar for all lung cancer

histological types, as well as early (up to 1b) and later (stage 2a and above) stages. Conventional cytology detected 16% of cancers with a specificity of 99%. Approximately 12% of cytospins contained insufficient epithelial cells for cytometric analysis. The inadequacy rate for conventional cytology was 43%.

Conclusions: In a high risk population, the LungSign test provides an effective means of detecting those patients most likely to have lung cancer. It is a continuous measure, permitting different cut points to be set. This flexibility allows the test to be used in multiple contexts such as screening patients for lung cancer or evaluating suspicious lesions detected on CT, thereby directing efficient use of other, more expensive and invasive diagnostic modalities.
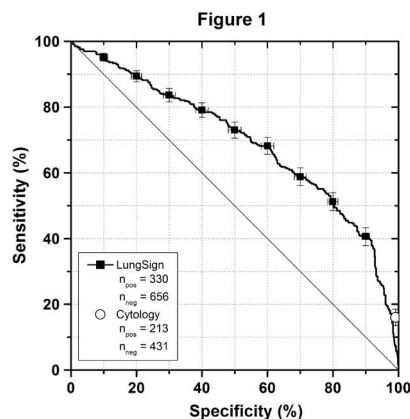

Figure 1; ROC curve for the LungSign score, with conventional cytology (circle). Sensitivity and specificity standard error bars representing one standard deviation are shown.

A7-04   **Prevention & Early Detection, Mon, 13:45 - 15:30**

Update on the use of oral iloprost for the chemoprevention of lung cancer

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Background: Pre-clinical studies have shown that the majority of NSCLC have decreased expression of prostacyclin synthase (PGIS) and genetically modified mice with selective pulmonary PGIS overexpression are chemoprevented from developing lung cancer in a variety of models, including cigarette smoke exposure. Based on these promising results, a multi-center, double-blind, placebo controlled, phase II trial of iloprost (an oral prostacyclin analogue) in subjects at increased risk for lung cancer was initiated and continues to enroll.

Methods: Subjects are selected for the trial if they meet the following criteria: current or former smoker (> 20 pack years); at least mild cytologic atypia on sputum cytology; no previous history of cancer. Fluorescent bronchoscopy is then performed with 6 standard sites biopsied, along with all other abnormally appearing areas. Subjects are then randomized to iloprost (in escalating doses) or placebo for 6 months and then undergo a repeat fluorescent bronchoscopy with repeat biopsy of all the central airway areas sampled on the first bronchoscopy. The primary endpoints for the study are bronchial histology and Ki-67

labeling index. Additional markers in the eicosanoid pathway are also being analyzed.

Results: To date, a total of 119 subject have been enrolled in the trial (the majority of these at the University of Colorado, n=82), with the investigators remaining blinded in terms of treatment groups. Fluorescent bronchoscopy was able to successfully identify areas of mild dysplasia or worse in the majority of patients (90/119, 76%). A total of 17 (14%) patients had normal bronchial epithelium at all biopsy sites. A wide range of endobronchial pathology has been observed in the biopsies, with average baseline histology being significantly elevated in current smokers. When biopsy sites are matched from the first and second bronchoscopies, changes in histologic grade have been observed, with more individual sites showing improvement (n=116) than progression (n=72). The subjects are all being scored on histologic outcomes and there have been subjects with partial responses, stable disease, and progression. In the subset of subjects with Ki-67 analysis (n=49), a significant increase in labeling index was associated with smoking status (current > former, p=0.006) and worsening histologic grade (p<0.001). Immunohistochemical analyses for other markers in the eicosanoid pathway (including COX-2, mPGES, PGIS) are currently in progress.

Conclusions: The iloprost chemoprevention trial continues to progress and has proven that our recruitment model can enrich for a population of subjects with endobronchial dysplasia. Results are still blinded. The treatment has been well tolerated and histologic improvement has been observed at specific biopsy sites in many of the subjects. Analysis of endobronchial biopsies for primary and secondary endpoints continues, and we are assessing different methodologies for evaluating clinical responses in chemoprevention trials (particularly those that could serve as a framework for current and future trials).

A7-05   **Prevention & Early Detection, Mon, 13:45 - 15:30**

Outcomes in bronchial dysplasia: persistence of lesions and associations with development of invasive non-small cell lung cancer

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Background: Patients at high-risk for the development of lung cancer frequently harbor atypical airway epithelium (bronchial dysplasia). These lesions are believed to be precursors of invasive lung cancer, especially squamous type. However, previous studies of the natural history of these lesions have been limited by small numbers of specimens and short periods of follow-up. We performed an exhaustive analysis of all patients enrolled in Colorado SPORE bronchoscopy protocols who underwent multiple bronchoscopies to characterize the long-term outcome of these lesions.

Methods: SPORE tissue bank records of histologic diagnosis, date of bronchoscopy and biopsy site for 121 patients (2,711 biopsies from 720 unique biopsy sites), who had between two and eight bronchoscopies over as many as 11 years were collected. Within subjects, biopsy sites from different bronchoscopies were matched and each site was

classified into progression, persistent or regression groups. Each site was assigned an initial diagnosis according to WHO classification criteria. Progression, persistence and regression rankings were assigned according to whether the biopsy site showed change, or lack thereof, from the initial diagnosis to either of four diagnosis groups defined as: Non-dysplastic (normal, basal cell hyperplasia and squamous metaplasia without atypia); Dysplastic (mild, moderate and severe); Carcinoma-in-situ and Invasive carcinoma. In addition to progression score, individual bronchoscopies were scored for dysplasia index (DI), defined as the number of dysplastic biopsies (including CIS) divided by the total number of biopsies per bronchoscopy. Thirty patients with multiple brochoscopies had cancer diagnosed at some point during their enrollment. In analyses of relationships between bronchial histology and development of invasive cancer, an additional 28 patients with a single pre-carcinoma bronchoscopy were included.

Results: High grade (HGD: moderate, severe dysplasia and CIS) bronchial lesions were more frequently persistent than low grade (LGD: mild) lesions (68.5% vs 43.7%, respectively; Chi-square <0.001). In addition, both initial and mean DI was higher in persistent versus non-persistent (regression) groups for HGD and LGD groups. Angiogenic squamous dysplasias (ASDs), a subset of dysplastic lesions, similarly showed increased persistence of HGD versus LGD lesions but additionally showed increased length of persistence in HGD-ASDs as compared non-ASD HGDs (25.5 vs. 17.1, respectively; p=0.024). A similar, non-significant trend toward increased length of persistence was seen for LGD-ASDs versus non-ASD LGDs. A trend toward increased persistence of HGD lesions in patients with all types of NSCLC versus no carcinoma patients (80% vs. 65%) was found, and this percentage was even higher, though still not statistically different, for the subset of patients with squamous cell carcinoma (85%). In addition, a trend toward increased mean DI in squamous cell carcinoma versus no carcinoma was seen (43% vs 31%).

Conclusions: Analysis of high risk patients undergoing multiple bronchoscopies shows a relationship between persistence and degree of atypia in bronchial dysplasia. An increase in the length of persistence is associated with presence of ASD histology. An association between squamous cell carcinoma development and high DI and/or persistence of HGD is suggested. These data support the role of bronchial dysplasias as pre-malignant lesions in non-small cell lung cancer.

A7-06   **Prevention & Early Detection, Mon, 13:45 - 15:30**

The prevalence and persistence of premalignant lesions: a report of a high risk lung cancer cohort

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Background: Lung cancer causes more deaths than all of the other major cancers combined with little improvement in the survival rate over the last 25 years. While premalignant lesions for central airway have been identified, the natural history of these lesions is unclear, especially for metaplastic lesions. Lung cancer screening of high-risk subjects with autofluorescent bronchoscopy (AFB) can reliably detect the premalignant lesions that are precursors to squamous cell carcinoma.

Methods: A cohort of 350 patients underwent AF bronchoscopy (AFB) screening based on lung cancer risk factors at the Lung Cancer Screening Clinic at the Roswell Park Cancer Institute in Buffalo NY