B4-05 Prevention & Early Detection + Epidemiology, Tue, 13:45 - 15:30

Development of a serum marker panel for early lung cancer detection and treatment response assessment using the proximity-ligation assay (PLA)

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Background: Most lung cancer patients are diagnosed with advanced disease, underscoring the need of early diagnosis. To date, there is no known clinically useful blood-based biomarker for lung cancers. Nevertheless protein based biomarkers in the blood hold a great promise as diagnostic markers for disease states and outcomes in clinical cancer management.

We hypothesize that tumor cells and the reactive host stroma secrete several specific proteins that can be readily detected by methods more sensitive than the presently available clinical methods. Furthermore, radiation and chemotherapy treatments can cause tumor lysis and release of intracellular proteins that can be detected in the blood with a highly sensitive approach and the level of such proteins can provide immediate response information for clinical decision.

Methods: Multiplexed and sensitive detection technologies with low sample consumption are required for validating large sets of biomarker candidates. Conventional immunoassays are hampered by limited sensitivity and relative high sample consumption. Our group has developed a new method named proximity ligation assay that is based on PCR-methods for protein analysis. In short, the assay employs a pair of proximity probes each composed of an antibody linked to an oligonucleotide. As these two antibody-based probes bind the protein analyte in solution, the two corresponding oligonucleotides gain a significant increase in local concentration. This enables the hybridization of a connecting oligonucleotide, leading to the formation of a unique target reporter amplicon that is quantified by real-time PCR. Several proteins (seven in each assay-set) are analyzed in one parallel reaction, and levels of different proteins are standardized against a known amount of controlled added protein. Each 7-plex assay requires only one microliter of either plasma or serum. When compared to standard ELISA methodology, the detection limits of the described method are up to three orders of magnitude lower (Fredriksson et al, Nature Methods in press).

Results: We have generated antibody-oligonucleotide set for 27 candidate proteins to be used in our assays. These include markers of hypoxia (osteopontin, HIF1), growth factors and cytokines (PDGF, IGF-2, CTGF, VEGF, MIF, TNFa, IL4, IL7, IL10), oncogenes and known tumor markers (erb-B2, CEA, CA125, CA19-9), extracellularly active molecules (ADAM8, SLPI, galectin 1, mesothelin, EpCam) and others.

Preliminary results indicate the feasibility and sensitivity of the method, and complete results on all markers will be presented.

Conclusion: We have successfully generated several new antibody-oligonucleotide sets for candidate serum markers. We have shown that it is feasible to detect these proteins in NSCLC patient blood and that PLA was more sensitive than conventional ELISA in detecting a subset of markers. Future directions will focus on generating more antibody-oligonucleotide sets for new proteins, specifically those that are induced in NSCLC in gene-expression datasets. In addition, we will apply this method to a larger set of serum samples from NSCLC patients and matched healthy controls to identify a diagnostic circulating protein signature for NSCLC.

B4-06 Prevention & Early Detection + Epidemiology, Tue, 13:45 - 15:30

Lung Cancer Survival in Relation to Histologic Subtype: An Analysis based upon Surveillance Epidemiology and End Results (SEER) Data

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Background: We have demonstrated through an analysis of SEER data on 307,797 lung cancer patients diagnosed between 1975 and 2003 that adenocarcinoma of the lung is now the most common form of lung cancer in the US. Indeed, adenocarcinoma is currently the most common histologic subtype of lung cancer in both women and men, blacks and whites, and in all age groups. The objective of this analysis is to examine relative survival in relation to histologic subtype.

Methods: SEER data on 234,273 lung cancer patients diagnosed in the US between 1980 and 2002 were analyzed. The primary endpoint was five-year survival of the four major lung cancer histologies: adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large-cell carcinoma. Five-year survival was measured for patients diagnosed during four time intervals (1980-1984, 1985-1989, 1990-1994, 1995-2002). Comparisons were drawn based upon stage (localized, regional, and distant) gender, race (white versus black) and age group (<40-yrs; 40-49-yrs, 50-59-yrs, 60-69-yrs, 70-79-yrs, >80-yrs).

Results: Stage of disease was clearly the most powerful predictor of five-year survival. However, histologic subtype was also a major predictor of survival. When all stages are combined, survival was highly statistically significantly superior in adenocarcinoma compared to other histologies ($p<0.0001$). Among patients with localized disease, 5-year survival in adenocarcinoma was far superior to that of squamous cell carcinoma, the second most favorable histology. For example, for cases diagnosed in 1995-2002, 5-year survival for localized disease was 60.1% for adenocarcinoma, 44.2% for squamous cell, 41.3% for large cell and 20.1% for small cell carcinoma. Interestingly, five-year survival within histologic subgroups has improved very little over this 22-year period. For example, 5-year survival for localized adenocarcinoma of the lung in 1980-1984 was 58.3% compared to 60.1% in 1995-2002. Among patients with regional disease, 5-year survival for adenocarcinoma was slightly better than the other histologies. For cases with regional disease diagnosed from 1995-2002, five-year survival was 20.1% for adenocarcinoma, 16.3% for squamous cell, 15.9% for large cell and 10.7% for small-cell. Significantly superior survival in adenocarcinoma was probably related to more favorable stage distribution in adenocarcinoma. For example, 38.2% of adenocarcinoma patients had localized disease compared to 25.2% of squamous cell patients ($p<0.0001$). Among those with distant disease, five-year survival was approximately 2% for all histologies. Overall, when adjusted for stage and histology, 5-year survival was significantly superior in females compared to males. However, the survival advantage was most striking for women with localized adenocarcinoma. When comparing blacks to whites, survival was inferior for blacks, when adjustments were made for stage and histology. For those diagnosed between 1995-2002, sur-

vival for blacks and whites with localized adenocarcinoma was 52.0% and 60.6%, while survival was 44.5% and 41.5% for blacks and whites with squamous cell carcinoma, respectively. With regard to age group comparisons, there was a significant decrease in 5-year survival with advancing age among those with localized disease.

Conclusions: Differences in relative survival of lung cancer was strongly dependent upon histologic subtype. Indeed, five-year survival for adenocarcinoma of the lung is significantly superior to that of other histologic subtypes. Improved survival in adenocarcinoma appears to be related to a more favorable stage distribution, which facilitates curative therapies

B4-07 **Prevention & Early Detection + Epidemiology, Tue, 13:45 - 15:30**

Sputum Methylation Analysis Detects Patients With Lung Cancer

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Background: Sputum methylation analysis is feasible and by some studies suggested to show an increased risk for lung cancer. The aim of this study was to examine 6 methylation markers in sputum of patients with lung cancer and controls.

Methods: In the Canisius Wilhelmina Hospital Nijmegen sputum has been prospectively collected for several years of patients with lung cancer and controls. From this bank 102 cases with lung cancer and 102 controls, most of them with COPD DNA was retrospectively extracted. Samples were subsequently randomized and blinded (TdB). Coded samples were exchanged between collaborators unaware of its origin. Quantitative MSP was performed for APC, Cytoglobin, MGMT, 3-OST-2, p16, RASSF1A, and TCF21. Follow-up was retrieved independently (MvdD). Pathology diagnosis was based on biopsy or only cytology. After submission of methylation data (to ZF with subsequent information of TdB) statistical analysis was performed (WY,ZF).

Results: The pathology diagnosis was squamous cell carcinoma (n=23), adenocarcinoma (n=23), SCLC (n=16) or NSCLC not further specified. The 77 of the 102 controls had a Gold score for COPD >0. Sputum samples were collected within 6 months of lung cancer diagnosis. Follow-up of COPD cases was minimally 2 years.

Reproducibility of methylation analysis between two labs was poor for MGMT.

RASSF1A showed hypermethylation in 81 % of the SCLC and 69% of the NSCLC lung cancer cases with a specificity of 94 and 74%, respectively. For two markers the sensitivity showed a decrease in sensitivity for SCLC and NSCLC to 50%, and 57%, respectively. The specificity increased for SCLC and NSCLC to 99 and 95%, respectively.

Conclusions: This blinded study shows that hypermethylation in sputum is with two markers detects 50% of the lung cancer patients.

Session B5: NSCLC: New Paradigm in Radiation Therapy
Tuesday, September 4

B5-01 **NSCLC: New Paradigm in Radiation Therapy, Tue, 13:45 - 15:30**

Phase III trial of cisplatin (P) plus etoposide (E) plus concurrent chest radiation (XRT) with or without consolidation docetaxel (D) in patients (pts) with inoperable stage III non-small cell lung cancer (NSCLC): HOG LUN 01-24/USO-023

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Background: Concurrent chemoradiation is standard treatment for pts with inoperable stage III NSCLC. A previously reported single-arm, phase II study by SWOG (Gandara et al JCO 2003) suggested D following EP/XRT further improved survival. We report results from a randomized, prospective phase III trial comparing EP/XRT with or without consolidation D.

Methods: Eligible pts had inoperable, stage IIIA/B NSCLC, PS 0-1 at study entry, FEV-1 > 1 L, and < 5% wt loss in the preceding 3 mos. Pts (n=243) received P 50 mg/m^2 iv d 1,8,29,36 and E 50 mg/m^2 iv d1-5, 29-33 concurrently with chest XRT to 5940 cGy. Non-progressing pts (PS 0-2) were randomized to D 75 mg/m^2 iv every 21 d for 3 cycles vs observation (O). The primary endpoint was to compare OS (Kaplan-Meier analysis). A multivariate parametric accelerated failure time model was performed to identify factors that affected survival. Accrual of 259 pts to randomize 180 was planned to demonstrate a difference in MST of 25 vs 15 mos (5% 2-sided alpha, 80% power). Based upon evidence of futility (predefined as p>0.7271), a DSMB recommended early termination after an analysis of the initial 203 pts.

Results: Median f/u 25.6 mos. Pt characteristics (n=203): 34%/66% F: M; median age 63; 39.4%/60.6% IIIA/B; staged with PET 66.5%; FEV-1 > 2 46.7%; current smoker 41.9%. G3/4 toxicities during EP/XRT included 9.8% febrile neutropenia (FN), 17.2% esophagitis. 147 of 203 pts (72.4%) were randomized to D (n=73) or O (n=74). 82.2% randomized to D received 3 cycles. G3/4 toxicities during D included: 10.9% FN, 8.2% pneumonitis. 28.8% of pts were hospitalized during D (vs 8.1% in O arm) and 5.5% died due to D. Factors predictive for improved survival included age < 70, FEV-1 > 2, and hemoglobin > 12 at baseline. PFS for D was 12.3 vs 12.9 mos for O (p=0.9412). The MST for all pts (n=203) was 21.15 mos; MST for D was 21.6 mos (95% C.I. 17.7-35) vs 24.2 mos for O (95% C.I. 18.1-34.4) (p=0.9402).

Conclusions: The MST with EP/XRT was higher than historical controls; however, consolidation D does not further improve survival, is associated with significant toxicity including an increased rate of hospitalization and premature death.