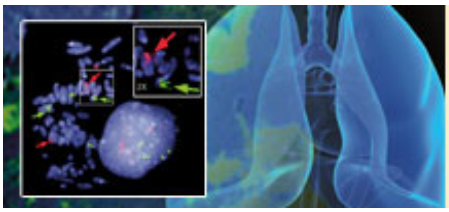

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Proceedings Supplement

Additional Invited Speaker Abstract

Concurrent Session 6: Biological Implications of Circulating Tumor Cells and Biomarkers in Lung Cancer Progression

A systems approach to the discovery of lung cancer biomarkers. Samir Hanash. Fred Hutchinson Cancer Research Center, Seattle, WA.

We have implemented a systems approach for the discovery of lung cancer biomarkers. Applications include risk assessment, early detection, and predicting response to treatment. The approach which includes genomic, proteomic, metabolomic, and immune profiling integrates data from human biospecimens, a large number of lung cancer cell lines grown under various conditions and several genetically engineered mouse models of lung cancer. An important component of the human studies consists of profiling of prediagnostic plasmas available through longitudinal cohort studies, particularly useful for the discovery and validation of blood based risk and early detection markers. Findings include molecular signatures and networks for lung cancer sub-types. Mouse model studies yielded an NKX2.1 signature relevant to early detection, an EGFR network signature for EGFR driven lung cancer and a neuroendocrine signature for small cell lung cancer which were validated in human samples. Integration of proteome and transcriptomic profiling of lung cancer cell line yielded several signatures and a marker panel that predict survival for early stage lung cancer. Mining of the large and continuously expanding sets of data currently being collected benefits from an organized collaborative effort.

Poster Session A: Corrections

A12 High-throughput mutation analysis of NSCLC circulating tumor cells. Heidi S. Erickson[1], Nana E. Hanson[1], Hai Tran[1], Gordon B. Mills[1], Ed S. Kim[1], John V. Heymach[1], Ignacio I. Wistuba[1], Hector G. Galindo[1], Katherine Stemke-Hale[2], Uma Giri[1], Christina McDowell[1], Luc Girard[1], Jack J. Lee[3], Roy Herbst[1], John Minna[1], Farideh Z. Bischoff[3]. [1]University of Texas MD Anderson Cancer Center, Houston, TX, [2]Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX, [3]Biocept, Inc., San Diego, CA.

Background: Circulating tumor cells (CTC) associated with solid tumors are being studied for their diagnostic and prognostic value. In patients with metastatic tumors, CTC presence in the blood has been putatively associated with short survival. Since blood collection is relatively noninvasive, CTC molecular analysis opens up the possibility of monitoring genotypic changes during cancer treatments. Unfortunately, CTCs are not present in large numbers, often at rates as low as one cell per 106-107 leukocytes. Thus, to perform genotypic biomarker analysis on CTCs, methodologies must be developed to using highly specific and sensitive technologies and an enrichment step to increase analytes to detectable levels.

Methods: We developed a methodology for detecting mutations in multiple oncogenes and chemotherapy resistance genes in non-small cell lung cancer (NSCLC) CTC specimens using high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) single nucleotide polymorphism (SNP) analysis (MASSarray; Sequenom, Inc.) to determine cancer-associated

genetic mutations in lung cancer specimens. This system allows for up to 10 different somatic mutations to be assayed per well in a 384-well format; requires very little DNA; can be used with whole genome amplified (WGA) DNA; and is sensitive enough to use with small samples such as core needle biopsies (CNB), fine needle aspirates (FNA), and CTCs. We developed a lung cancer assay panel of 13 genes/135 mutations (including AKT1, BRAF, CTNNB1, EGFR, ERBB2, KRAS, MEK1, NRAS, PIK3CA, PIK3R1,PTEN, and STK11) to test for somatic mutations in genes representing multiple pathways known to be involved in lung cancer. All assays can detect a mutation in < 25% of a sample.

Results: In the effort to analyze CTCs, we first analyzed 57 NSCLC cell lines with known mutations and confirmed known mutation status. Next, we successfully analyzed DNA from 90 frozen and matched FFPE NSCLC resected tissues. Analysis of unamplified and matched WGA cell line DNA quantity CNB and FNA equivalents gave the same mutational status results. Moving to CTC equivalents, we successfully analyzed cell line DNA and matched WGA DNA equivalents of 100-1000 cells with known EGFR L585R and KRAS G34A mutations and negative control DNA (negative for all assays). Next, WGA methodology for direct CTC cell lysate DNA amplification was developed using CTC cell number equivalents (3 – 200 cells) obtained from a typical clinical blood sample CTC preparation. Then, we directly compared unamplified and matched amplified CTC cell equivalents (50, 100, and 200 cells). Analysis of both unamplified and amplified CTC cell equivalents reported identical mutation status results. We have applied this methodology to spiked blood sample and clinical blood sample CTC fractions. Thus, we demonstrated that we are able to study mutations in multiple genes using small amount of DNA from CTC cell numbers in a high-throughput manner.

Conclusion: We developed a robust method for accurately determine cancer-associated genetic mutations in NSCLC CTC cell number equivalent lysates using MALDI-TOF MS SNP analysis which can be applied to better understand the molecular characteristics of lung cancer during treatment and progression. As additional clinical NSCLC CTC samples are collected, we will continue applying this methodology to assess CTC mutation status as potential diagnostic and/or prognostic markers

A18 Spatial analyses reveal increased incidence of large cell lung carcinoma in specific regions of Maine that differ in men and women. Janet M. Hock[1], Christopher Farah[1], H. Dean Hosgood, III[2], Molly Schwenn[3], Candice C. Black[4] . [1]Maine Institute for Human Genetics and Health, Bangor, ME, [2]National Cancer Institute Occupational and Environmental Epidemiology Branch, Bethesda, MD, [3]Maine Cancer Registry, Augusta, ME, [4]Dartmouth Medical College, Dartmouth, NH.

Lung cancer is a major cause of death among cancers in the U.S. Environmental exposures to tobacco or radon are considered the two highest risks for lung cancer. Maine is one of the top 15 states with the highest rates of lung cancer in the U.S., with smoking rates that vary from 20-30% across counties. We recently reported on spatial variations in radon exposure. In this study, we investigated if there were spatial regions of excessive lung cancer by gender in Maine, and if these might overlay the high radon exposure regions. Age-adjusted lung cancer incidence rates in Maine were compared to those of the US overall, using NCI SEER and CDC databases. Data for all lung cancer cases in Maine reported during 1995-2006 were obtained from the Maine Cancer Registry. Population data used Base U.S. Census 2000 data. Lung cancer incidence was adjusted for age and population density. We assessed the spatial distribution of lung cancer by subtype, using spatial scan statistic, assuming a discrete Poisson distribution. We did not adjust for race as Maine is 95% white. We also examined spatial variations by pathology sub-types classified as adenocarcinoma, small cell carcinoma, large cell carcinoma.

Within Maine, Washington County, which also has the highest smoking rate, reported the highest lung cancer incidence rates [men: 131.7 (110.5,156.2); women: 73 (58.5, 90.5), compared to Maine overall (men: 97.2 (93.9,100.5); women: 66.6 (64.2, 69.1) or the US (men: 84.3 (84.1, 84.5); women: 55.8 (55.6, 55.9)]. Maine is a rural, mostly nonagrarian state, with significant health disparities due to poverty, access to healthcare and geographic isolation. In considering other disadvantaged populations, lung cancer incidence rates for Maine men and women were higher than those reported for U.S. blacks, Hispanics and American Indians.

Our results showed small “hotspots” for lung cancer overall with RR greater than 1 for females (RR 1.8, p 0.03) in eastern Washington County, and for males (RR 1.7, p 0.03) in northern Washington County and in the Pittsfield Region, Somerset County. No significant differences were detected in