AACR-IASLC Joint Conference on

Molecular Origins of Lung Cancer: Biology, Therapy, and Personalized Medicine

January 8-11, 2012 • San Diego Marriott Marina & Hotel • San Diego, CA

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The AACR and IASLC thank the following organizations for their generous support of this conference:

Award Supporters

The AACR and IASLC thank the following organizations for their generous support of the travel awards provided at this conference. A complete list of awardees and their affiliations is available on page 5.

The AACR-Axel Ullrich Travel Awards

Center to Reduce Cancer Health Disparities

Scholar-in-Training Awards

Five presenters of meritorious abstracts have been selected by the Conference Chairpersons to receive awards to attend this conference. All graduate and medical students, postdoctoral fellows, and physicians-in-training were eligible for consideration. The names of the Scholar-in-Training awardees, their affiliations, and the poster or proffered presentation numbers are provided below. AACR and IASLC sincerely thank Aflac, Inc., AstraZeneca, and Dr. Axel Ullrich for their support of these awards.

AACR-Aflac, Incorporated Scholar-in-Training Awards

Daiana D. Becker Santos, BC Cancer Research Center, Vancouver, BC, Canada, A4
Eric S. Kim, The University of Texas MD Anderson Cancer Center, Houston, TX, A21
Madhu S. Kumar, Cancer Research UK, London, United Kingdom, A26

AACR-AstraZeneca International Scholar-in-Training Awards

Mauricio S. Caetano, Brazilian National Cancer Institute, Rio de Janeiro, Brazil, B2

The Axel Ullrich Travel Awards

Xiaoling Song, Yale University, New Haven CT, B35

AACR Minority Scholar in Cancer Research Awards

The AACR is pleased to administer this important award program that is supported by a generous grant from the Center to Reduce Cancer Health Disparities of the National Cancer Institute, and provides funds for the participation of meritorious minority scientists in AACR meetings and conferences. These awards are intended to enhance the education and training of minority researchers and to increase the visibility and recognition of minorities involved in cancer research. Eligible scientists are full-time predoctoral (graduate or medical) students or residents, clinical or postdoctoral fellows, and junior faculty who are either engaged in cancer research or who have the training and the potential to make contributions to this field. This program applies only to racial/ethnic minority groups that have been defined by the NCI as being traditionally underrepresented in cancer and biomedical research (African American/Black, Alaska Native, Hispanic American, Native American, and Native Pacific Islander.) Only citizens of the United States or Canada or scientists who are permanent residents in these countries may receive one of these awards. The awardee name(s) will be announced onsite at the conference.

AACR Minority-Serving Institution (MSI) Faculty Scholar in Cancer Research Awards

The AACR MSI Faculty Scholar Awards program is supported by a generous grant from the Center to Reduce Cancer Health Disparities of the National Cancer Institute. The purposes of the award are to increase the scientific knowledge base of faculty members at MSIs, to encourage them in their research, and to assist in inspiring their students to pursue careers in cancer research. The AACR offers these awards for participation in its meetings and conferences to full-time faculty of minority-serving institutions (historically black colleges and universities [HBCUs], Hispanic-serving institutions [HSIs], Indian tribally controlled colleges and universities (ITCCUs), and other postsecondary institutions as defined by the U.S. Department of Education) who are scientists at the assistant professor level or above. Only citizens of the United States or Canada or scientists who are permanent residents in these countries may receive one of these awards.The awardee name(s) will be announced onsite at the conference.

Accreditation Statement

The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education activities for physicians.

Credit Designation Statement

AACR has designated this live activity for a maximum of 19.0 AMA PRA Category 1 Credit(s)™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Claiming (CME) Credit

Physicians and other healthcare professionals seeking AMA PRA Category 1 Credit(s)TM for this continuing medical education activity must complete the online CME Request for Credit Survey by Friday, February 24, 2012. The Request for Credit Survey will be available via a link on the AACR website at **www.aacr.org/lungcancercme** and via e-mail.

Your CME certificate will be sent to you via e-mail after the completion of the activity.

Statement of Educational Need, Target Audience, and Learning Objectives

Lung cancer remains the most common cause of cancer death in the United States. The American Cancer Society estimates there were 222,520 new cases of lung cancer in 2010 (an increase since 2009) with 158,080 deaths.[1] The last 15 years of research have provided insight into the molecular nuances that stratify different types of non-small cell lung cancer (NSCLC). In the past an approach to target all NSCLC with cytotoxic chemotherapeutic agents, a "one size fits all approach," provided a small measureable benefit but often with response rates of less than 20%.[2] Most recently, common molecular subtypes of NSCLC have been found, allowing for more targeted and subtype-based treatment selection. The most studied genetic variant in NSCLC is mutation in the gene encoding EGFR. Patients with this mutation have a better response rate to EGFR tyrosine kinase inhibitors, such as gefitinib or erlotinib, than those without the EGFR mutation. Similarly in 2010, the molecular subtype of NSCLC with ALK fusions emerged to the forefront as a clinically relevant and treatable oncogenic mutation. The ALK fusion subtype of NSCLC responds to a different drug than those lesions with an EGFR mutation (~57% response rate to crizotinib), highlighting the need to understand an individual's tumor genotype for optimal treatment.

Emerging advances in the field of lung cancer research that require multidisciplinary attention are: genetic mutations that confer resistance to treatment, the other molecular subtypes of lung cancer, and the possibility of screening for early detection reported in the results of the November 2010 NCI National Lung Screening Trial.

To best understand and personally treat the correct subtype of lung cancer, it is critical to continue to study alterations that can occur within the gene, by gene product fusion, and even from transcriptional regulation.

After participating in this CME activity, physicians should be able to:

1. To provide an overview of the current state of lung cancer research in the areas of prevention, diagnosis, and treatment, as well as recent developments and research through a series of lectures given by nationally and internationally renowned scientists from diverse backgrounds.
2. To educate more junior investigators and students in this field of research on advances and technologies, and to encourage them to initiate investigations in lung cancer research.

3. To accelerate translational research in the field and stimulate transdisciplinary information exchange by bringing together a broad range of scientists, including clinicians, basic, translational, and clinical researchers from academia and industry, as well as patient advocates.

4. To offer a forum for open discussion, scientific interaction, and collaboration among scientists with complementary expertise through the cooperation of the two associations, the American Association for Cancer Research (AACR) and the International Association for the Study of Lung Cancer (IASLC). The conference atmosphere and schedule will provide these opportunities. We expect approximately 300 participants to attend this meeting, similar to the last meeting in 2010, and take advantage of the networking opportunities.

Disclosure Statement

It is the policy of the AACR that the information presented at AACR CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, AACR will provide information that Scientific Program Committee members and speakers have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity. This disclosure information will be made available in the Program/ Proceedings of this conference.

Acknowledgment of Financial or Other Support

This activity is supported by educational grants from: Abbott, Daiichi Sankyo, Eli Lilly, Genentech, and Pfizer. Any other grants will be disclosed at the activity.

Questions about CME?

Please contact the Office of CME at (215) 440-9300 or cme@aacr.org.

Conference Program

Sunday, January 8

6:00 p.m-7:30 p.m. Opening Session

Welcome and Opening Remarks
David P. Carbone, Vanderbilt-Ingram Cancer Center, Nashville, TN
Roy S. Herbst, Yale Cancer Center, New Haven, CT

Keynote Address

microRNAs and lung cancer
Frank J. Slack, Yale University, New Haven, CT

7:30 p.m-9:00 p.m. Welcome Reception

Monday, January 9

7:00 a.m-8:00 a.m. Continental Breakfast

8:00 a.m-10:00 a.m. Plenary Session 1 Biology of Lung Cancer

Session Co-Chairpersons: Jonathan M. Kurie, The University of Texas MD Anderson Cancer Center, Houston, TX; and Eric B. Haura, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Network models in oncogene-addicted lung cancer
Eric B. Haura

Mechanisms of lung cancer metastasis
Jonathan M. Kurie

Lung cancer stem cells, acquired vulnerabilities, and molecular portraits: Translation to the clinic
John D. Minna, UT Southwestern Medical Center, Dallas, TX

Defining the mechanisms of tumorigenesis by mutant EGFR using mouse models
Katerina A. Politi, Yale University, New Haven, CT

Session Discussion

10:00 a.m-10:30 a.m. **Break**

10:30 a.m-12:30 p.m. **Plenary Session 2**

The Genomic Landscape of Lung Cancer

Session Co-Chairpersons: William Pao, Vanderbilt-Ingram Cancer Center, Nashville, TN; and Tetsuya Mitsudomi, Aichi Cancer Center Hospital, Nagoya, Japan

Gene fusions in lung cancer
David G. Beer, University of Michigan, Ann Arbor, MI

Somatic genome alterations in human lung cancers
Matthew L. Meyerson, Dana-Farber Cancer Institute, Boston, MA

Genetic and genomic difference in lung cancer based on ethnicity
Tetsuya Mitsudomi

Title to be announced
William Pao

Session Discussion

12:30 p.m-2:30 p.m. **Poster Session A and Lunch**

2:30 p.m-4:30 p.m. Concurrent Sessions 1-2

Concurrent Session 1: Combined Modality Treatment

Session Co-Chairpersons: David M. Jablons, University of California, San Francisco, CA; and David R. Gius, Vanderbilt School of Medicine, Nashville, TN

Stereotactic ablative radiotherapy (SABR) for lung cancer
Jeffrey D. Bradley, Washington University Medical School, St. Louis, MO

A genetic connection between aging, sirtuins, and carcinogenesis
David R. Gius

Molecular determinants of prognosis in resectable NSCLC
David M. Jablons

A prospective trial of SBRT boost for stage III non-small cell lung cancer
Ronald C. McGarry, University of Kentucky, Lexington, KY

Session Discussion

Concurrent Session 2: Immunology and Lung Cancer

Session Co-Chairpersons: David P. Carbone, Vanderbilt-Ingram Cancer Center, Nashville, TN; and Dmitry Gabrilovich, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Regulation of T-cell function in cancer by activation of Notch signaling
David P. Carbone

Molecular mechanisms regulating synergistic effect of immunotherapy and chemotherapy of cancer
Dmitry Gabrilovich

Causes and consequences of acid pH in tumors
Robert Gillies, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

TSLP-driven inflammation fosters development of epithelial tumors
A. Karolina Palucka, Baylor Institute for Immunology Research, Dallas, TX

Session Discussion

4:45 p.m-6:15 p.m. Concurrent Sessions 3-4

Concurrent Session 3: Transformational Research: Patient-Bench-Patient

Session Co-Chairpersons: Kim Norris, Lung Cancer Foundation of America, Los Angeles, CA; and Bonnie J. Addario, Bonnie J. Addario Lung Cancer Foundation, San Carlos, CA

Speakers:
Bonnie J. Addario
Michael Weitz, St. John's Health Center, Woodland Hills, CA
Shane Dormady, Valley Medical Oncology Consultants, Mountain View, CA
Steven Young, Addario Lung Cancer Medical Institute, San Carlos, CA
David P. Carbone, Vanderbilt-Ingram Cancer Center, Nashville, TN
Kim Norris

Concurrent Session 4: Short Talks from Proffered Abstracts

Integrating expression data improves mutational significance analysis of lung squamous carcinoma
Bryan Hernandez, Broad Institute, Cambridge, MA

The genetic status of the EGFR, K-ras, and EML4-ALK genes in multiple primary noninvasive lung adenocarcinomas
Kenji Sugio, National Kyushu Cancer Center, Fukuoka, Japan

NSCLC and SCLC mouse models mediated by lentiviral gene delivery
Yifeng Xia, Salk Institute for Biological Studies, La Jolla, CA

The tumor suppressor role of Wnt/β-Catenin pathway in development of small cell lung cancer
Kwon-Sik Park, Stanford University, Palo Alto, CA

A phase II study of sorafenib in patients with stage IV non-small cell lung cancer (NSCLC) with a K-Ras mutation
Wouter W. Mellema, VU University Medical Center, Amsterdam, The Netherlands

Phase 1b/2 trial of HER3 inhibitor U3-1287 in combination with erlotinib in advanced NSCLC patients (pts): HERALD study
Joachim von Pawel, Asklepios Fachkliniken Munich-Gauting, Gauting, Bavaria, Germany

Tuesday, January 10

7:00 a.m-8:00 a.m. **Continental Breakfast**

8:00 a.m-10:00 a.m. **Plenary Session 3**
Lung Cancer Screening: Imaging and Molecular Approaches
Session Co-Chairpersons: Denise Aberle, UCLA David Geffen School of Medicine, Los Angeles, CA; Christine Berg, National Cancer Institute, Rockville, MD; and Gabriella Sozzi, Fundacion IRCCS Instituto Nazionale dei Tumori, Milan, Italy

Implications for lung cancer of the NLST results
Christine Berg

Cost effectiveness of CT screening
Peter Bach, Memorial Sloan-Kettering Cancer Center, New York, NY

Role of biomarkers in the early detection of lung cancer
Pierre P. Massion, Vanderbilt University Medical Center, Nashville, TN

The inflammation-EMT-cancer initiating cell axis in the pathogenesis of lung cancer
Steven M. Dubinett, David Geffen School of Medicine at UCLA, Los Angeles, CA

Session Discussion

10:00 a.m-10:30 a.m. **Break**

10:30 a.m-12:30 p.m. **Plenary Session 4**
Molecular vs. Histological Classifications of Lung Cancer
Session Co-Chairpersons: Paul A. Bunn, Jr., University of Colorado Denver, Aurora, CO; and Fred R. Hirsch, University of Colorado Denver, Aurora, CO

The molecular pathology of adenocarcinoma versus histopathological classification
Elisabeth M.P. Brambilla, Hôpital A. Michallon, Grenoble, France

Pathology of lung neuroendocrine tumors: In search of evidence for classification
Ilona Linnoila, National Cancer Institute, Bethesda, MD

Molecular pathology of squamous cell carcinoma of the lung
Ignacio I. Wistuba, The University of Texas MD Anderson Cancer Center, Houston, TX

Molecular and histological classifications of lung cancer inform each other
Marc Ladanyi, Memorial Sloan-Kettering Cancer Center, New York, NY

Session Discussion

12:30 p.m-2:30 p.m. Lunch on own

2:30 p.m-4:30 p.m. Concurrent Sessions 5-6

Concurrent Session 5: Lung Cancer Prevention

Session Co-Chairpersons: Waun Ki Hong, The University of Texas MD Anderson Cancer Center, Houston, TX; and Avrum Spira, Boston University School of Medicine, Boston, MA

Targeted lung cancer chemoprevention through reverse migration
Waun Ki Hong

New opportunities to the personalization and management of early lung cancer
James Mulshine, Rush University, Chicago, IL

Molecular field cancerization
Avrum E. Spira

Risk prediction models for lung cancer
Margaret Spitz, Baylor College of Medicine, Houston, TX

Session Discussion

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

Session Co-Chairpersons: Katerina A. Politi, Yale University, New Haven, CT; and Pierre P. Massion, Vanderbilt University Medical Center, Nashville, TN

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

miRNA and lung cancer: Early detection in high-risk subjects
Gabriella Sozzi, Fndn. IRCCS Inst. Nazionale dei Tumori, Milan, Italy

Elucidating cancer biomarkers using aptamers
Weihong Tan, University of Florida, Gainesville, FL

Multiplexer mutational profiling of CTCs in NSCLC
John V. Heymach, The University of Texas MD Anderson Cancer Center, Houston, TX

Session Discussion

4:30 p.m-6:30 p.m. Poster Session B and Reception

Wednesday, January 11

7:00 a.m-8:00 a.m. **Continental Breakfast**

8:00 a.m-10:00 a.m. **Plenary Session 5**
New Molecular Targets and Novel Therapy
Session Co-Chairpersons: Garth Powis, The University of Texas MD Anderson Cancer Center, Houston, TX; and Roy S. Herbst, Yale Cancer Center, New Haven, CT

Title to be announced
Jeffrey A. Engelman, Massachusetts General Hospital, Boston, MA

Dual inhibitors of FT and GGT-1 as novel therapeutic agents
Said Sebti, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Redox pathways as platforms for drug development
Kenneth D. Tew, Medical University of South Carolina, Charleston, SC

Mechanisms and inhibition of mutant KRAS in non-small cell lung cancer
Garth Powis

10:00 a.m-10:15 a.m. **Break**

10:15 a.m-12:15 p.m. **Plenary Session 6**
Bringing Personalized Therapy into the Clinic
Session Co-Chairpersons: Joan H. Schiller, UT Southwestern Medical Center, Dallas, TX; and Paul A. Bunn, Jr., University of Colorado Denver, Aurora, CO

Lessons learned from the BATTLE trials: a step towards personalized medicine
Roy S. Herbst, Yale Cancer Center, New Haven, CT

Bringing personalized therapy into the clinic: Can pathology deliver?
Keith M. Kerr, Aberdeen University Medical School, Aberdeen, United Kingdom

Integration of genomic testing into the management of NSCLC
Bruce Johnson, Dana-Farber Cancer Institute, Boston, MA

Cetuximab in NSCLC and predictive markers
Fred R. Hirsch, University of Colorado Cancer Center, Aurora, CO

12:15 p.m. **Departure**

Keynote Address

microRNAs and lung cancer. Frank J. Slack. Yale University, New Haven, CT.

microRNAs are small noncoding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. let-7 encodes a microRNA implicated in human cancer. Specifically, human let-7 is poorly expressed or deleted in lung cancer, and over-expression of let-7 in lung cancer cells inhibits their growth, demonstrating a role for let-7 as a tumor suppressor in lung tissue. let-7 is expressed in the developing mammalian lung and regulates the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for let-7's involvement in cancer. We are focused on the role of let-7 and other oncomiRs in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis.

Plenary Session 1: Biology of Lung Cancer

Network models in oncogene-addicted lung cancer. Eric B. Haura[1], Takeshi Yoshida[1], Bin Fang[1], Alexey Stukalov`[2], Steven Eschrich[1], Eric Welsh[2], Jacques Colinge[2], John Koomen[1], Giulio Superti-Furga[2], Jiannong Li[1], Uwe Rix[1], Guolin Zhang[1], Jae-Young Kim[1], Keiryn Bennett[2], Lanxi Song[1], Ann Chen[1], Yun Bai[1]. [1]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, [2]CeMM Research Center for Molecular Medicine, Vienna, Austria.

Lung cancer is a devastating worldwide disease yet enthusiasm exists for treatment of subsets of the disease with molecularly targeted agents. Mutations in the epidermal growth factor receptor (EGFR) or translocation of echinoderm microtubule associated protein like 4 – anaplastic lymphoma kinase (EML4-ALK) define two unique subsets of lung cancer characterized by sensitivity to tyrosine kinase inhibitors (TKI). Despite striking results with TKI, not all patients respond, the drugs are non-curative, and resistance is universal. Mutations in KRAS also define a group of patients awaiting therapeutic opportunities. We are characterizing signaling networks using tandem affinity purification (TAP) and liquid chromatography-mass spectrometry (LC-MS/MS) to map protein-protein interactions (PPI) and anti-phosphotyrosine immunoprecipitation coupled with LC-MS/MS to map tyrosine phosphorylation. In PC9 cells with mutated EGFR, we characterized a physical EGFR network consisting of 266 proteins by integrating both TAP and pTyr MS data. In H3122 cells harboring EML4-ALK, we identified a PPI network consisting of 113 proteins and using pTyr MS identified changes in tyrosine phosphorylation in 120 proteins (58 decreased, 62 increased) following exposure to ALK tyrosine kinase inhibitor. Functional proteins are being discovered from these networks using siRNA and inhibitor screens. In parallel studies, we have exploited the use of chemical proteomics to discern targets of promiscuous kinase inhibitors and enable optimal combination approaches. In this way, we view network mapping linked to chemical proteomics as one approach to discern novel drug combination studies for in vivo validation and ultimately translation to early phase clinical trials. To translate PPI based mass spectrometry studies to clinic, we are developing in situ assays that identify and quantify PPI using proximal ligation assays (PLA). Pilot studies identify EGFR:Grb2 interactions in formalin fixed human lung cancers. These approaches have the potential to enable 'network medicine' by identifying novel combination approaches as well as through identifying subtypes of cancers through network views of cancer.

Lung cancer stem cells, acquired vulnerabilities, and molecular portraits: Translation to the clinic. John D. Minna[1], Chunli Shao[1], Amit Das[1], Chris Desevo[1], Patrick Dospoy[1], Boning Gao[1], Rachel Greer[1], Suzie Hight[1], Kenneth Huffman[1], Jill Larsen[1], Michael Peyton[1], Misty Shields[1], James P. Sullivan[1], Laura Sullivan[1], David Gerber[1], David Mangelsdorf[1], Joan Schiller[1], Jerry Shay[1], Alexander Pertsemlidis[2], Kevin Coombes[3], John V. Heymach[3], Jonathan Kurie[3], Jack Roth[3], Luc Girard[1], Xifeng Wu[3], Carmen Behrens[3], Heidi Erickson[3], Ignacio Wistuba[3], Bruce Posner[1], Michael Roth[1], Adi Gazdar[1], Michael White[1], Yang Xie[1], Alex Augustyn[1], Rolf Brekken[1], Rebecca Britt[1], Robin Frink[1], Ryan Carstens[1]. [1]University of Texas Southwestern Medical Center, Dallas, TX, [2]University of Texas Health Science Center San Antonio, San Antonio, TX, [3]University of Texas MD Anderson Cancer Center, Houston, TX.

Recent advances in detailed molecular understanding of lung cancer including complete genome sequences affords the possibility of developing molecularly targeted therapy for potentially all lung cancers. Currently the Lung Cancer Mutation Consortium (LCMC) has developed an integrated approach and network for identifying lung cancers with specific mutations and funneling these patients into clinical trials targeting the specific mutations identified. However, another approach is to use genome wide and chemical library screens to identify genetic and epigenetic changes that have been created in lung cancer cells during tumor pathogenesis that are absolutely required for the activated oncogenic pathways to function. These are often referred to as "synthetic lethal" changes and represent adaptations the cancer cell has to make to allow the "oncogene addictions" to drive tumor growth and survival. They are present in tumor but not normal cells and thus represent "acquired vulnerabilities" that can be therapeutically targeted. The NCI's "Cancer Target Discovery and Development Network" (CTD2N) exemplifies this combined genetic and pharmacologic approach. A most important subgroup of these vulnerabilities are changes that are required for the continued function and survival of a subpopulation of tumor cells that have acquired tumor initiating and often metastatic and drug resistant characteristics including many of the properties of stem cells that are referred to as cancer stem cells (CSCs) or "cancer initiating cells." To achieve these goals we have developed a large integrated research platform. We have also: developed assays for identifying lung CSCs, "molecular portraits" (clades) that group lung cancers into subsets of clinical and molecular relevance; genome wide siRNA and chemical library functional screens to test for portrait/clade specificity. From these efforts we have learned that: There are a subset of cells within lung cancers (ranging from 0.1-30% of non small cell lung cancers, NSCLCs) and 50% + of small cell lung cancers, SCLCs) identified by elevated aldehyde dehydrogenase (ALDH) activity that have dramatically enhanced clonogenic, tumorigenic, and self renewal capacity; Patients whose tumors are enriched is such ALDH+ tumor cells have significantly impaired prognosis; The notch pathway (particularly Notch3) and ALDH1A3 are major vulnerabilities in lung CSCs; NSCLCs can be subdivided by mRNA expression profiles into "molecular portraits" (or "clades") that have relevant clinical, oncogenotype, and drug response phenotypes; Genome wide siRNA and large chemical library screens identify targets that are specific for lung cancer over normal lung epithelial cells; The identified targets show dramatic clade and oncogenotype specificity as well as specificity for lung cancers with different responses to available chemotherapy and targeted therapy for lung cancer; The newly identified vulnerabilities provide coverage of essentially all lung cancers and as such, provide a new functional "vulnerability classification" of lung cancer. All of these findings set the stage for the development of a rational approach to developing therapy targeted at lung cancer acquired vulnerabilities including those in lung CSCs that will include personalizing the therapy for each patient.

(Supported by Lung Cancer SPORE P50CA70907, DOD Prospect, CPRIT, and NCI CTD2N CA148225.)

Defining the mechanisms of tumorigenesis by mutant EGFR using mouse models. Katerina A. Politi. Yale University, New Haven.

Mutations in exons encoding the epidermal growth factor receptor (*EGFR*) are found in 10-15% of lung adenocarcinomas. The two most common mutations: a point mutation in exon 21 that leads to substitution of an arginine for a leucine at position 858 and small in-frame deletions in exon 19 are associated with sensitivity to the specific tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Approximately 70% of tumors harboring *EGFR* mutations respond to treatment with these drugs, however 30% of these tumors exhibit primary resistance to treatment with these agents. Moreover, responses are transient and, on average, within a year of initiating treatment patients who initially respond eventually develop TKI-inhibitor-resistant disease. Drug resistance, in most cases, is due to a secondary mutation in *EGFR* (EGFR T790M). Other less frequent mechanisms of resistance include *MET* amplification, *PIK3CA* mutations and transformation to small cell lung cancer. However, the mechanism of resistance is still unknown in approximately 30% of cases. More effective treatment of patients with *EGFR* mutant lung cancer requires a better understanding of the mechanisms of primary and acquired resistance to EGFR TKIs and the development of strategies to overcome this resistance.

To study these problems in vivo, we developed tetracycline-inducible transgenic mouse models of *EGFR* mutant lung cancer. Expression of lung cancer associated *EGFR* mutants gives rise to lung adenocarcinomas with bronchioloalveolar carcinoma features that are dependent upon the continuous activity of mutant EGFR for survival. Thus, treatment of the mice with erlotinib leads to tumor regression and long-term treatment with the drug gives rise to drug-resistant tumors that harbor some of the alterations observed in human TKI-resistant tumors such as the T790M mutation. Current efforts to use these mouse models to: 1) study mechanisms of primary and acquired resistance to EGFR TKIs and 2) evaluate new therapies to overcome resistance to first generation TKIs will be discussed.

Plenary Session 2: The Genomic Landscape of Lung Cancer

Gene fusions in lung cancer. David G. Beer. University of Michigan, Ann Arbor, MI.

Gene fusions represent one type of genetic alteration recently detected in lung cancer that results from chromosomal rearrangements. A fusion joining the echinoderm microtubule-associated protein-like 4 (*EML4*) and anaplastic lymphoma kinase (*ALK*) genes was detected as a transforming gene in a small subset of lung adenocarcinomas. Importantly, patients with these fusions show responsiveness to ALK kinase inhibitors. Additional studies have identified multiple *EML4-ALK* gene fusion variants and the histopathological characteristics of the lung tumors identified. Various methods utilized to detect lung gene fusions have included retrovirus-mediated expression screening with 3T3 cell transformation assays, FISH, mRNA/protein expression, RACE-coupled PCR sequencing, massively parallel paired-end and transcriptome sequencing. Additional novel gene fusion events in lung cancer have recently been identified using these and newly defined approaches in small percentages of primary lung tumors. Potential mechanisms underlying the production and triggers for gene fusion events will be discussed.

Somatic genome alterations in human lung cancers. Matthew Meyerson. Harvard Medical School, Boston, MA.

Cancer is a disease of the genome. High-throughput genome analysis tools now enable the detection of somatic alterations in cancer cells including point mutations, copy number alterations, translocations, and infections. Our approaches include next-generation sequencing of cancer genomes, exomes, and transcriptomes as well as single nucleotide polymorphisms (SNP) array analysis of copy number.

As part of "The Cancer Genome Atlas" or TCGA project of the National Institutes of Health, which aims to characterize the genomes of 10,000 human cancers, we are performing genomic analysis of lung carcinomas. In this presentation, I will discuss analysis of the genomes of squamous cell lung carcinomas. New results regarding mutations, genomic structure, and classification will be presented.

Genetic and genomic difference in lung cancer based on ethnicity. Tetsuya Mitsudomi[1], Kenichi Suda[1], Kenji Tomizawa[1], Yasushi Yatabe[1], Keitaro Matsuo[2]. [1]Aichi Cancer Center Hospital, Nagoya, Japan, [2]Aichi Cancer Center Research Institute, Nagoya, Japan.

Since the discovery of somatic mutation of the gene for the epidermal growth factor receptor (EGFR) in the non-small cell lung cancer (NSCLC), it has been known that EGFR mutation tends to occur in a certain subset of patients with a distinct clinical-pathologic feature. Namely, EGFR mutations are predominantly found in female, non-smoking patients with adenocarcinoma of East Asian origin. According to the data of 2880 patients complied from the literature, EGFR mutations are highly dependent on ethnicity (East Asians, 32% compared with Caucasians, 7%), sex (male, 10% compared with female, 38%), smoking history (never smoked, 47% compared with smoked, 7%), and histologic type (adenocarcinoma, 30% compared with other types of lung cancer, 2%) (1). In contrast, KRAS mutations predominantly occur in Caucasian patients with smoke exposure. KRAS mutation is present ~30% of adenocarcinoma of Caucasian patients, while it is present in ~10% of Asian patients (2). There appears to be no ethnic difference for incidence of ALK gene translocation although it is more frequent in non-smoking population similar to EGFR mutation.

According to Shigematsu and Gazdar, incidence of EGFR mutations is significantly different between East Asian patients (33%) and non-Asian patients (6%) ($P < 0.001$) in their analysis of 2,347 cases (3). When limited to adenocarcinoma, this difference is also present (48% vs. 12%). They also reported that 4 of 5 Asian patients with lung cancer in the United States and Australia had EGFR mutations, suggesting that genetics may be more important than geographic factors for affecting EGFR mutations (3).

The remarkable difference in prevalence of EGFR mutation in NSCLC patients between East Asian and Western population suggests that there may be differences in background risk/protective factors between these two populations.

Several polymorphic variations in EGFR gene including a CA repeat in intron 1 (CA simple sequence repeat 1 (CA-SSR1) (lower number of repeats) or single nucleotide polymorphisms in the promoter region (-216(G/T or T/T) and -191 (C/A or A/A) have been identified. All these polymorphisms are associated with increased expression of the EGFR protein and are less frequent in East Asians (4). Therefore, East Asians in general make less EGFR protein. To make lung cancer to occur, EGFR gene should be mutated and amplified because mutation of the EGFR gene is a prerequisite for amplification, if a certain amount of mutated EGFR protein is necessary to activate EGFR pathway signaling. This may partly explain ethnic difference in incidence of EGFR gene mutation described above (4).

We are now conducting Genome-Wide Association Study in Japanese to identify genetic locus associated with the risk of EGFR mutated and non-mutated NSCLC. In a part of the phase I study at Aichi Cancer Center [341 lung cancer cases (125 EGFR mutated and 216 EGFR wild-type) and 972 non-cancer controls] using Illumina platform-based genome scanning data, we have identified two loci showing genome-wide significance ($p<10^{-8}$) and two loci suggestive significance ($10^{-8\sim-5}$) loci for the risk of EGFR mutation. For EGFR wild-type NSCLC, three loci showed genome-wide significance and four loci showed suggestive significance. Some of the loci partly explain difference in the prevalence of EGFR mutation. A risk-allele frequency of one of the loci showing

association with EGFR mutated NSCLC was high in HapMap JPT (0.972) and low in HapMap CEU (0.438). We are now validating our finding in other Japanese cohorts. We believe that difference in EGFR mutation among NSCLC patients between East Asian and Western population can be explained by genetic difference.

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Concurrent Session 1: Combined Modality Treatment

A prospective trial of SBRT boost for stage III non-small cell lung cancer. Ronald C. McGarry, Jonathan M. Feddock. University of Kentucky, Lexington, Kentucky.

Purpose/Objective: Escalation of radiation dose in stage III lung cancer has been problematic and no prospective studies have confirmed a survival benefit for patients receiving >60Gy concurrent with chemotherapy. Nonetheless, conventional treatment approaches may not deliver adequate doses to the primary tumor. We report on the feasibility of using SBRT as a means of dose escalation of the primary tumor mass for patients found to have resolution of mediastinal nodal disease following conventional chemoradiotherapy (CRT). The goal was to deliver a biological effective dose (BED) of more than 100 Gy to the primary tumor mass (CRT + SBRT boost doses combined).

Methods: Patients with biopsy proven stage II/III NSCLC underwent CT or PET-CT scanning one month following completion of conventional CRT (59.4 Gy or greater). Eligible patients were required to have achieved either negative or only minor FDG residual uptake in nodal disease within the mediastinum. Patients with PET positive residual disease (≤7 cm) within the site of the primary tumor were included. Tumor volumes were defined using 3d planning based on the location of the initial tumor and PET uptake. The prescription dose was 10 Gy per fraction X 2 (20 Gy total dose, BED_{10} =112Gy combined CRT and SBRT boost dose) and later patients with tumors touching the mediastinum as per RTOG definition received SBRT to the residual tumor of 650cGy X 3 (1950 total dose). Patients underwent routine follow-up with repeat CT scans every 3 months or PET/CT as indicated. The primary endpoints were toxicity and tumor response. Target enrollment is 37.

Results: 35 patients (33 reviewed here) have been enrolled with a median age 63. Stages included were IIB (n=4), IIIA (n=18), and IIIB (n=13). Additionally, 9 patients were screened by 1 month post-CRT PET/CT and found to be not eligible: 2 patients with a CR after initial therapy and 7 with significant residual uptake in the mediastinum or metastatic disease. Pathologies included squamous cell carcinoma (75%), adenocarcinoma (11%), large cell carcinoma (10%) and lung cancer NOS (4%). Median combined BED was 110 Gy (range 110-120). Mean PTV volume was 51.3 cm^3 (range 46-2571cc) and average treatment coverage to 20 Gy prescription dose was 96.8%. Grade <3 toxicities have been very low. Only 4 local failures have occurred. Of these, two presented approximately 12 months after treatment with grade 5 toxicity, both of whom had large cavitary recurrences of their malignancies which involved the hilum. One patient had an SBRT boost volume encompassing the hilum, the other did not, but both anecdotally died of pulmonary hemorrhage. Of the 33 patients

reported here, 16 patients had "medial" tumors of which 10 received 1000cGy X 2 boosts to the hilum. As a safety precaution, for patients with tumor volumes touching the hilum, a revised SBRT dose of 650cGy X 3 (1950cGy total boost, BED_{10} =102Gy) was delivered. Thus far, 6 patients received 650cGy X 3 boost that included the hilum in most cases. No toxicities have been noted in these patients.

Conclusions: The use of linac-based SBRT boost as a means of dose escalation for limited residual NSCLC following definitive chemoradiotherapy is a feasible treatment approach with minimal toxicity.

Concurrent Session 2: Immunology and Lung Cancer

Molecular mechanisms regulating synergistic effect of immunotherapy and chemotherapy of cancer. Dmitry Gabrilovich, Rupal Ramakrishnan, Chun Huang. H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

Despite recent advances in the development of new chemotherapeutic drugs and improvements in radiation therapy, conventional cancer therapy often falls short of the goal of controlling tumor progression. Therapeutic cancer vaccines and adoptive T-cell transfer are long considered as very attractive therapeutic options in the treatment of cancer. However, clinical trials of different cancer vaccines performed in recent years demonstrated rather weak clinical efficacy. It has become apparent that therapeutic cancer vaccines given as a single agent may not produce substantial clinical benefits and combination with conventional methods of treatment will be necessary. The use of conventional cancer chemotherapy in combination with cancer vaccines was previously not considered as very attractive due to the potent immunosuppressive effect usually associated with chemotherapy. This paradigm was challenged in recent years by serendipitous observations made in a number of phase I/II clinical trials that reported high rates of objective clinical responses when cancer vaccines were combined with chemotherapy. The paradox is that the types of chemotherapy that are used in lung cancer are known to suppress the immune system. We have recently demonstrated that chemotherapy and cytotoxic T cells (CTL) have a synergistic cytotoxic effect that may be mediated by mannose-6-phosphate receptor (MPR). We have shown that when cancer vaccines or adoptive T-cell transfer are used as a single modality, only limited numbers of T cells are able to penetrate the tumor parenchyma. CTLs exert their cytotoxic effect only against tumor cells that express specific antigen, since it requires a direct cell-cell contact, and release of perforin and granzymes (GrzB). If chemotherapy is administered immediately after immunotherapy, it causes disruption of tumor stroma that allows for better penetration of antigen-specific T cells. Chemotherapy via autophagy causes substantial increase in MPR expression on tumor cells. Small number of activated CTLs interacting with tumor cells expressing tumor antigen can release GrzB that can penetrate into neighboring tumor cells without requirement for cell-cell contact. Therefore large number of tumor cells including those that do not express specific antigen would be susceptible to the effect of CTLs. This may explain substantial enhancement of antitumor effect of combined treatment. It is likely that this effect would not be long-lasting since CTL activity will be eliminated by chemotherapy and the immune suppressive tumor microenvironment. However, it may provide a sufficient window to achieve a significant antitumor effect. Since memory T cells are more resistant to chemotherapy than effector T cells, it is possible that subsequent immunization would be able to boost antitumor immunity and thus provide a longer lasting effect of combined therapy.

TSLP-driven inflammation fosters development of epithelial tumors. Anna Karolina Palucka, Kangling Xu, Alexander Gonzalez Pedroza, Te-Chia Wu, Sandra Zurawski, Florentina Marches, Gerard Zurawski, Yong-Jun Liu, Jacques Banchereau. Baylor Institute for Immunology Research, Dallas, TX.

Solid tumors are often associated with aseptic inflammation. There are two types of inflammation that have opposing effects on tumors, chronic inflammation that promotes cancer cell survival, and metastasis, and acute inflammation which triggers cancer cell destruction. Chronic inflammation is often linked with the presence of type 2-polarized macrophages (M2), which are induced by Th2 cytokines, IL-4 and IL-13.

Our recent studies have demonstrated the presence in breast cancer tumors of inflammatory Th2 cells, which produce IL-13, IL-4, and TNF. These CD4+ T cells appear to play a key role in the disease as they accelerate breast tumor development in a xenograft model through the production of IL-13. Breast tumors appear to play a critical role in conditioning the infiltrating myeloid DCs (mDCs) to induce such inflammatory Th2 cells. Our most recent results suggest that thymic stromal lymphopoetin (TSLP) secreted by cancer cells plays a role in mDCs conditioning. Breast cancer cell lines and primary tumors from patients show TSLP protein expression. TSLP-neutralizing antibodies block the upregulation of OX40L by mDCs exposed to tumor supernatant and consequently block mDCs capacity to generate inflammatory Th2 cells in vitro. The TSLP production is mediated by PAR2-signaling in cancer cells. PAR2 is expressed by all 4 breast cancer cell lines, and TSLP production can be induced upon PAR2-agonist peptide treatment. Similar observations were made by us and others in pancreatic cancer as well as lung cancer. Thus, TSLP could serve as therapeutic target in tumors of epithelial origin.

Plenary Session 3: Lung Cancer Screening: Imaging and Molecular Approaches

Role of biomarkers in the early detection of lung cancer. Pierre P. Massion. Vanderbilt Ingram Cancer Center, Nashville, TN.

While lung cancer remains the leading cause of cancer-related death in the world with a 16% overall survival rate, we now know that early detection by low dose chest CT saves lives. The implementation of screening programs represents a huge challenge. Research is needed to address who will benefit from screening, for how long, and with what frequency we should screen. Using biomarkers to select the most at-risk population, to detect the disease while measurable and yet not clinically apparent, and to predict survival, has been the goal of many investigations. Recent advances in molecular strategies and analytical platforms including genomics, epigenomics, proteomics, and metabolomics, have identified increasing numbers of potential biomarkers. These molecular approaches are applied to a variety of samples collected by non invasive methods including blood, urine, exhaled breath condensate, bronchial specimens, saliva, and sputum. None have yet moved to the clinical setting. In this presentation, we will define clinical contexts where diagnostic biomarkers may have utility in the management of lung cancer, we will review the state of development of candidate diagnostic biomarkers, and finally, we will discuss critical aspects of study design in biomarkers research.

Plenary Session 4: Molecular vs. Histological Classifications of Lung Cancer

The molecular pathology of adenocarcinoma versus histopathological classification. Elisabeth Brambilla, Grenoble University Hospital, Inserm U823, Institut Albert Bonniot, Grenoble, France

Recent therapeutic advances have led to a revolution in the lung cancer field in discovering therapeutically tractable

oncogene dependency with major implications for patient evaluation and approach to diagnosis. A recently published IASLC/ATS/ERS classification of lung adenocarcinoma addresses these issues fully based on the molecular features of histological subtypes of adenocarcinoma (1). As deduced from their molecular signatures, pathological patterns are the most achieved translation of the full genomic space (expression profiles, gene copy number alterations, and likely epigenetics space …). Therefore the functional clusters of genes defining a phenotypic population dictates the survival and contains targets of therapies in adenocarcinoma supporting the contention that each of the pathological and molecular features contains this information (2, 3). The new classification of adenocarcinoma has a high prognostic interest validated on further independent series of the literature (4). Molecular pathology recapitulates both differentiation markers and biomarkers of therapeutic sensitivity.

A stepwise accumulation of genetic and molecular abnormalities is sustaining the morphological transition between preinvasive and invasive and metastatic process in adenocarcinoma.

Now that lung cancer therapies personalized for individual patients is based on the histologic type of lung cancer and molecular status, the pathologist role using the knowledge on molecular pathology is to allow the histomolecular classification on small biopsies and cytology, the only specimens available in advanced treatable cases of adenocarcinoma (5).

A few differentiation markers (TTF-1, P63, P40, mucines) met the need for subclassification of cases with adenocarcinoma components (adenocarcinoma per se, large cell carcinoma, sarcomatoid carcinoma and NSCLC/NOS) driving their molecular analysis (6).

TTF-1 positive solid and cribriform tumor with signet ring are strongly associated with susceptibility for EML4-ALK fusion for ALK positivity candidate for crizotinib therapy, whereas Ras mucinous adenocarcinoma (previously mucinous BAC) are essentially TTF-1 negative, amphiregulin positive with IGFR activated pathway which are strongly resistant to gefitinib alone but are restored to sensitivity by IGFR inhibitors (7). Articulation between molecular pathology and histopathological features will in the future be the first organizers of therapeutical decisions (7).

Conclusion: As the molecular changes directly influence the therapy today and even more in the future, we think this should be reflected in the taxonomy as well as in the term given in the WHO classification. Histopathological classification would tend to predict that the molecular characteristics of the tumors together with their immunohistochemical and morphological appearance will dictate therapy in the future. Gefitinib is less effective in non adenocarcinoma NSCLC harboring EGFR mutations (8).

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Pathology of lung neuroendocrine tumors: In search of evidence for classification. Ilona Linnoila. National Cancer Institute, Bethesda, MD.

Neuroendocrine (NE) tumors comprise approximately 25% of all tumors in human lung. As a group they continue to provide challenges at biological, molecular and pathological level for uniform classification. The prototype of NE tumors is the highly aggressive small cell lung cancer (SCLC) that accounts 15% of human lung cancers. For

years lung cancers were clinically classified mainly into SCLCs and non-SCLCs (NSCLCs) while it has become evident that lung NE tumors actually form a complex spectrum from SCLC to carcinoid with shifting boundaries. The current WHO classification recognizes SCLC, its variant form combined SCLC, typical and atypical carcinoids as well as large cell NE carcinoma (LCNEC) and its variant form combined large cell carcinoma, the last two cancers under the main NSCLC category of large cell carcinoma. With the exception of the LCNEC, it is expected that the diagnoses will be rendered by routine hematoxylin-eosin stain according the established criteria. Benign entities include diffuse idiopathic pulmonary NE cell hyperplasia which is described in the chapter of preinvasive lesions. Over time there have been several efforts to place lung NE tumors into two to four-tier categories according to their malignant potential (high versus low grade) or degree of differentiation often leaving behind confusing terminology that is later misused. Unlike surgically resected NSCLCs, most NE tumors are frequently diagnosed from biopsies which remains a seldom recognized fact that may create its own biases. The diagnosis that experts most often agree with is SCLC, while distinctions between atypical carcinoid and LCNEC that are in the middle of the spectrum are often disputed. Many molecular studies have also “correctly” classified the endpoints of the NE spectrum while grouping in the middle of it has produced varied results. Moreover, several studies have suggested that NSCLCs with NE features (NSCLC-NEs) is a distinct carcinoma type with worse prognosis than ordinary non-NE NSCLC, while others disagree. Biologically this category is very interesting as NSCLCs and SCLCs are ultimately thought to be derived from the same cell. The regulation of NE differentiation is poorly understood. The proneural achaete-scute homolog 1 (ASCL1), a lineage-dependent oncogene may be a key factor for NE differentiation. On the other hand, it was recently shown that the deletion of the tumor suppressor genes Rb1 and p53 specifically in NE cells, a subpopulation of cells in normal airways, or in alveolar non-NE type2 pneumocytes of mice resulted in SCLCs closely mimicking human disease. These results provide experimental evidence for the close relationship of NE and non-NE epithelial cells in carcinogenesis.

Concurrent Session 5: Lung Cancer Prevention

Targeted lung cancer chemoprevention through reverse migration. Waun Ki Hong. The University of Texas MD Anderson Cancer Center, Houston, TX.

The hypothesis that lung cancer can be prevented with agents selected on the basis of epidemiologic data has, unfortunately, been disproven in many large randomized trials conducted in the last 25 years.

The results of these trials have overwhelmingly demonstrated not only the lack of benefit of most chemopreventive agents selected in this manner but also, importantly, the harmful effects of some of these agents in subsets of patients including current smokers.

Despite limited progress in the multimodality treatment of lung cancer, the cure rate for this disease is very disappointing; however, more recent discoveries of molecular “driver mutation” targets in lung cancer and treatment approaches with “matched” targeted agents in selected patient cohorts has been highly promising. This latter approach demonstrates that progression of even heterogeneous cancers can be intercepted by “molecularly relevant” targeted agents for the treatment of advanced disease.

A proposed new strategy for lung cancer prevention, therefore, is to apply a “reverse migration” of agents from the advanced disease to the chemopreventive setting; that is, to first identify the molecular drivers in the premalignant target tissue, and then to match these “hallmark” changes with molecularly targeted agents seen to be effective in advanced disease. This approach can be applied in the adjuvant as well as prevention settings, as has been demonstrated by the use of tamoxifen in breast cancer.

Thus, the major points I am going to present include the current landscape of chemoprevention in lung cancer and a new strategy of “reverse migration” for future chemoprevention trials in the 21st century.

New opportunities to the personalization and management of early lung cancer. James L. Mulshine. Rush University, Chicago, IL.

Lung cancer is the most lethal cancer across the globe with 5-year mortality rates from 80 to over 90% related to the frequency of metastatic disease at initial diagnosis. While metastatic disease is generally incurable, early lung cancer when found still localized to the airways, can frequently be cured. A recent NCI-sponsored, randomized trial of helical CT compared to chest X-ray screening in a high risk cohort reported that the CT arm resulted in a 20% reduction in lung cancer mortality. Coupled with the recent comprehensive revision of lung cancer staging classification, there is a much clearer understanding of the relationship between primary tumor size and lung cancer outcome. The finding that smaller lung cancers are more frequently curable provides a firm conceptual framework for population-based early lung cancer detection strategies as a productive approach to significantly improve lung cancer outcomes.

As was demonstrated by reports from both I-ELCAP and the NELSON clinical trials groups, detection rates of stage I lung cancer with helical CT could exceed 70%. Further, the expense and morbidity of invasive diagnostic work-up strategy could be efficient focused based on the suggestion by Yankelevitz and co-workers to use measurement of nodule growth rate on serial CT scans as a biomarker to identify clinically aggressive lung cancers. In this fashion as reported by van Klaveren and co-workers, volumetric determination of suspected lung cancers could enable efficient and accurate lung cancer case detection.

The surgical management of early stage lung cancers is increasingly employing video-assisted thoroscopic surgery. Recent studies demonstrate lower complications and more favorable operative mortality rates compared to standard, open thorocotomy approaches. The net effect of these developments is to reduce the possibility of over-treatment in the lung cancer screening setting. These surgical procedures do provide sufficient primary tumor tissue which allows comprehensive molecular analysis of the tumor to identify critical signaling pathways.

As the evolution of effective and efficient early lung cancer management unfolds, opportunities exist to better define the relevant at-risk population for screening approaches with the ability to calibrate the frequency of screening relative to the measured risk profile. Information from the imaging and tumor tissue evaluation of detected cases may also provide insight as to the molecular underpinnings of the cancer. This characterization of the primary tumor may subsequently guide the development of more tailored adjuvant therapies and eventually chemopreventive strategies that would be targeted to the specific pathogenic mechanisms of lung carcinogenesis.

The continuous improvement of early lung cancer management is an appropriate area to apply the process improvement strategies proposed by the Institute of Medicine in their Learning Health System approach. In this way, the component elements of the screening process can be optimized and personalized to allow overall progress in advancing public health benefit with CT-based lung cancer screening.

Risk prediction models for lung cancer. Margaret R. Spitz[1], Carol Etzel[2]. [1]Baylor College of Medicine, Houston, TX, [2]The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: There are an estimated 45 million current smokers in the US and 49 million former smokers. The challenge is to construct reliable risk prediction models to identify that fraction of smokers most likely to get lung cancer. High-risk individuals could undergo a program of screening surveillance

that might not be appropriate for a lower risk population and receive the most intensive smoking cessation interventions. Models with improved discriminatory ability will also have clinical benefits. In the US, 150,000 patients are diagnosed with focal pulmonary lesions annually, with the prevalence of malignancy ranging from 10 to 70%. Current guidelines are to employ best clinical judgment and/or validated models. Finally, risk prediction tools could be incorporated into the design of smaller, more powerful, and “smarter” prevention trials. Cardiovascular and type 2 diabetes risk profiles using epidemiologic data have been effectively used for nearly two decades. In the cancer arena, risk prediction models for breast cancer have the longest history, although models have also been generated for prostate, lung, melanoma, ovary, colorectal, and bladder cancers. The advent of genome-wide association studies to identify low-penetrance common susceptibility alleles now heralds the possibility of incorporating panels of gene variants into existing models and to assess improvement in model performance. However, to date, the expanded models for many cancer sites have shown only modest incremental improvements in discrimination.

Lung Risk Models: We and others have explored various approaches for lung cancer prediction. Such tools hold promise, but their interpretation is complex.

Epidemiologic/clinical: Bach published the original risk prediction model based on data from the Carotene and Retinol Efficacy Trial (CARET) of 14,000 heavy smokers and >4,000 asbestos-exposed men, mostly white (1). The variables included age, gender, asbestos exposure, smoking history, cigarettes per day, duration of smoking and of cessation. The Bach model has been validated in an independent data from the placebo arm of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study. Our baseline model was based on data derived from an ongoing lung cancer case control study (2). Variables included were environmental tobacco smoke (for never and former smokers only), family cancer history, asbestos and dust exposures, prior respiratory disease, history of hay fever, and smoking history variables. All variables have strong biologically plausible etiologic roles, and are relatively easy to ascertain through patient interview. The validated area under the curve (AUC) statistics for the former and current smoker models were modest (0.63, and 0.58, respectively), although consistent with those from other risk prediction models. If age and smoking status (case-control matching variables) had been included in the models, the models would likely have performed far better.

The Liverpool Lung Project (LLP) variables (3) include smoking duration, prior diagnosis of pneumonia, occupational exposure to asbestos, prior cancer diagnosis and family history of lung cancer. Most recently a carefully constructed model based on data from 70,962 control subjects in the Prostate, Lung, Colorectal, Ovarian cancer screening trial (PLCO) was published (4). This model includes age, SES (education), BMI, family history of lung cancer, COPD, recent chest x-ray, smoking status (never, former, or current), pack-years smoked, and smoking duration. A second model also included smoking quit-time. External validation was performed with 44,223 PLCO intervention arm participants. In the external validation sample, models 1 and 2 had area under the curves of 0.841 and 0.784, respectively. These models had high discrimination in women, men, whites, and nonwhites.

Extended Models:

Functional Data: We have estimated the improvement in model performance by incorporating two measures of DNA repair capacity that have been shown in case-control analyses to be associated with increased lung cancer risk (5). Addition of the biomarker assays does improve the sensitivity of the models over epidemiologic and clinical data alone. These in vitro lymphocyte culture assays, however, are time-consuming and require some level of technical expertise. Therefore while feasible in a controlled academic setting, they are not applicable for widespread population-based implementation.

Genetic Data: Genetic data are stable, inexpensive to ascertain, accurate and amenable to high-throughput analysis. However, it remains uncertain whether enriched multimarker models give better discrimination. We have added three SNPS that were most significant in GWAS of lung cancer to the baseline lung epidemiologic model. These include a replicated SNP in the 15q25 chromosomal region that encompasses the nicotinic acetylcholine receptor subunit genes, CHRNA3 and CHRNA5, that have a defined role in nicotine dependence, and a hypothesized direct role in downstream signaling pathways that promote carcinogenesis. We also included two SNPs from the 5p15.33 locus (rs2736100 and rs401681) that contains two known genes: the TERT (human telomerase reverse transcriptase) gene and the CLPTM1L. The AUC showed only modest improvement. Such small increases in discriminative accuracy are unlikely to be of diagnostic or predictive utility.

Young (6) developed a risk model using a 20-SNP panel including metabolizing, inflammation, DNA repair, anti-oxidant, apoptosis and addiction genes. The final model included the 20 SNPs, age, history of COPD, family history of lung cancer and gender. When numeric scores were assigned to both the SNP and demographic data, and sequentially combined by a simple algorithm in a risk model, the composite score was found to be linearly related to lung cancer risk with a bimodal distribution.

Spira et al (7) have advanced the notion that upper airway gene expression in smokers may serve as a relatively noninvasive surrogate marker of the physiologic response of the lung to tobacco smoke and could be used in large-scale screening and chemoprevention studies for lung cancer. Gene expression profiles in cytologically normal large airway epithelium were obtained via bronchoscopic brushings and were predictive of cancer status in a combined clinicogenomic model ($P < 0.005$). There was a significant improvement in performance of the clinicogenomic relative to the clinical model ($P < 0.05$). Use of the clinicogenomic model may reduce invasive diagnostic procedures for individuals without lung cancer.

The ROC curve may not be sensitive to differences in probabilities between models, and therefore insufficient to assess the impact of adding a new predictor. A substantial gain in performance may not yield a substantial increase in AUC, and only a very large independent association of the new marker with risk will yield a meaningful larger AUC. As an example, the Framingham Risk Score that is widely applied has an AUC of about 0.80. New metrics need to be developed to compare nested models.

In summary, the ability to accurately predict risk of lung cancer among former and current smokers has public health, clinical and financial implications for primary prevention, surveillance programs, screening programs and chemoprevention trials.

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

miRNA and lung cancer: Early detection in high-risk subjects. Gabriella Sozzi[1], Mattia Boeri[1], Carla Verri[1], Luca Roz[1], Paola Suatoni[1], Carlo M. Croce[2], Ugo Pastorino[1]. [1]Fondazione, IRCCS Istituto Nazionale Tumori, Milan, Italy, [2]The Ohio State University, Columbus, OH.

Background: Lung cancer remains the major cause of cancer mortality in the world. In addition to primary prevention, earlier detection and more targeted treatments, tailored on the biological characteristics of the tumor and its microenvironment, could significantly reduce morbidity and mortality for this disease. The real efficacy of lung cancer screening by spiral-computed tomography (CT) in heavy smokers is still to be defined since, in spite of a proved capacity to detect small asymptomatic nodules, the frequency of false positive CTs as well as unnecessary treatments is very high if compared to the limited mortality reductions. The development of biomarkers able to identify tumors in a pre-clinical phase and to track the different aggressiveness of lung tumors is of paramount importance. microRNAs (miRNAs) represent a recently identified class of regulatory molecules and several studies showed that miRNA are involved in lung tumor development and progression and also circulate in plasma and serum of lung cancer patients.

Materials and Methods: We analyzed miRNA profiles of lung tumors, normal lung tissues and plasma samples from cases with variable prognosis identified in two independent spiral-CT screening trials where multiple plasma samples, collected from one to four years before radiological detection of the disease were available.

Results: We found miRNA expression profiles associated with aggressiveness of the disease and poor survival in tumors and also in normal lung tissues of the patients, thus proving the critical influence of a smoking-related lung microenvironment on tumor progression. Specific microRNA signatures were identified in plasma samples collected up to two years before spiral-CT detection of the disease, thus able to catch the earlier biological phases of disease development. We also defined a plasma signature that discriminates subjects according to aggressiveness of their future tumors, and in particular the occurrence of early metastatic but spiral-CT invisible lung tumors or small spiral-CT detected lesions with aggressive potential. Of interest plasma miRNas involved in the signatures of lung cancer risk and of aggressive disease more closely reflected miRNAs expressed in the normal lung rather than those characterizing the tumor samples of patients, supporting the concept that normal lung microenvironment has a critical influence on tumor development and aggressiveness.

Conclusion: These results open up the prospective of using plasma miRNAs as non invasive lung cancer biomarkers.

Plenary Session 5: New Molecular Targets/Novel Therapy

Dual inhibitors of FT and GGT-1 as novel therapeutic agents. Said Sebti. H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

Inhibition of mutant H-Ras farnesylation with farnesyltransferase inhibitors (FTIs) blocks its binding to membranes and its ability to activate oncogenic signaling. In contrast, inhibition of K-Ras farnesylation with FTIs leads to its prenylation by geranylgeranyltransferase I (GGT-1), and therefore, inhibition of K-Ras prenylation and oncogenic function requires blocking both FT and GGT-1. Furthermore, several proteins downstream of K-Ras that mediate its malignant transforming activity also require farnesylation (e.g.Rheb) or gernaylgeranylation (e.g. Ral). In addition, the ability of mutant K-Ras to induce lung cancer in mouse models is severely hampered when both FT and GGT-1 are conditionally deficient. These observations prompted us to design small molecule dual FT and GGT-1 inhibitors.

In this presentation, the development of FGTI-2734 as a novel therapeutic for lung cancer will be described. The presentation will focus on the effect of this dual inhibitor as compared to the selective FTI-2148 and GGTI-2418 on K-ras prenylation, oncogenic signaling and malignant transformation in lung cancer cells that depend on K-Ras.

Redox pathways as platforms for drug development. Kenneth D. Tew. Medical University of South Carolina, Charleston, SC.

While oxygen based metabolism is the most evolved and efficient method for producing energy, the generation of reactive oxygen electrophiles creates physiological stress. Sulfur has variable valencies, and as a consequence its nucleophilicity allows it to react with electrophilic species and exist in a variety of oxidation states. Lung cancer cells, in common with most cancers have aberrant redox homeostasis, a property that has provided a platform for drug development for both therapeutic and prevention approaches. There are >200,000 cysteines encoded by the human genome and the most common non-protein form of sulfur in cells is glutathione (GSH). Glutathione S-Transferase P (GSTP) is one member of the family of GST's that contribute to cellular thiol and redox balance. GSTP is characteristically expressed at high levels in some tumors (including lung) and in drug resistant cancer cells. Targeting of GSTP with small molecules has identified a specific inhibitor (Telintra) that has myeloproliferative properties and may be useful in combination drug regimens. There are also a number of preclinical and clinical (e.g. Telcyta) GSTP activated pro-drug candidates that are at various stages of testing. A recently completed Phase III trial in NSCLC used NOV-002 (a glutathione disulfide mimetic) to alter the toxicity profiles of standard anticancer drugs by modulating intra- and extracellular redox balance. Changes in cellular redox homeostasis play an essential role in numerous signaling cascades, including those associated with proliferation, inflammatory responses, apoptosis, and senescence. Moreover, GSTP has been implicated in catalyzing S-glutathionylation (a post-translational modification adding glutathione to cysteine residues) of a number of proteins that fall into functional clusters important to these signaling events. Recent studies suggest that S-glutathionylated proteins may serve as clinical biomarkers of drug response. Finally, many chemoprevention drugs are designed to stimulate the production of protective Phase II detoxification enzyme systems in target normal tissues. Many of these agents are sulfur based and most contain electrophilic centers that show selectivity for the Nrf2 transcription factor and genes that contain antioxidant response elements (ARE). Chronic, low dose exposure to these agents may have short-term prevention potential, but the down sides of long term impact of low levels of electrophilic stress remains to be answered.

Mechanisms and inhibition of mutant KRAS in non-small cell lung cancer. Garth Powis. The University of Texas MD Anderson Cancer Center, Houston, TX.

KRAS is the predominant form of mutated RAS (mut-KRAS) and is found in 25% of patient tumors across many cancer types. Mut-KRAS is well known to play a critical role in driving tumor growth and resistance to therapy, and its effects are so powerful that it overrides the activity of many of the new molecularly targeted signaling drugs such that they cannot be used in patients with mut-KRAS. However, despite extensive effort, there is no effective treatment for mut-KRAS. The effects of mut-KRAS are mediated through multiple downstream signaling pathways which have been independently associated with tumorigenesis, including Raf1, RalGds, and Pi3k. The recently completed BATTLE-1 clinical trial, a prospective, multi-arm, biopsy-mandated, biomarker-driven, clinical trial in advanced refractory NSCLC, found that mut-KRAS did not accurately predict patient outcome (progression-free survival) to targeted intervention. This finding contradicted published evidence for such a relationship from colon cancer and some

previous NSCLC studies. We explored more specifically the nature of the KRAS mutations, which are primarily found at codons 12 and 13, where different base substitutions lead to alternate amino acid substitutions. NSCLC has a much higher proportion of mut-KRAS G12C(cysteine) substitutions (47%) due to carcinogens in tobacco smoke, and only 15% mut-KRAS have G12D(aspartate). Other solid tumors, such as colon or pancreas, predominantly manifest mut-KRAS G12D (50%) and only 9% mut-KRAS G12C. In a subset analysis of the BATTLE-1 data, we showed significantly worse progression-free survival in patients with mut-KRAS G12C, versus other mut-KRAS including G12D and who were treated with erlotinib, vandetanib or sorafenib. We have found that mut-KRAS G12D activates both Pi3k and Mek signaling, while mut-KRAS G12C does not and alternatively activates Pkcζ and Ral signaling. This finding was confirmed in immortalized human bronchial epithelial (HBEC) cells stably transfected with wt-KRAS or different forms of mut-KRAS. Our molecular modeling studies show that the different conformation imposed by mut-KRAS G12C could lead to altered association with downstream signaling transducers, compared to mut-KRAS G12D. Because there is no effective treatment for mut-KRAS we have also searched for genes that when inhibited will block the growth of mut-KRAS cancer cells without affecting wt-KRAS cell growth using isogenic pairs of cells with and without oncogenic KRAS. We have identified CNKSR1 (connector enhancer of kinase suppressor of Ras 1) as a top hit. CNKSR1 is found associated with KRas in the Ras membrane-associated signaling nanocluster. Knockdown of CNKSR1 with siRNA inhibited the growth of a panel of NSCLC cell lines with mut-KRAS but not NSCLC cell lines with wt-KRAS, and inhibited KRas dependent phosphorylation of Raf1. CNKSR1 is a multidomain protein that has a potentially druggable pleckstrin homology (PH) domain responsible for binding to membrane phosphatidylinositols. We have targeted the PH domain of CNKSR1 and have developed first generation inhibitors that block mut-KRAS signaling and give selective inhibition of mut-KRAS cell growth compared to wt-KRAS cell growth, thus creating a therapeutic potential for patients with oncogenic KRAS.

Plenary Session 6: Bringing Personalized Therapy into the Clinic

Bringing personalized therapy into the clinic: Can pathology deliver? Keith M. Kerr. Aberdeen University Medical School, Aberdeen, United Kingdom.

The rapidly evolving area of personalized medicine in the treatment of non-small cell lung cancer (NSCLC) raises many questions, not the least of which is posed in the title of this abstract. The short answer is ‘yes’, but there are, inevitably, some caveats. But it is essential that Pathology does deliver, in support of this vital and exciting development.

The recognition that some drugs have differential efficacy (pemetrexed) or toxicity (bevacizumab), depending on the histological subtype of NSCLC was a key factor in focusing the lung oncology community on the importance of pathological diagnosis in NSCLC. The rapid emergence of molecular targeted therapies, which appear to be primarily effective against tumors which bear, and are driven by, particular molecular / genetic changes has increased the demand upon Pathology to deliver a molecular as well as morphological diagnosis. This demand will only increase, as more targets are identified. Whilst the assessment of some such predictive markers is a clinical reality right now, the development of clinically applicable prognostic markers, primarily to determine adjuvant therapy is less advanced, but ‘on the agenda’.

The portfolio of possible tests is thus rapidly expanding. In NSCLC, the accuracy and specificity of morphological diagnosis is aided by immunohistochemically (IHC) determined molecular profiles. IHC also has a potential role in identifying some predictive markers, such as ALK, EGFR, MET, mutant EGFR and ERCC1 proteins. Some have advocated an alternative approach to some of these, and other markers; the assessment of

mRNA rather protein. In some instances, the potentially important predictive information appears to be gene copy number (PI3K, C-MET, possibly EGFR or HER2, FGF2 etc), making in situ hybridization (ISH) approaches an important part of testing strategy. ISH is also a key factor in detecting ALK gene rearrangements. Gene mutations are, of course, of key importance; activating mutations of several genes may give rise to tumors ‘addicted’ to a hyperactive tyrosine kinase which can be targeted. Multiplex approaches, allowing the parallel assessment of several gene sequence or copy number alterations are being developed. Multi-gene expression signatures have been used to generate both predictive, but more usually prognostic data which may yet find a secure clinical niche. Multiple different testing modalities may be required, on the same tissue sample.

‘Tissue is the issue’. Tissue diagnosis of lung cancer is particularly challenging, not only because of the complexity and variability in NSCLC morphology, but also because the tissue samples on which diagnosis is made are frequently very small, contaminated by non-neoplastic tissue and sometimes of poor quality. Poor patient cardiorespiratory fitness and tumor location often conspire to limit access to tumor tissue; most patients present with advanced disease so that whole tumor resection is inappropriate. One of the greatest challenges in Pathology is the most efficient and appropriate utilization of this very limited tissue resource. A culture shift around tissue acquisition may be required; away from minimal intervention producing only the bare minimum for diagnosis, towards maximizing tissue yield as safely as possible in each patient. Repeat biopsy may be indicated. Nonetheless, it will not be possible to ‘test for everything’ on these limited samples, for some patients testing will be suboptimal due to inadequate material for testing. Testing algorithms are urgently needed to address the needs of individual patients. Allied to these are questions around who decides which tests should be prioritized? At which point in the investigation cycle should they be performed? Where should these tests be performed and by whom? These points raise further issues relating to communication between oncologists, pathologists and patients. Reimbursement is also an issue.

Does the ‘result’ of the test adequately and accurately reflect the relevant marker status of the patient’s entire tumor burden? The uncomfortable truth is that it may not. Both pathologists and oncologists have a responsibility to understand the limitations of the tests, make certain that the patient is given the best possible chance of an informative diagnosis, and ensure the highest quality in testing methodology and outcome. Tumor heterogeneity and sampling error may conspire to render a non-representative test outcome. Tissue handling and processing may compromise the outcome of the test. Unless great care is taken, the tissue used for extraction of DNA or RNA may not contain sufficient cellular tumor tissue to allow the test methodology in use to provide a relevant result. External quality assurance measures are important to ensure and maintain the highest standards in laboratory practice. Validated, reliable and meaningful testing methods must be employed.

Pathology can and must deliver on this important new aspect of lung cancer diagnosis. Education and training of ALL those involved in the diagnostic process is required – awareness of the issues will help eliminate errors and improve outcomes for patients. Time, technological and tissue resources may be in short supply; collaboration and communication between all parties is essential for the most efficient and effective use of available diagnostic material.

Cetuximab in NSCLC and predictive markers. Fred R. Hirsch. University of Colorado, Aurora, CO.

The role of the EGFR humanized monoclonal antibody cetuximab (Erbitux®, Bristol-Myers Squibb) in the treatment of patients with advanced non-small cell lung cancer (NSCLC) has until recently been controversial. While it is clear that the EGFR pathway plays an important role in NSCLC

development and progression, the clinical data with cetuximab in NSCLC has not been consistent from the different studies including the two large phase III studies (BMS -099 and FLEX), despite the overall survival in the FLEX study was statistically significant in favor the combination of chemotherapy plus cetuximab (HR 0.87 [0.76-1.0]) (1, 2). The FLEX study comparing gemcitabine/ cisplatin with and without cetuximab required EGFR positive tumor (IHC > 1% positive cells) as inclusion criteria, while the non-significant results from BMS -099 was based on "all comers". However, most recently a more detailed analysis of the EGFR protein expression in the FLEX study, by using the H-score (intensity:0-3 x percentage of cells within each intensity category :0-100% for a final H-score of 0-300) was presented (3). Patients having tumors with EGFR H-score≥ 200 (about 30% of the patients) had a significant better survival from chemotherapy plus cetuximab compared to cetuximab alone (HR=0.73 [0.58-0.93]), and even better for the largest subgroup of patients; the Caucasians with H-score ≥ 200 (HR=0.64 [0.49-0.83]) with a 3.5 months difference in median survival (11.4 months vs. 7.9 months HR= 0.49 [0.29-0.81]). Patients with high EGFR expression and adenocarcinoma had a median survival of 20.2 months on the combined therapy versus 8.0 months for chemotherapy alone (3). A reproducibility study of the H-score classification showed a high concordance (91%) among 10 pathologists after specific assessment training (4). A previous reported randomized phase II study comparing chemotherapy with concomitant cetuximab versus sequential cetuximab from SWOG (0342) demonstrated a doubling of PFS and OS in the EGFR FISH positive patients compared to the FISH negative patients (5), while he EGFR FISH assay did not show a significant impact on outcomes in the large randomized BMS -099 study or the FLEX study.

Thus, very encouraging biomarker results are achieved retrospectively for selection of patients to chemotherapy plus cetuximab therapy, and these biomarkers are currently being prospectively validated in the large prospective randomized phase III study SWOG 0819, which compare chemotherapy (+/- bevacizumab) with and without cetuximab.

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Proffered Abstracts

PR1 Integrating expression data improves mutational significance analysis of lung squamous carcinoma. Bryan Hernandez[1], Peter Hammerman[1], Marcin Imielinski[1], Michael Lawrence[1], Petar Stojanov[1], Gad Getz[1], Matthew Meyerson[2]. [1]Broad Institute, Cambridge, MA, [2]Dana-Farber Cancer Institute, Boston, MA.

In contrast to lung adenocarcinoma in which a driving and potentially targetable genetic aberration can be identified in excess of 50% of tumors, only 10% can be found in squamous cell lung cancer, the second most common type of lung cancer, none of which are druggable by an approved targeted therapy. Our limited knowledge regarding targetable genetic alterations in squamous cell lung cancer is the result of a lack of detailed genomic study of this disease and the lack of experimental validation of identified putative therapeutic targets. We have analyzed exome sequencing data to identify novel driving mutations in 184 lung squamous cell carcinomas using previously successful methods (Integrated genomic analyses of ovarian carcinoma, Nature 2011), but these analyses are confounded by an unusually high mutation rate, 8-9/Mb on average, some reaching as many as 80/Mb. Many of the most significantly mutated genes in the analysis are unlikely related to the pathogenesis of the disease based on a lack of expression in lung and known role in cancer, suggesting that genes which are not expressed (or minimally expressed) escape negative selection and result in a higher mutation density as compared with expressed genes.

Here, we present a method integrating expression and mutation data in order to more accurately determine the significance of a mutated gene based on the expression subclass in which it falls and the frequency at which it's mutated relative to our expectation; unexpressed genes are likely much more tolerant of mutations and should therefore have a higher expected background mutation rate. Analyses of this kind have removed many of the genes putatively irrelevant in this cancer type and have identified many biologically plausible novel genes that to date are unlinked with lung squamous cell carcinoma. We believe this method refines an already powerful analysis for identifying significantly mutated genes in cancer with a particularly high potential for separating signal and noise in the most highly mutated cancer types.

This abstract is also presented as Poster A17.

PR2 The genetic status of the EGFR, K-ras, and EML4-ALK genes in multiple primary noninvasive lung adenocarcinomas. Kenji Sugio, Takashi Seto, Yukito Ichinose, Kenichi Taguchi, Kaname Nosaki, Taro Ohba, Gohshi Toyokawa, Fumihiko Hirai, Masafumi Yamaguchi, Riichiroh Maruyama, Motoharu Hamatake. National Kyushu Cancer Center, Fukuoka, Japan.

Backgrounds: The development of high-resolution CT (HRCT) has enabled the detection of small-sized lung tumors and sometimes multiple tumors. In resected specimens, preinvasive lesions proposed as adenocarcinoma in situ (IASLC/ATS/ERS 2011) are often observed, and these lesions are recognized as primary lung cancer. Formerly, these lesions were classified as non-mucinous bronchioloalveolar carcinoma (BAC), of which non-invasive tumors were classified as Noguchi's type A or B. The mutations of EGFR, K-ras and ALK are defined as so-called driver mutations, which drive tumor formation and maintenance. We analyzed the genotype of the EGFR and K-ras genes, and the expression of EML4-ALK fusion gene in synchronous multiple non-invasive adenocarcinomas, to evaluate the possibility of multicentric carcinogenesis.

Patients and Methods: There were 9 patients with synchronous multiple adenocarcinomas, 20 mm in diameter or less, who underwent

surgery from March 2005 to July 2009. In total, 26 lesions were diagnosed as non-invasive adenocarcinoma. In these 9 patients, 2 tumors were detected in 5 cases, 3 tumors in 2 cases, and 5 tumors in 2 cases. A PCR-based fragment analysis was used as a screening method to detect, and direct sequencing was used to confirm the presence of EGFR (exon 19, 21) and K-ras mutations in macro-dissected materials from paraffin-embedded sections. The expression of EML4-ALK fusion gene was examined by an immunohistochemical analysis.

Results: Seventeen of the 26 tumors (65%) had an EGFR or K-ras mutation, and nine tumors had wild type. In the EGFR gene, an exon 19 deletion (ΔE746-A750) was detected in 11 tumors, and an exon 21 L858R mutation was detected in 4 tumors. A K-ras codon 12 mutation (G12D) was detected in 2 tumors. Two patients had the same EGFR 19 deletion in all of their tumors, and one had the same K-ras codon 12 mutation in all of their tumors. In one patient, 2 of their 3 tumors had the same EGFR 19 del mutation. In another patient with 5 tumors, 2 tumors had the L858R mutation, 2 had a different type of 19 del mutation, and one showed wild-type of EGFR. In one patient, one tumor showed a 19 del mutation and the other showed a L858R mutation. In 3 cases, same mutation was not detected in their tumors. Therefore, 5 of 9 cases (56%) had the same type of mutation in their multiple tumors. The expression of EML4-ALK, as determined by immunohistochemistry, was not detected in any of the tumors.

Conclusion: The EGFR and K-ras genes, which are mutually exclusive, can be used to define clinically relevant molecular subsets of lung adenocarcinoma, and can also define tumor clonality. In several patients in this study, multiple tumors which were defined as pathologically non-invasive tumors, showed the same genetic mutation. These findings demonstrate that multicentric carcinogenesis under the same genetic backgrounds occurs in lung adenocarcinoma.

This abstract is also presented as Poster A40.

PR3 NSCLC and SCLC mouse models mediated by lentiviral gene delivery. Yifeng Xia, Narayana Yeddula, Mathias Leblanc, Reuben Shaw, Inder Verma. Salk Institute for Biological Studies, La Jolla, CA.

Lung cancer, the leading cause of cancer deaths, is responsible for 1.3 million lives lost worldwide every year. Good animal models that can faithfully recapitulate the human disease are in great need for pre-clinical studies. Based on the different responses to treatment, lung cancer is divided into two major classes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). We have developed a novel lentiviral gene delivery system to study the initiation and development of both types of lung cancer. We use lentiviral vectors to establish lung tumors in mice for the following reasons: 1) lentiviruses infect almost any type of cells, and transgene expression can be controlled by a tissue-specific promoter, which allows a more precise tracing of the origin of the cancer cell; 2) lentiviruses integrate into genomic DNA so that it is possible to stably deliver oncogenes and shRNAs against tumor suppressors, and bypass the requirement of numerous conventional genetic crossings; 3) viral titers can be controlled so as to infect only a few cells, in order to more precisely recapitulate human cancer initiation.

We used CA2Cre-shp53 lentiviral vector to initiate lung adenocarcinoma in KrasG12D mice and studied the role of NF-κB/IKK2 in tumor development. We found that IKK2 depletion in tumor cells significantly attenuated tumor proliferation and significantly prolonged mouse survival. We identified Timp-1 (a tissue inhibitor of metalloproteinases), one of the NF-κB target genes, as a key mediator for tumor growth. Timp-1 binds to its receptor CD63, activates Erk signaling pathway and stimulates cell proliferation. Knockdown of either IKK2 or Timp-1 by shRNAs reduced tumor growth in both xenograft and lentiviral models, suggesting the possible application of IKK2 and Timp-1 inhibitors in treating lung cancer. Due to the safety concerns of systemic NF-κB inhibition, IKK2 inhibitors haven't been applied to the

clinical trials. Our identification of Timp-1 may serve as an alternative target for blocking tumor proliferation. Timp-1 neutralizing antibodies are currently being tested to treat mice carrying Kras adenocarcinomas.

SCLC is the most malignant form of lung cancer with a five-year survival less than 6%. A mouse model with p53/Rb deletions activated by Adeno-Cre has been established in Berns lab. To improve the model and study other genes that are frequently mutated in human SCLC, we designed a single lentiviral vector to deliver oncogenes (L-myc) and shRNAs against tumor suppressors (p53, Rb etc) and successfully initiated SCLC in wild-type mice. Furthermore, we included luciferase or fluorescence protein genes in the same vector to follow tumor growth and metastasis in the live animal. Using this new model, we are now studying the crosstalk of multiple mutations in the SCLC development.

We hope that our unique lentiviral tool can simplify and accelerate the lung cancer study to understand the big signal pathway complex leading to tumorigenesis in the lung and search for new therapies for this deadly human disease.

This abstract is also presented as Poster A45.

PR4 The tumor suppressor role of Wnt/β-Catenin pathway in development of small cell lung cancer. Kwon-Sik Park, Jing Shan Lim, Andrew Singh, Julien Sage. Stanford University, Palo Alto, CA.

Lung cancer is the leading cause of cancer deaths in the United States and worldwide. Among the different types of lung cancer, small cell lung cancer (SCLC) is the deadliest form with a 5-year survival rate of less than 5%. No efficient therapeutic strategy exists for SCLC except for the rare cases when the disease is detected early. Although some of the genes commonly mutated in human SCLC are known, including the *RB* and *p53* tumor suppressor genes, the molecular mechanisms of SCLC initiation and progression remain still only partially understood thus hampering the development of novel targeted therapies as well as the ability for early detection. Growing evidence shows that signaling pathways important for organ development are frequently (re-) activated and play crucial roles during tumorigenesis. Because Wnt/β-Catenin signaling plays important roles in lung development and has been implicated in non-small cell lung cancer, we have investigated the potential role of the Wnt/β-Catenin pathway in SCLC using a mouse model, *Rb/p53* mutant mice. In this model, the inactivation of *Rb* and *p53* genes conditionally in the lung epithelium results in the development of tumors that recapitulate the histopathological and molecular features of human SCLC. Using the compound Rb/p53 mutant mice crossed with the Wnt pathway reporter line, Axin2$^{lacZ/+}$ mice, we found that Wnt signaling is active in Rb/p53 mutant SCLC cells, including the earliest detectable lesions. We also found that nuclear localization of β-Catenin, indicative of the pathway activity, was increased in early lesions and fully-grown tumors, as well as in mouse primary SCLC cells. To determine the roles of the Wnt/β-Catenin pathway in tumor progression, we performed an in vivo gain-of-function experiment in which activity of Wnt/β-Catenin signaling was increased by deletion of *Axin2*, a negative regulator (and also a target gene) of the canonical Wnt pathway. We observed reduced tumorigenesis in *Rb/p53/Axin2* mutant mice compared to *Rb/p53* mutant mice. Similarly, treatment with LiCl, a chemical known to increase Wnt/β-Catenin activity, reduced the growth of SCLC cells in culture. We are currently conducting various loss-of-function and gain-of-function experiments to modulate Wnt pathway activity in mice to further determine the role of this pathway in SCLC. Together, these findings support the novel concept that the Wnt/β-Catenin pathway, unlike its oncogenic role in many tumors, may play a tumor suppressor role in SCLC development and maintenance. These findings identify a novel therapeutic strategy to treat this devastating cancer.

This abstract is also presented as Poster B25.

PR5 A phase II study of sorafenib in patients with stage IV non-small cell lung cancer (NSCLC) with a K-Ras mutation. Wouter W. Mellema[2], Anne-Marie C. Dingemans[1], Harry J.M. Groen[3], Atie van Wijk[2], Sjaak Burgers[4], Peter W.A. Kunst[5], Frederik B. Thunnissen[2], Daniëlle A.M. Heideman[2], Egbert F. Smit[2]. [1]Maastricht University Medical Center, Maastricht, The Netherlands, [2]VU University Medical Center, Amsterdam, The Netherlands, [3]University Medical Center Groningen, Groningen, The Netherlands, [4]Netherlands Cancer Institute, Amsterdam, The Netherlands, [5]Academic Medical Center, Amsterdam, The Netherlands.

Background: In a pilot study (1) we found sorafenib to display clinical activity against patients with K-Ras positive NSCLC, sufficient for formal phase II testing. Methods: Patients with K-Ras mutated NSCLC that progressed after at least 1 platinum containing regimen with adequate organ reserve, ECOG 0-2, who provided written informed consent according to local IRB regulations were eligible. A tumor biopsy confirming the presence of a K-Ras mutation was mandatory. Treatment consisted of sorafenib 400 mg BID until disease progression or unacceptable toxicity. Dose reductions and delays were specified per protocol in the face of CTC toxicities grade 3 and 4. Primary endpoint: Rate of No Progression (NPR) at 6 weeks. Secondary endpoints: duration of response, progression free survival (PFS), overall survival and treatment related toxicities. A 2-stage design was implemented (Simon's optimal design; p0=40%, p1=60%, alpha=0.05, beta=0.20) for a total number of 48 pts. Results: 59 patients were entered between May 1st 2010 and February 18 2011. Median age was 58 (range 46-79) years, 17 Male / 42 Female, ECOG PS 0/1/2 23/32/4. 57 patients started treatment. At 6 weeks 5 PR, 25 SD, 27 PD were observed; NPR 52.6%. Fifteen patients stopped treatment before 6 weeks, of which 10 patients stopped due to (clinical) progression. Median duration of treatment was 9 (range 0-62) weeks, 2 patients are still on treatment. Median duration of response was 32 (range 5-58) weeks. Median PFS was 2.3 months, median OS was 4.9 months, 14 patients still alive. Dose modifications were realized in 21 patients, of whom 4 discontinued treatment. Most reported adverse events were fatigue (6.4%), hand-foot reaction (5.7%), dyspnea (5.6%), anorexia (3.7%) diarrhea (3.6%) and cough (3.6%). Sorafenib related grade 3-4 toxicity was reported in 10 patients. Five patients with grade 3 skin toxicity, 4 patients with grade 3 gastrointestinal toxicity and 1 patient with both grade 3 metabolic abnormalities and a grade 3 pneumonitis. Conclusion: Treatment with sorafenib has relevant clinical activity in patients with K-Ras mutational status. Further randomized study with this agent is warranted.

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This abstract is also presented as Poster B18.

PR6 Phase 1b/2 trial of HER3 inhibitor U3-1287 in combination with erlotinib in advanced NSCLC patients (pts): HERALD study. Joachim von Pawel[1], Barbara Lueps[1], Jennifer Tseng[2], Catherine Copigneaux[3], Robert Beckmann[3]. [1]Asklepios Fachkliniken Munich-Gauting, Gauting, Bavaria, Germany, [2]MD Anderson Cancer Center Orlando, Orlando, FL, [3]Daiichi Sankyo Pharma Development, Edison, NJ.

Background: EGFR is frequently overexpressed in NSCLC, and while many advanced NSCLC tumors initially respond to EGFR tyrosine kinase inhibitors (TKIs), development of therapeutic resistance often follows. HER3 is a key dimerization partner of HER family members, including EGFR, and activates oncogenic signaling pathways. HER3 overexpression occurs in many solid tumors and is associated with poor prognosis in lung cancer pts. Data indicate that HER3 expression may play a role in EGFR TKI resistance, suggesting that simultaneous inhibition of HER3 and EGFR may be beneficial. U3-1287 is a fully human anti-HER3 monoclonal antibody with synergistic anticancer activity in combination with anti-EGFR inhibitors in preclinical models. The phase 1b/2 HERALD trial was initiated to

investigate the combination of U3-1287 with erlotinib in the treatment of advanced NSCLC pts after failure of at least 1 prior chemotherapy. Results as of August 12, 2011 are reported here.

Methods: Eligible pts had stage IIIB/IV NSCLC that progressed on ≥1 prior chemotherapy treatments and were EGFR treatment-naive. In the open-label, phase 1b portion, pts received erlotinib 150 mg/day orally and U3-1287 18 mg/kg intravenously every 3 weeks (Q3W). In the event that 18 mg/kg was not tolerated based on DLT assessment, sequential cohorts were to receive de-escalating doses of U3-1287. As no DLTs were reported, the recommended phase 2 dose is 18 mg/kg Q3W for U3-1287 in combination with 150 mg/day erlotinib. The phase 2 portion of the study is a randomized, placebo-controlled, double-blind study assessing the efficacy and safety of U3-1287 combined with erlotinib relative to erlotinib alone. It is a 3-arm study of 150 mg/day erlotinib with U3-1287 high-dose (18 mg/kg Q3W), U3-1287 low-dose (18 mg/kg loading dose followed by 9 mg/kg Q3W), or placebo. Study end points include adverse event (AE) incidence, pharmacokinetics, human antihuman antibody (HAHA) formation, tumor response, and progression-free survival (PFS).

Results: The phase 1b portion of the trial enrolled 7 pts (4 male), with a median age of 68 years (range, 48–78). There were no reported DLTs. Erlotinib-related AEs reported in ≥2 pts were rash (6 pts), diarrhea (4), dry skin (3), decreased appetite (3), stomatitis (3), dehydration, dermatitis acneiform, dysgeusia, mucosal inflammation, nausea, and skin exfoliation (2 each). The only U3-1287–related AE reported in ≥2 pts was decreased appetite (2 pts). AEs grade ≥3 occurred in 2 pts: one grade 3 case each of pain, fatigue, headache, dehydration, diarrhea, and blood creatinine increase; none were related to U3-1287. Three pts had 3 serious AEs: grade 3 pain (unrelated to study treatment), grade 3 dehydration (erlotinib-related), and grade 1 decreased appetite (erlotinib- and U3-1287-related). All seven pts tested negative for HAHA formation after drug administration. As of Aug 12, 3 pts have ended study treatment due to disease progression. Four pts had best responses of stable disease lasting 86, 87, 90, and 117 days. As of Aug 12, in the phase 2 portion, 11 pts (4 male) have been screened; first study treatment was June 21. Pts had a median age of 70 years (range, 49–83). There have been 2 serious AEs in 1 pt, both unrelated to study treatment: grade 2 cardiac disorder requiring hospitalization and death due to multi-organ failure. Treatment-related AEs reported were diarrhea (3 pts), rash (3), cardiac disorder, decreased appetite, nausea, and vomiting (1 each); all were grade 1 or 2.

Conclusions: Results across the phase 1b and 2 portions indicate that U3-1287 in combination with erlotinib is generally well tolerated.

This abstract is also presented as Poster B38.

A1 microRNAs expression profile associated with radioresistance in lung cancer. Elena Arechaga-Ocampo[1], Yamilet Noriega-Reyes[1], Cesar Lopez-Camarillo[2], Perla Lopez-Moreno[2], Carlos Perez-Plasencia[3], Oscar Angeles-Zaragoza[1], Luis A. Herrera[3]. [1]Instituto Nacional de Cancerologia, Mexico City, Mexico, [2]Universidad Autonoma de la Ciudad de Mexico, Mexico City, Mexico, [3]Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.

Lung cancer is the neoplasia with highest incidence and mortality worldwide. One of the strategies of treatment for this tumor type is based on ionizing radiation. Radiation induces cytotoxicity through some mechanisms, including gene expression changes. Recently, microRNAs (miRNAs) have been described as one of the molecules that regulate expression genes and may contributes to radioresistance in several tumor types. Global miRNAs expression profiles of radiosensitive and radioresistant lung tumor cells may elucidate the molecular mechanism related to tumoral radioresistance. In order to identify miRNAs associated with innate and acquired radioresistance, we will evaluate the miRNAs expression profiles in two lung carcinoma cell lines and in the same cell lines with acquire radioresistance by fractionated doses radiation treatment. For establish the median lethal dose radiation, H1944 and Calu-1 lung carcinoma cell lines were treated with increasing doses of X-rays (from 2 to 12 Gy). Cell survival, proliferative ability and clonogenic potential postradiation were evaluated. Moreover, we will generate a model of acquired radioresistance by treating lung tumor cells with fractionated doses of 2 Gy X-rays up to total dose of 60 Gy for to evaluate the expression of miRNAs in cells with acquired radioresistance. Results showed differential sensitivity to radiotherapy from lung tumors cells, whit 8 Gy as median lethal dose radiation. For to evaluate the expression of miRNAs during innate response to radiotherapy, total RNA was obtained from tumor cells treated with 8 Gy of X-rays. Additionally, it has been obtained lung tumor cells clones with treatment total doses of 28 Gy in a fractionated radiation scheme. These results will allow compare miRNAs expression during immediate cell response to radiation and those whose expression will modified during fractionated radiation treatment and may be associated with radioresistance acquired, which contribute to understanding the molecular mechanism to radioresistance in lung cancer.

A2 Cells of origin of the different subtypes of lung cancer. Caitlin Filby[1], Kati Viitaniemi[1], Jonathan McQualter[2], Philip Antippa[3], Lou Irving[3], Ivan Bertoncello[2], Marie-Liesse Asselin-Labat[1]. [1]The Walter and Eliza Hall Institute, Parkville, Australia, [2]The University of Melbourne, Parkville, Australia, [3]The Royal Melbourne Hospital, Parkville, Australia.

Lung cancer is the leading cause of cancer death worldwide. Five-year lung cancer survival is only 15% and lung cancer is responsible for more deaths than prostate, colon, pancreas, and breast cancers combined.

Understanding the cellular and molecular mechanism at the origin of lung cancer will generate great insights for better management of the disease. However, the lack of cell surface markers to identify and isolate early progenitor or stem cells in the normal lung represents a gap of knowledge that needs to be filled to identify cells of origin of lung cancers. By macroscopic isolation of different morphological region of the human lung and staining with cell surface markers, we have isolated distinct cell subpopulations by flow cytometry. Interestingly, these markers are expressed in some types of lung cancers but not others. We are characterizing the different subpopulations by immunostaining of cytospun cells and evaluating their in vitro colony formation capacity. In order to address

potential cells of origin for the different lung tumor subtypes, gene signatures of the different normal epithelial subpopulations will be compared to the gene expression profiles of lung carcinomas available from public database.

These cell surface markers may be critical tools to identify stem/progenitor cells in the normal lung that could be the cell of origin of one type of lung cancer and not others.

A3 Fibroblast-specific Foxm1 is essential within premetastatic niche for lung cancer metastasis. David Balli, Yufang Zhang, Vladimir V. Kalinichenko, Tanya V. Kalin. Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

The lung microenvironment is essential for supporting the colonization and survival of disseminating tumor cells. Lung stromal fibroblasts produce various growth factors and extracellular matrix components that support the formation of a pre-metastatic niche required for successful colonization. However, the transcriptional regulation of these fibroblast functions remains largely unexplored. We have previously identified that the transcription factor Foxm1 has important roles within the lung tumor microenvironment. Whether Foxm1 within Fsp1+ fibroblasts is required for metastatic seeding of tumor cells has not been determined. In this study, we generated transgenic mice in which Foxm1 was deleted from lung fibroblasts (*Fsp-cre*$^{tg/-}$/*Foxm1*$^{fl/fl}$: Fsp-Foxm1 -/-). To model metastatic colonization of the lung, B16-F10 melanoma cells were injected into experimental and control mice either subcutaneously or intravenously. In these models, metastatic lesions develop in the lung 28 days and 21 days after injection, respectively. Preliminary studies determined that deletion of Foxm1 from Fsp1+ fibroblasts decreased metastatic seeding and tumor formation following both subcutaneous and intravenous injection of melanoma cells. Expression of *Sdf-1* and *Tenascin-C*, both important regulators of lung metastasis, were significantly down regulated in lungs of Fsp-Foxm1 -/- mice. These data demonstrate that Foxm1 is novel regulator of the pre-metastatic niche and is required within fibroblasts to promote metastatic colonization.

A4 An expression map of long noncoding RNAs in human lung and non-small cell lung cancer. Daiana D. Becker-Santos, Ewan E. Gibb, Emily A. Vucic, Katey S.S. Enfield, Greg L. Stewart, Calum E. MacAulay, Stephen Lam, Wan L. Lam. BC Cancer Research Centre, Vancouver, BC, Canada.

Background: Although still largely unexplored, long non-coding RNAs (lncRNAS) are emerging as an integral functional component of the human transcriptome. LncRNAs are mRNA-like transcripts of at least 200 nucleotides (nts) with no protein-coding capacity. Similar to their protein-coding counterparts, lncRNAs are frequently spliced and polyadenylated, but act at the RNA level. The range of functions described for lncRNAs is extensive, and includes key biological roles in chromatin remodeling, alternative splicing and mRNA degradation. Given their biological functions, dysregulation of lncRNAs is rising as an important feature of many disorders, including malignant transformation. However, the extent of the contribution of differential lncRNA expression to normal lung tissue and lung cancer has not been investigated in a comprehensive manner.

Hypothesis: We hypothesized that lncRNAs are expressed in a lung tissue-specific manner and that non-small-cell lung cancer (NSCLC) exhibits aberrant lncRNA expression patterns.

Methods: Serial Analysis of Gene Expression (SAGE) libraries were used to characterize polyadenylated transcripts in lung tissue compared to a panel of 25 different normal human tissues, and to a cohort of 12 NSCLCs. To generate lncRNA expression profiles, we developed a lncRNA discovery pipeline to map-tag-to-lncRNA matches. To identify differentially expressed lncRNAs we used a permutation test based statistical analysis. Expression pattern in lung tumors were compared to profiles from a variety of cancer types in order to identify lncRNA

changes prominent in lung cancer.

Results: Here we show that large-scale expression profiling through SAGE, is an effective resource for investigating the expression pattern of polyadenylated lncRNAs. Applying a novel lncRNA discovery pipeline we reveal extensive, tissue-specific lncRNA expression in normal lung compared to a panel of several different normal human tissues. Importantly, our study reveals that NSCLC demonstrate significantly altered lncRNA expression patterns and identify highly dysregulated transcripts associated with this malignancy as oppose to other types of cancer.

Conclusion: Collectively, our findings support an important role for tissue-specific lncRNAs in lung cancer. Characterization of the functional role of these transcripts will have a considerable impact on our understanding of lung cancer development and progression, and may reveal clinically important biomarkers.

A5 Characterizing and targeting B cells in lung cancer patients. Tullia C. Bruno[1], Jeffrey Kern[2], Jill E. Slansky[1]. [1]University of Colorado--Denver, Denver, CO, [2]National Jewish Health, Denver, CO.

Lung cancer is the leading cause of cancer death in men and women, both in the United States and the world. The current 5-year survival rate for lung cancer in the United States for all races is a discouraging 16% and has not changed dramatically during the past 30 years. The biggest reason for the discouraging survival statistics is that the majority of lung cancer subjects present with late-stage disease that is not curable by current therapies i.e. chemotherapy and radiation. However, in the last several years novel therapies have emerged to make lung cancer therapy better tolerated and more effective. As tumor immunologists, we are particularly interested in developing an immunotherapy that will help to engage the immune system in targeting and destroying tumor cells. In a comprehensive survey of the immune infiltrate in non-small cell lung cancer (NSCLC) patients, we discovered that the frequency of the B cell infiltrate in the tumor versus the normal tumor-adjacent tissue was significantly increased in comparison to other immune subsets, specifically, CD4, CD8, and regulatory T cells as well as NK and NKT cells. Further preliminary studies on the characterization of these B cells suggest that they have proliferated and acquired a memory phenotype. We will continue to characterize the B cells in the tumor microenvironment to determine if they are activated as well as functional. If the B cells in the tumor microenvironment are activated, we will test if the cells are responsive to antigens currently in clinical trial like MUC-1 as well as new antigens in the cancer-testis classification like XAGE-1b. We will test if the antigens are stimulating the B cells through the BCR and if these antigens will be presented to CD4 T cells in vitro, which would lead to the stimulation of the CD4 T cells. If the B cells are not activated, we will then query whether or not the B cells could have a suppressive phenotype, which will be determined by assaying the types of cytokines produced by the B cells using a cytokine array. As we continue to further characterize the B cells, we will also continue to monitor the various immune subsets in the three subtypes of NSCLC: adenocarcinoma, squamous cell carcinoma and large cell carcinoma. This immunophenotyping of the immune subsets will aid us in the identification of the appropriate immune cell populations to target for immunotherapy in the three subtypes of NSCLC. Ultimately, we aim to identify a novel target for an immunotherapy clinical trial in lung cancer patients.

A6 Transcriptomic analysis of the lung cancer stem/initiating cells from patient with lung adenocarcinoma. Huei-Wen Chen[1], Wan-Jiun Chen[1], Chao-Chi Ho[2], Hsuan-Yu Chen[3], Sung-Liang Yu[1], Jeremy JW Chen[4], Pan-Chyr Yang[1]. [1]National Taiwan University Medical College, Taipei, Taiwan, [2]National Taiwan University Hospital, Taipei, Taiwan, [3]Academia Sinica, Taipei, Taiwan, [4]National Chung-Hsing University, Taichung, Taiwan.

Cancer stem/initiating cells (CSICs), with stem cell-like properties in self-renewal and differentiation, have been suggested to be responsible for carcinogenesis, drug resistance, tumor recurrent, and metastasis, these matters strongly affect the clinical outcome. Accumulated evidences indicated the importance of CSICs in tumor formation and successfully isolated the CSICs from patients or cell lines with different cancer types; however, this has not been well explored in lung cancer. Not alike previous isolation through specific markers and quiescent condition, we use autologous intramural tumor-associated stroma cells as the feeder cells to create the microenvironment for culturing and maintaining the tumor sphere-like lung CSICs from patient with lung adenocarcinoma. Here, we show that the cultured lung CSICs (LCSICs) (Nanog+/Oct4+/Sox2+/ Klf4+ cells) with relative higher percentages of side population (27.4%), ALDH activity (14%), and colony formation ability as comparing to the other lung cancer cell lines (A549, CL1-5, H522, H23, EKVX and Hop62). Also, the LCSICs could generate adenocarcinoma with highly metastatic ability both in orthotropic and subcutaneous models in severe combined immunodeficient (NOD/SCID) mice as less as 50 cells. The stemness and tumorigenecity could be reduced after removing the feeder cells; whereas, the LCSICs might differentiate into cancer cells with adenocarcinoma types. These “differentiated” adenocarcinoma cells with less chemo-resistance to docetaxel, cisplatin, etoposide, and vinorelbine distrate; the ALDH activity was reduced to only 1.2% (14% in LCSICs), and cannot form the tumor in NOD/SCID mice under 1,000 cells. Through transcriptomic analysis, several stemness factors and pathways are highly regulated on LCSICs comparing to the differentiated cancer cells, including Notch, WNT, EGF, TGF-beta, bFGF and BMPs signaling. Several key regulators (kinectin 1, interleukin 6 signal transducer, jagged 2, Notch homolog 2 N-terminal like, Nipped-B homolog, transforming growth factor beta regulator 1, EGR-1, E2F, POU class 2 homeobox 1, SMAD family member 2, transferrin receptor 2, Oct-4, and nanog) and related signaling (Akt and PI3K) were confirmed and validated via Q-PCR and in vitro analysis. Our studies established a model of lung cancer stem/initiating cells in vitro and this system could be maintained with the feeders. This model should be very useful and helpful for studying the roles of LCSICs on carcinogenesis in lung cancer and also for developing novel therapeutic strategies targeting on lung cancer stem cells.

A7 NOTCH 1 and NOTCH 3 expressions for early stage of non-small cell lung cancer. Eun Kyung Cho, Jae Hoon Lee, Seung Yeon Ha, Sanghui Park, Jae-ik Lee, Junshik Hong, Minkyu Jung, Sun Jin Sym, Jinny Park, Dong Bok Shin. Gachon University Gil Hospital, Inchon, Republic of Korea.

Backgrounds: Notch gene Encode 300kDa single-pass transmembrane receptors. It can variously serve as an oncogene or a tumor suppressor and a repressor or inducer of terminal differentiation. It also regulates cell division, differentiation and survival of stem and/or progenital cells in wide range tissues. We examined Notch 1 and 3 to study the correlation of Notch expression and prognosis for non-small cell lung cancer.

Methods: Paraffin-embedded tissue microarrays were constructed. We analyzed Notch 1 and 3 by immunohistochemical stain on surgical tumor specimens.

Results: Results: One hundred fifty-nine of 185 resected tissues for lung cancer

(median age 62 years) were available for immunohistochemical stain. The types of tumor histology were adenocarcinoma; 54, squamous cell carcinoma:79, others;26. 121 patients were taken lobectomy and 38 were done pneumonectomy. Notch 1 and 3 receptors were detected in 119 (74.8%) and 130 (81.7%) of total 159 (M:F=114:45) tumor tissues, respectively. Notch 1 receptors showed higher expressions on squamous cell carcinoma than non-squamous cell carcinoma (58.8% vs. 37.8%, p=0.03). However, it was not correlated with sex, smoking status or advanced stages. Five year-relapse free survival (RFS) and overall survival (OS) were 57.4 % and 59.3 % in 159 patients, respectively. Patients with any expression of Notch1 or 3 have correlated with neither RFS nor OS. 5 year OS for patients who showed Notch 3 expression were 62 % and the others were 47.8%, however, it was not statistically significant (p= 0.165). Both of their expressions were detected in 106 of 159 (66.7%) tumor tissues. Sixteen (10 %) had negative expressions in both of them. The patients with both positive expressions had the tendency of longer OS than both negative patients. Their 5 year OS were 60.8 % vs. 38.2% (p=0.068) and 5 year RFS were 56.5% vs. 49.7 % (p=0.35).

Conclusions: Notch 1 showed higher expression on squamous cell carcinoma. Neither Notch 1 nor 3 have correlated with RFS or OS, but patients with both expressions had the tendency of longer overall survival.

A8 Mutation mapping in non-small cell lung cancer: A meta-analysis by geography and histology (MutMap). Simon P. Dearden[1], James Stevens[1], Yi-Long Wu[2], David Blowers[1]. [1]AstraZeneca, Macclesfield, United Kingdom, [2]Guangdong Lung Cancer Institute, Guangzhou, China.

This study comprises a meta-analysis of publicly available and newly generated mutation data in non-small cell lung cancer patients to identify differences between histological subtypes and geographical populations. In addition to mutations in individual genes, comparisons are also made between mutation co-occurrences in these subgroups. These patterns of mutation co-occurrence have been termed The MutMap.

Publications reporting mutation data on two or more genes, in histologically and geographically defined populations were identified. Assessments of single gene mutation frequencies and 2-gene mutation co-occurrences were performed in Western and Eastern populations. These were further separated into adenocarcinoma and squamous cell carcinoma histologies.

In total, 21 studies were identified which matched the criteria above. In addition, data were generated at AstraZeneca and the Guangdong Lung Cancer Institute for inclusion in the meta-analysis. Mutation data were collated for 8 genes (EGFR, KRAS, TP53, LKB1, BRAF, PIK3CA, PTEN and ERBB2) in the 4 subgroups. Overall, mutations were more commonly reported in adenocarcinoma cases than squamous cell carcinoma cases. The most commonly mutated gene in NSCLC was TP53, however, mutation frequencies varied between Eastern and Western populations and between adenocarcinoma and squamous cell carcinoma cases. Mutations in EGFR were common (46.3%) in Eastern adenocarcinoma patients but rarer in Western cases (14.8%). Conversely KRAS mutations were more common in Western adenocarcinoma patients (25.7%) than Eastern cases (11.2%). Mutations in LKB1 were also more common in Western patients. Mutations in BRAF, PIK3CA and ERBB2 were rare in all NSCLC subgroups. Mutations in PTEN were common in Eastern squamous samples (9.8%) but rarer in the other 3 subtypes.

Co-occurrences between mutations in 2 genes were also examined. As reported in other studies, mutations in EGFR and KRAS were exclusive to each other. In addition, in Western adenocarcinoma patients, mutations in both EGFR and LKB1 occurred significantly less often than would be expected by chance. There were several 2-gene mutation co-occurrences which were detected at levels >=5% of the NSCLC population, including TP53 with either KRAS or EGFR

in adenocarcinoma and TP53 with LKB1 mutation in squamous cell carcinoma.

Overall, this study confirms and expands the observation that mutation prevalence in NSCLC is complex, with variation not only between histological subtypes, but also between geographical populations. The co-occurrence of mutations may also be important in future treatment paradigms as clinically relevant segments of NSCLC possess both activating oncogenic driver mutations and mutations in tumour suppressor genes.

A9 Pharmacological stimulation of DLL1-Notch signaling as an antitumor immunotherapy. Yuhui Huang[1], Luping Lin[2], Asel K. Biktasova[2], Anshu Malhotra[3], Anil Shanker[3], David P. Carbone[2], Mikhail M. Dikov[2]. [1]Harvard University, Boston, MA, [2]Vanderbilt University, Nashville, TN, [3]Meharry Medical College, Nashville, TN.

Deficiencies in immune function are one of mechanisms for tumor escape from host immune surveillance, and therapies aimed at overcoming these defects are of major importance in cancer immunotherapy. However, the underlying causes of these defects are multiple and remain not fully understood. We report a previously undescribed mechanism for tumor-associated defects in T cell function mediated by reducing levels of Delta-like (DLL) 1 and DLL4 in the hematopoietic microenvironment, and show that elevated levels of circulating vascular endothelial growth factor (VEGF) characteristic for cancer patients is one of the mediators of this effect. Remarkably, selective activation of DLL1-Notch signaling alone by over-expression of DLL1 in bone marrow precursors or by soluble multivalent DLL1 treatment significantly enhances tumor-specific immune responses and inhibits tumor growth in multiple tumor models. The data suggest that tumor growth suppresses T cell differentiation and function via inhibition of DLL-mediated Notch signaling in the hematopoietic compartment. Our findings uncover a novel molecular mechanism of immune suppression and suggest a pharmacologic intervention based on activation of DLL1-Notch signaling to reverse this effect, stimulate immune responses and suppress tumor growth. Given that recently DLL1 was also implicated in abnormal tumor vessel formation and growth inhibition [Zhang JP, ea. Cancer Lett. 2011;309:220-227], systemic stimulation of Notch by multivalent DLL1 might represent an efficient therapeutic strategy targeting multiple mechanisms of tumor growth.

A10 Prognostic significance of CXCL10/CXCR3 overexpression in mucinous bronchioloalveolar carcinoma. Michael Duruisseaux[1], Martine Antoine[2], Nathalie Rabbe[1], Virginie Poulot[1], Etienne Giroux Leprieur[1], Armelle Lavolé[2], Jacques Cadranel[1], Marie Wislez[1]. [1]Université Pierre et Marie Curie, Paris, France, [2]Hôpital Tenon, Paris, France.

Introduction: The 2004 WHO Pathological classification distinguishes two cytological subtypes of bronchioloalveolar carcinoma (BAC): mucinous (M) and non-mucinous (NM). This distinction appears to be relevant in terms of clinical characteristics, prognostic and treatment effectiveness. We attempted to better characterize the molecular profile of M relative to NM.

Experimental procedures: Bronchoalveolar lavage fluid (BALF) supernatants from M-BACs (n = 23) and NM-BACs (n = 11) were analyzed for 30 cytokines (IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, FGF, PDGF, HGF, VEGF, Eotaxin, G-CSF, GM-CSF, IFNγ, CXCL-1, CXCL-8, CXCL-10, MCP-1, MIP-1α, MIP-1β, TNF-α, RANTES, VCAM-1) by Bio-Plex multiplex bead-based assay (BIORAD, Marne la Coquette, France). Cytokines of interest expression was studied on paraffin embedded specimens from surgical specimens (n = 45) using immunochemistry. EGFR and K-ras mutation analysis using direct PCR and Alk overexpression detection using immunochemistry were also performed. Univariate and multivariate analysis by Cox model was undertaken to identify the variables associated with a risk for shortened survival.

Summary of results: Among the 30 chemokines analyzed, CXCL10 was the only one for which concentration was significantly higher in BALF supernatants from M-BAC (n = 23) compared to NM-BAC (n = 11) and control (n = 4) (p = 0.041). The cellular source of CXCL10 was not yet identified while CXCR3, its receptor, was expressed by tumor cells. CXCR3 was more frequently and more intensively expressed in M-BAC (n = 21) than in NM-BAC (n = 24) (52% vs 17%, p=0.023 and 52±16 vs 12±6, p = 0.047, respectively). EGFR mutation was never found in M-BAC (0%, 0/21) while present in 21% (5/24) of NM-BAC (p = 0.026). K-ras mutation and Alk overexpression did not differ between the two subtypes (2/21 vs 1/24, p > 0,05 and 3/21 vs 2/24, p > 0,05, respectively). The expression of CXCR3 was not associated with EGFR or K-ras mutation or Alk overexpression. In univariate analysis, factors associated with shorter survival were CXCL10 BALF concentration (HR=2.615, 95% CI: 1.102-6.206), PS > 0 (HR=1.308, 95% CI: 1.131-1.726) and bilateral extension (HR=2.914, 95% CI: 1.208-7.031). Multivariate analysis did not show any independent prognostic factor.

Conclusions: Our results show a CXCL10/CXCR3 overexpression in M-BAC compared to NM-BAC and suggest an association between CXCL10 overexpression and shorter survival.

A11 STRADα regulates cell polarity and invasion through PAK1 signaling in LKB1 null cells. Carrie M. Eggers, Erik R. Kline, Diansheng Zhong, Wei Zhou, Adam I. Marcus. Winship Cancer Institute of Emory University, Atlanta, GA.

The kinase LKB1 is mutated in multiple tumor types with a high mutation rate in non-small cell lung cancer. LKB1 activity is regulated by the pseudokinase, STE20-related adaptor alpha (STRADα), and the STRADα-LKB1 pathway plays critical roles in cell polarity and metastasis. Though much attention is given to the STRADα-LKB1 pathway, the function of STRADα itself, including a role outside of the LKB1 pathway, has not been well-studied. Data in C. elegans suggest that STRADα has an LKB1-independent role in regulating cell polarity, and therefore we tested the hypothesis that STRADα regulates cancer cell polarity and motility when wild-type LKB1 is absent. These results show that STRADα protein is reduced in LKB1-null cell lines (mutation or homozygous deletion) and this partial degradation occurs through the Hsp90-dependent proteasome pathway. The remaining STRADα participates in cell polarity and invasion, such that STRADα depletion results in misaligned lamellipodia, improper Golgi positioning, and reduced invasion. To probe the molecular basis of this defect, we show that STRADα associates in a complex with PAK1, and STRADα loss disrupts PAK1 activity via thr423 PAK1 phosphorylation. When STRADα is depleted, PAK1-induced invasion could not occur, suggesting that STRADα is necessary for PAK1 to drive motility. Based upon these data, we conclude that STRADα regulates PAK1 in LKB1-null cells to oversee cancer cell polarity and invasion.

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[1], Uma Giri[1], Christina McDowell[1], Luc Girard[2], Jack J. Lee[1], Roy Herbst[1], John Minna[2]. [1] The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Hamon Center for Therapeutic Oncology Research, The University of Texas Southwestern Medical Center, Dallas, TX.

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 non-invasive, 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 10^6-10^7 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 $\leq$ 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

A13 Lung adenocarcinoma subtypes based on the expression of airway basal cell genes. Tomoya Fukui[1], Renat Shaykhiev[1], Neil R. Hackett[1], Robert J. Downey[2], William D. Travis[2], Ronald G. Crystal[1]. [1]Weill Cornell Medical College, New York, NY, [2]Memorial Sloan-Kettering Cancer Center, New York, NY.

Purpose: Lung adenocarcinoma, the most common subtype of lung cancer, is a heterogeneous disease. Biologic heterogeneity of cancer is determined, at least in part, by specific cell populations of the original tissue that contribute to the development of the particular tumor subtypes. Airway epithelium, the primary site of molecular changes leading to the development of lung cancer, is composed of various cell types, including ciliated, intermediate, secretory (mucus-producing and Clara), neuroendocrine, and basal cells (BC). The specific contribution of individual cell types of the airway epithelium to lung cancer heterogeneity to lung adenoCa is unclear. Based on the knowledge that airway BC are

the stem/progenitor cells of the human airway epithelium and that molecular features of local stem/progenitor cells contribute to a distinct molecular subtype of various tumors, we hypothesized that a subset of lung adenoCa is characterized by activation of unique molecular features of airway BC linked to a distinct phenotype of lung adenoCa.

Methods: The normal human BC transcriptome was used to define an airway "BC signature" (Hackett et al., PLoS ONE 2011; 6:e18378). The primary data set included the transcriptomes of 182 lung adenoCa (Chitale et al, Oncogene 2009;28:2773). Two independent lung adenoCa transcriptome validation sets included 58 (Bild et al, Nature 2006;439:353) and 327 (Shedden et al, Nat Med 2008;14:822) subjects. Squamous cell carcinoma transcriptomes (n=53) were from Bild et al (Nature 2006). Survival analyses were performed using the Kaplan-Meier method (univariate) and Cox proportional hazard model (multivariate). Differential expression of selected genes between tumor subtypes was validated by immunohistochemistry.

Results: Gene expression analysis identified a subset of lung adenoCa that expressed high levels of airway BC signature genes ("BC-high adenoCa"). Compared to "BC-low adenoCa" (lung adenoCa expressing low levels of the airway BC signature genes), this subset of lung adenoCa exhibited more aggressive features with poorer tumor grade ($p<0.001$), more advanced pathological tumor stage ($p<0.05$), larger tumor size ($p<0.05$), higher frequency of vascular invasion ($p<0.004$), lymph node metastasis ($p<0.04$) and a 43 month shorter median survival (log rank $p<0.001$). Multivariate analysis, in which age, gender, smoking status, pathological stage, adenoCa with lepidic pattern (formerly BAC), and adjuvant therapy were included, demonstrated that high expression of the airway BC signature was an independent prognostic factor associated with shorter survival in lung adenoCa (hazard ratio; 1.59, 95% confidence interval; 1.14-2.22, $p<0.008$). The strikingly shorter survival of BC-high vs BC-low adenoCa was verified in the 2 independent validation data sets. At the molecular level, BC-high adenoCa displayed higher frequency of *KRAS* mutations ($p<0.04$), lower frequency of *EGFR* mutations ($p<0.03$) and a distinct differentiation pattern characterized by a suppression of genes related to ciliated and Clara cells, accompanied by a broad activation of genes related to the epithelial-mesenchymal transition program (SNAIL, SLUG, TWIST1, CDH2). Compared to the lung SqCa characterized by overexpression of classical BC genes related to squamous differentiation (TP63, keratin KRT5, KRT6B), BC-high adenoCa exhibited up-regulation of novel BC genes including KRT7, members of the EGFR pathway (amphiregulin, ERBB receptor feedback inhibitor 1), and tissue factor pathway inhibitor 2. In total, 24% of the airway BC signature genes were expressed differentially between these 2 subtypes of lung cancer.

Conclusion: Activation of a unique airway BC program contributes to a distinct, more aggressive, BC-high subtype of human lung adenoCa, a novel subtype of lung adenoCa, which may originate from airway BC.

A14 Small tumor diameter at cure threshold (< 20mm) among aggressive lung cancers predicts both chest x-ray and CT screening outcomes in a novel simulation framework. Deborah Lynn Goldwasser, Marek Kimmel. Rice University, Houston, TX.

Background: The population distribution of lung cancer growth and tumor diameter at cure threshold is an important component of a model of the natural course of lung cancer.

Methods: We simulate outcomes in the Mayo CT lung screening study using an existing simulation model of lung cancer progression calibrated to the Mayo Lung Project (males only) and evaluate the goodness of fit.
We perform a likelihood-based analysis to determine whether the conditional probability of five-year lung cancer survival given tumor diameter at detection depends significantly on

detection modality. A novel probabilistic model characterizes growth and tumor progression after transformation of a single malignant cell, governed by two parameters f and μ, the branching fraction and cellular mutation rate, respectively. We identify distinct sets of parameter pairs (f,μ), each of which satisfies a method of moments condition given a survival function governing detection in the absence of screening. We examine their goodness of fit with respect to simulated tumor size and five-year lung cancer survival among detected incident lung cancers in the MLP and Mayo CT studies.

Results: An existing model of lung cancer progression under-predicts the number of advanced-stage incident lung cancers among males in the Mayo CT study (p-value = 0.004). The probability of five-year lung cancer survival given size at detection depends significantly on detection modality (p-value = 0.0312). Selected models having a median tumor diameter at cure threshold ranging from 16.7-27.0 mm predict both MLP and Mayo CT outcomes.

Conclusions: The median lung tumor diameter at cure threshold among aggressive lung cancers may be small (< 20 mm).

A15 Snail upregulation of SPARC leads to increased invasion in models of both early-and late-stage NSCLC. Jeanette L. Grant[1], Tonya C. Walser[1], John D. Minna[2], Jerry W. Shay[2], Steven M. Dubinett[1]. [1]University of California, Los Angeles, Los Angeles, CA, [2]UT Southwestern Medical Center, Dallas, TX.

The zinc-finger protein Snail is upregulated in human non-small cell lung cancer (NSCLC) tissues and is associated with poor patient prognosis. We have shown that Snail upregulation in lung cancer cell lines leads to morphologic changes indicative of epithelial-mesenchymal transition (EMT) in vitro and tumor progression in vivo. Snail overexpression is associated with differential gene expression related to diverse aspects of lung cancer progression. One gene upregulated by Snail in both NSCLC and histologically normal human bronchial epithelial cell lines is secreted protein, acidic and rich in cysteine (SPARC). Expression of SPARC modulates reversible interactions between cells and their extracellular matrix and is known to play an important role in the wound healing process of normal epithelial cells. Its upregulation in several cancer types is associated with increased migration, invasion, and poor patient prognosis. Here, we show that overexpression of Snail leads to increased invasion in a modified Boyden chamber assay in models of both early and late stage NSCLC. Knockdown of SPARC by shRNA leads to a reversal of invasion, indicating that SPARC is at least partially responsible for the effect of Snail on invasion. Computational analysis indicates that Snail does not directly enhance the transcription of SPARC; therefore, we have investigated the indirect mechanism for this relationship. Both ERK1/2 and the chaperone protein HSP27 have emerged as potential components of the pathway. Snail overexpression also leads to activation of ERK1/2, and chemical inhibition of this pathway leads to a decrease in SPARC protein. Snail-overexpressing cell lines show increased transcription of HSP27 mRNA. As Snail has been shown to be overexpressed in the inflammatory microenvironment of premalignancy and in established tumors, SPARC-driven invasion supports both the parallel and linear models of tumor progression. Delineating pathways involved in Snail-dependent and SPARC-mediated tumor progression may yield new targets for lung cancer prevention and treatment.

A16 Aberrant signaling pathways in squamous cell lung carcinoma. Nooshin Hashemi Sadraei[1], Ivy Shi[1], Zhong-Hui Duan[2], Ting Shi[1]. [1]Cleveland Clinic Foundation, Cleveland, OH, [2]University of Akron, Akron, OH.

Lung cancer is the second most commonly occurring non-cutaneous cancer in the United States with the highest mortality rate among both men and women. In this study, we utilized three lung cancer microarray datasets generated by previous researchers

to identify differentially expressed genes, altered signaling pathways, and assess the involvement of Hedgehog (Hh) pathway. The three datasets contain the expression levels of tens of thousands genes in normal lung tissues and squamous cell lung carcinoma. The datasets were combined and analyzed. The dysregulated genes and altered signaling pathways were identified using statistical methods. We then performed Fisher's exact test on the significance of the association of Hh pathway downstream genes and squamous cell lung carcinoma. 395 genes were found commonly differentially expressed in squamous cell lung carcinoma. The genes encoding fibrous structural protein keratins and cell cycle dependent genes encoding cyclin-dependent kinases were significantly up-regulated while the ones encoding LIM domains were down. Over 100 signaling pathways were implicated in squamous cell lung carcinoma, including cell cycle regulation pathway, p53 tumor-suppressor pathway, IL-8 signaling, Wnt-β-catenin pathway, mTOR signaling and EGF signaling. In addition, 37 out of 223 downstream molecules of Hh pathway were altered. The pvalue from the Fisher's exact test indicates that Hh signaling is implicated in squamous cell lung carcinoma. Numerous genes were altered and multiple pathways were dysfunctional in squamous cell lung carcinoma. Many of the altered genes have been implicated in different types of carcinoma while some are organ-specific. Hh signaling is implicated in squamous cell lung cancer, opening the door for exploring new cancer therapeutic treatment using GLI antagonist GANT 61.

A17 Integrating expression data improves mutational significance analysis of lung squamous carcinoma. Bryan Hernandez[1], Peter Hammerman[1], Marcin Imielinski[1], Michael Lawrence[1], Petar Stojanov[1], Gad Getz[1], Matthew Meyerson[2]. [1]Broad Institute, Cambridge, MA, [2]Dana Farber Cancer Institute, Boston, MA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR1) of the conference *Proceedings*.

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]. [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.

Lung cancer is a major cause of death among cancers in the US. 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 US, 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 US 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 subtypes 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 non-agrarian 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 US 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 spatial distribution for adenocarcinoma or small cell carcinoma. The most striking geospatial regions with RR greater than 1 were detected for large cell lung cancer in Washington County, one of the poorest counties in the US. Women exhibited very high rates (RR 5.3, p 0.002) along the southern coastal region of Washington County while men exhibited very high rates (RR 3.7, p 0.04) within a smaller region in northern coastal Washington county where we have mapped use of pesticide sprays. Published literature linking large cell carcinoma with smoking is ambivalent. Washington County and the Pittsfield Region have air and water radon exposures below 4 pCi. There are no heavy industry or highways that could implicate particulate matter. In conclusion, we found statistically significant spatial regions (p<05; RR greater than 1) of high large cell lung carcinoma incidence in coastal regions of Washington County, but “hotspots” for men and women differed geographically. Environmental risk factors commonly correlated with lung cancer did not appear to explain the distribution of large cell lung cancer cases. More research is needed to explain the high incidence of large cell lung cancer in a non-agrarian, mostly white population with cancer and health disparities.

A19 Lung cancers taken from autopsies express more tumor antigens than those taken from surgical resections: Implications for immunoprevention. Lisheng Ge, Nils Lambrecht, Yi Ouyang, Neil Hoa, Longchaun Chen, Xiamong Deng, Maria Dacosta-Iyer, Sandor Szabo, Martin Robert Jadus. Veterans Affairs Medical Center, Long Beach, CA.

Lung cancers are fatal cancers, even if caught early, since some cancer cells have spread by the time they are detected. Some lung cancer patients treated by immunotherapy have improved their survival. A limited number of tumor-associated antigens (TA), both humoral-based and cell-mediated antigens, are known for lung cancers. The identification of more relevant antigens will allow better immunotherapies to be developed. We analyzed adenocarcinomas and small cell lung cancers taken from surgical resections or from autopsies to determine the prevalent antigens by using quantitative real time PCR techniques for 43 putative tumor antigens. We compared these tumor-derived profiles with normal lung cell expression. The data fell into 3 profiles where: 1) both tumors were positive for 18 TA; 2) only SCLC expressed 14 TA; 3) a group of 10 TA that had no differences. The lung tumor samples taken at autopsy, expressed a higher number of TA and expressed more mRNA TA than those taken from surgical resections. Blotting techniques using the specimens from the lung cancer autopsies confirmed increased expression of the tumor antigen precursor proteins when compared to the normal lung. Use of two lung cancer cell lines also independently confirmed the presence of these proteins. These data suggest that human lung cancers at the time of death are more antigenic than those taken at an earlier stage. This type of tumor taken at autopsy might provide more targets for vaccine generation and allow the concept of immunoprevention to be used in lung cancer patients.

A20 Downregulation of the LRIG3 interacting proteins LMO7 and LIMCH1 in human lung cancer. Terese Karlsson, Camilla Holmlund, Roger Henriksson, Håkan Hedman. Radiation Sciences, Umeå, Sweden.

The purpose of the study was to identify proteins interacting with leucine-rich repeats and immunoglobulin-like domains protein 3 (LRIG3), and to study their role in human lung cancer. LRIG3 is a transmembrane protein suggested to be a serum biomarker for NSCLC. In a yeast two-hybrid screen we identified LMO7 and LIMCH1 as interacting partners of LRIG3. LMO7 and LIMCH1 are highly homologous proteins. Reportedly, LMO7 interacts with cytoskeletal proteins and deletion of the gene in mice results in spontaneous lung adenocarcinomas. Confocal microscopy revealed that overexpressed LRIG3 colocalized with both LMO7 and LIMCH1 and using a proximity ligation assay we showed that endogenous LRIG3 interacted with LMO7 in several cell lines. Using quantitative RT-PCR, the mRNA expression of LMO7 and LIMCH1 was analyzed in a panel of human tissues. High mRNA expression of both LMO7 and LIMCH1 was found in human lung. In human lung tumors, mRNA levels of LMO7 and LIMCH1 were reduced compared to normal lung. These experiments show that LRIG3 interacts with both LMO7 and LIMCH1, and that LMO7 and LIMCH1 are down-regulated in human lung cancer.

A21 Tissue platinum concentration and tumor response in non-small cell lung cancer. Eric S. Kim, Guangan He, Chi-Wan Chow, Junya Fujimoto, Jack J. Lee, Neda Kalhor, Stephen G. Swisher, Ignacio I. Wistuba, David J. Stewart, Zahid H. Siddik. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Platinum resistance is a major limitation in the treatment of advanced non-small cell lung cancer (NSCLC). Reduced intracellular drug accumulation is one of the most consistently identified features of platinum-resistant cell lines, but clinical data are limited. We assessed effects of tissue platinum concentrations on tumor response, time to recurrence, progression-free survival (PFS) and overall survival (OS) in patients with early-stage NSCLC following neoadjuvant platinum-based chemotherapy.

Methods: We measured total platinum concentrations in 44 archived fresh frozen NSCLC specimens from patients who received neoadjuvant platinum-based chemotherapy. Samples were analyzed by flameless atomic absorption spectrophotometry to assess absorbance reading associated with platinum concentration. Four specimens from patients who underwent surgery only and one patient who received pemetrexed as neoadjuvant chemotherapy were analyzed as negative controls. Absorbance value per mg of tissue was correlated with percent reduction in tumor size on post- vs prechemotherapy CT scans. Kaplan-Meier curves and log-rank tests were used to evaluate differences in time to recurrence and survival between two groups divided by median platinum concentration.

Results: Platinum absorbance values in 44 neoadjuvant specimens were easily detectable and ranged from 0.6 to 6.7 x 10^{-3} per mg of tissue while five negative controls demonstrated absorbance readings similar to 0.1N HCL. Platinum absorbance correlated significantly with percent reduction in tumor size ($R^2=0.48$, $P<0.00001$). The same correlations were seen in cisplatin ($P=0.0003$), carboplatin ($P=0.0013$), adenocarcinoma ($P=0.0003$) and squamous cell carcinoma ($P=0.019$) groups. Furthermore, there was no significant impact of potential variables such as the type of platinum compound, number of cycles, pre-treatment tumor diameter and time lapse from last chemotherapy on platinum concentration. Patients with lower Pt concentration also had shorter time to recurrence ($P=0.0494$), progression free survival ($P=0.0366$) and overall survival ($P=0.035$).

Conclusion: This is the first tissue-based study to establish a relationship between tissue platinum concentrations and response in NSCLC. Reduced intratumoral platinum

accumulation may constitute a significant mechanism of platinum resistance even in clinical specimens. Further studies investigating factors that modulate intracellular platinum concentration are warranted (Supported by National Foundation of Cancer Research 90088436, Department of Defense W81XWH-07-1-0306, National Institute of Health CA127263, CA160687 and CA16672).

A22 Clinical implication of microscopic anthracotic pigment in mediastinal staging of non-small cell lung cancer by endobronchial ultrasound-guided transbronchial needle aspiration. Young Whan Kim[1], Young Sik Park[1], Jinwoo Lee[1], Sang-Min Lee[1], Jae-Joon Yim[1], Sung Koo Han[1], Jin Chul Pang[2], Doo Hyun Chung[2], Seok-Chul Yang[1]. [1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine and Lung Institute, Seoul, Republic of Korea, [2]Seoul National University College of Medicine, Seoul, Republic of Korea.

Introduction: Microscopic anthracotic pigment is frequently observed in EBUS-TBNA specimen from non-small cell lung cancer, but the clinical interpretation is not known. The aim of this study was to evaluate the clinical implication of microscopic anthracotic pigment in mediastinal staging of non-small cell lung cancer by EBUS-TBNA.

Methods: From May 2010 to July 2011, Consecutive potentially operable non-small cell lung cancer patients who underwent EBUS-TBNA for mediastinal staging were recruited in this retrospective study.

Results: Total 133 patients, male were 102 (76.7%). Median age was 68. Squamous cell carcinoma (59, 44.4%) and adenocarcinoma (57, 42.9%) were most common. Clinical stages after EBUS-TBNA were as followed; IA (18.8%), IB (17.3%), IIA (6.0%), IIB (9.0%), IIIA (36.8%), IIIB (12.0%). Total 281 mediastinal lymph nodes were sampled by EBUS-TBNA, station 4R (100, 35.8%) and station 7 (86, 30.8%) were most common sites. Malignant lymph nodes were 100 (35.8%). Microscopic anthracotic pigment was observed in 61 (21.7%) lymph nodes, and among them malignant lymph nodes were only 3 ($P<0.001$). The lymph nodes with microscopic anthracotic pigment were small (9.0 vs. 10.8 mm, $P=0.001$) and low SUV on FDG-PET (5.4 vs. 6.7, $P=0.120$). In multivariable analysis, microscopic anthracotic pigment was statistically significant associated with benign lymph node (adjusted OR, 14.3; 95% CI, 3.6-57.3; $P<0.001$).

Conclusion: In potentially operable non-small cell lung cancer patients, microscopic anthracotic pigment in endobronchial ultrasound-guided transbronchial needle aspiration specimen was strongly associated with benign mediastinal lymph node.

A23 Detection of K ras mutations in Korean non-small cell lung cancer patients. Hee Joung Kim, Sun Jong Kim, Gwang Ha Yoo, Won Dong Kim, Seo Young Oh, Wan Seop Kim, Kye Young Lee. Konkuk University School of Medicine, Seoul, Republic of Korea.

Background: Point mutation of the K ras gene is known to be a poor prognostic marker of an adenocarcinoma of the lung. This study aims to define frequency of K ras mutations and evaluate clinical features of patients with Non small cell lung cancer (NSCLC) who harbor K ras mutation.

Methods: NSCLC patient who were diagnosed from Sep. 2005 to Aug. 2011 at Konkuk University Hospital were included in this retrospective study. K ras mutation was assessed by pyrosequencing on biopsy or cytology specimen. Medial chart review was performed and clinical characteristics were compared according to K ras mutation status of their tumors.

Results: K ras mutation was found in 25 (9.0%) of 277 patients and which was similar to those in East Asian countries, but lower than those in western countries. Fifteen mutations (60.0%) were point mutation at codon 12 (GGT), 7 mutations (28.0%) were point mutation at codon 61 (CAA), and 3 mutations (12.0%) were found at codon 13

(GGC) in exon 2. Median age of 25 patients with KRAS mutation was 6 years (range 43-85 years). There were 20 males (80.0%) and 3 never-smokers (12.0%). The majority of histological diagnoses were adenocarcinomas (21 cases; 84.0%) and squamous cell carcinomas were followed (3 cases; 12.0%). Interestingly, 2 patients had both mutations of EGFR and KRAS unlike previous studies, they showed favorable response to EGFR-TKIs .

Conclusions: The data indicate that KRAS mutation is less frequent in Korean than Caucasian patients. Although it is suggested that EGFR and KRAS mutations occur in mutually exclusive, very few of the patients with KRAS mutation showed also EGFR mutation and relatively favorable response to EGFR-TKIs. Additional large scale prospective studies are needed in order to validate the clinical significance of Kras mutation in Asian patients.

A24 CpG island methylation of microRNAs is associated with tumor size and the recurrence of non-small cell lung cancer. Kentaro Kitano, Akiteru Goto, Masashi Fukayama, Yutaka Yatomi, Nobuya Ohishi, Daiya Takai, Kousuke Watanabe, Noriko Emoto, Hidenori Kage, Emi Hamano, Takahide Nagase, Atsushi Sano, Tomohiro Murakawa, Jun Nakajima. The University of Tokyo Hospital, Tokyo, Japan.

We investigated whether the CpG island methylation of certain microRNAs was associated with the clinicopathological features and the prognosis of non-small cell lung cancer. The methylation of *mir-152*, *-9-3*, *-124-1*, *-124-2*, and *-124-3* was analyzed in 96 non-small cell lung cancer specimens using a combined bisulfite restriction analysis. The median observation period was 49.5 months. The methylation of *mir-9-3*, *-124-2*, and *-124-3* was individually associated with an advanced T factor independently of age, sex, and smoking habit. Moreover, the methylation of multiple microRNA loci was associated with a poorer progression-free survival in a univariate analysis. Our result enlightens the accumulation of aberrant DNA methylation which occurs in concordance with the tumor progression.

A25 Compromised LKB1 function promotes exploratory invasive behavior through FAK activation. Erik R. Kline, Adam I. Marcus. Winship Cancer Institute of Emory University, Atlanta, GA.

The tumor suppressor LKB1 is the third most frequently mutated gene in lung adenocarcinoma and has been implicated in regulating tumor cell metastasis, which is the leading cause of death in cancer patients. To understand the role LKB1 loss plays in metastatic disease, we employed a number of cell biology, pharmacological, and advanced microscopy techniques in lung cancer cells with compromised LKB1 function.

Lung cancer cells that are stably depleted of either LKB1 or its activating cofactor STRADα are significantly more adhesive to a fibronectin matrix than isogenic wild-type control cells. This enhanced adhesion in LKB1-compromised cells occurs with a concomitant increase in phosphorylation of Focal Adhesion Kinase (FAK) at Y861 and Y397 as determined by Western blot and immunofluorescence. LKB1 and FAK co-immunoprecipitate and also exist in spatial proximity to one another at the leading edge of motile cells. On the contrary, LKB1 and pY861/pY397-FAK are mutually excluded from one another at the leading edge, further suggesting that LKB1 serves as a FAK repressor. Sub-toxic doses of the pharmacological FAK inhibitor PF-573228 repress FAK phosphorylation, and inhibit cell adhesion in LKB1-compromised cells to a greater extent than in cells with wild-type LKB1. Live-cell imaging reveals that motile LKB1- and STRADα-depleted cells have the same velocity as control cells, but have significantly impaired directionality as evidenced by their meandering indices. Lamellipodia in LKB1-compromised cells are also aberrantly shaped and form at a much higher frequency than in control cells. These defects in directionality and lamellipodia formation are restored in LKB1-

compromised cells by treatment with either FAK-specific siRNA or a pharmacological FAK inhibitor. Lastly, over-expression of GFP-FAK shows that focal adhesion sites have longer duration and are larger in size in wild-type cells compared to LKB1-compromised cells. Collectively, these findings implicate the tumor suppressor LKB1 as a negative regulator of FAK phosphorylation. Loss of LKB1 or STRADα results in rapid focal adhesion turnover and in a more exploratory cancer cell phenotype that can be restored via FAK inhibition.

A26 GATA2 is necessary for oncogene-mutant non-small cell lung tumorigenesis. Madhu S. Kumar, Julian Downward. Cancer Research UK London Research Institute, London, United Kingdom.

Non-small cell lung cancer (NSCLC) is the most common histological cancer type in terms of both prevalence and mortality worldwide. In NSCLC, KRAS and EGFR are the most frequently mutated oncogenes, constituting half of all patients. Thus, there is a profound need to develop therapies targeting these lesions. To address this need, we previously performed an RNA interference screen for factors required in KRAS mutant cells. Through this screen, we found KRAS mutant cells depend on the transcription factor GATA2. Subsequent analysis of a broad panel of NSCLC lines revealed GATA2 depletion suppressed mutant cell viability. We then explored the role of GATA2 in NSCLC cells with a variety of oncogene mutations. We observed lethality with GATA2 loss in mutant NSCLC cells, with no effect in wild type NSCLC cells. Moreover, transplanted mutant NSCLC cells depleted for GATA2 exhibited abrogated tumor growth, while wild type tumors were unaffected, suggesting GATA2 is needed in oncogene-mutant NSCLC.

To elucidate the mechanism of GATA2 function in NSCLC gene expression, we performed gene expression analysis of mutant cells with GATA2 suppression. With GATA2 loss, we observed global down-regulation of the proteasome and subsequent inhibition of proteasome activity. This inhibited proteasome function is biologically salient, as restoration of proteasome activity rescued growth in response to GATA2 depletion. We further explored the role of GATA2 in NSCLC by integrating the above gene expression data with global genome occupancy with chromatin immunoprecipitation coupled to next-generation sequencing (ChIP-seq). Through these approaches, GATA2 loss suppressed expression of the IL-1 signaling pathway in mutant cells, down-regulating pathway flux. Consequently, restored IL-1 signaling reestablished growth in response to GATA2 depletion. Moreover, GATA2 occupancy was enriched in Rho signaling pathway components, functionally inactivating Rho signaling. Accordingly, activated Rho signaling rescued viability in response to GATA2 loss. Analyzing the DNA motifs from our GATA2 ChIP of mutant NSCLC cells, we observed enrichment of the STAT5 response element at Rho target genes. We subsequently isolated a GATA2-STAT5 complex in mutant NSCLC cells. Overall, our integrative genomic analyses have revealed a transcriptional network governed by GATA2 in oncogene-driven NSCLC.

To examine the effect of GATA2 loss in genetically engineered mouse models (GEMMs) of NSCLC, we combined a conditional allele of Gata2 with Cre-inducible oncogenic Kras, where Gata2 deletion suppressed tumor growth compared to wild type animals. We then examined the role of whole-body loss of GATA2 in established lung tumors via systemic deletion of Gata2. In this system, GATA2 depletion caused complete regression of established lesions. We then explored whether inhibition of GATA2-regulated pathways with clinically approved compounds would recapitulate the effects of GATA2 loss in NSCLC GEMMs. To do so, we combined bortezomib, an inhibitor of the proteasome, and fasudil, an inhibitor of Rho signaling, in the Kras lung tumor model. Treatment of the Kras-mutant GEMM with these compounds caused a near-complete clearance of tumors. These combined genetic and therapeutic approaches reveal GATA2 is required for autocthonous oncogene-driven

tumorigenesis and that combined inhibition of GATA2-controlled pathways with licensed agents suppresses NSCLC growth.

In sum, these results demonstrate GATA2 is necessary for oncogene-mutant NSCLC cell survival. Most strikingly, these findings suggest that the functional pleiotropy of GATA2, not a traditional druggable target, represents a network of druggable pathways for therapeutic exploitation.

A27 Expression of the EGFR suppressor LRIG1 is associated with survival in non-small cell lung cancer. Samuel Kvarnbrink[1], Karolina Edlund[2], Johan Botling[2], Patrick Micke[2], Håkan Hedman[1], Mikael Johansson[1]. [1]Umeå University, Umeå, Sweden, [2]Uppsala University, Uppsala, Sweden.

Leucine-rich repeats and immunoglobulin-like domains (LRIG)-1 is an endogenous inhibitor of epidermal growth factor receptor (EGFR) signaling, that is down-regulated in several forms of human cancer. The LRIG gene family also includes LRIG2 and LRIG3. EGFR signaling is important in non-small cell lung cancer (NSCLC) and EGFR tyrosine kinase inhibitors are today considered standard treatment in patients with advanced disease. Since smoking recently was reported to down-regulate LRIG1 gene expression in lung epithelium and squamous cell carcinoma of the lung, the LRIG family of proteins may be important in lung cancer.

The objective of the present study was to investigate if LRIG is a prognostic factor in non-small cell lung cancer (NSCLC). Therefore, we conducted an in silico analysis of LRIG gene expression in publicly available datasets using the Oncomine database and compared LRIG expression to clinical outcome. We also analyzed the LRIG protein expression by immunohistochemistry in a tissue micro array (TMA, n=190) representing long- and short time survivors from a surgically treated NSCLC patient cohort.

In seven out of 11 datasets in the in silico analysis, high expression of LRIG1 correlated ($p<0.05$) with long patient survival. This correlation was apparent both in adenocarcinoma and squamous cell carcinoma. In the TMA, LRIG1 expression was significantly higher in the long-term survival group than in the short time survival group ($p<0.05$). LRIG2 and LRIG3 protein expression did not significantly correlate to survival.

In conclusion, both our in silico analysis of gene expression and TMA study of protein expression showed associations between high LRIG1 expression and longer NSCLC patient survival. This implicates that LRIG1 may be a prognostic factor in NSCLC and further studies on larger datasets have been initiated.

A28 Genome-scale analysis of DNA methylation in lung adenocarcinoma and integration with mRNA expression. Suhaida A. Selamat[1], Wan L. Lam[2], Stephen Lam[2], Adi F. Gazdar[3], Ite A. Laird-Offringa[1], Brian Chung[1], Luc Girard[3], We Zhang[3], Ying Zhang[1], Mihaela Campan[1], Kimberly D. Siegmund[1], Michael N. Koss[1], Jeffrey A. Hagen[1]. [1]University of Southern California, Los Angeles, CA, [2]British Columbia Cancer Agency, Vancouver, BC, Canada, [3]University of Texas Southwestern Medical Center, Dallas, TX.

Lung cancer is the leading cause of cancer death worldwide and adenocarcinoma is its most common histological subtype. Clinical and molecular evidence indicates that lung adenocarcinoma is a heterogeneous disease, which has important implications for treatment. Here we performed genome-scale DNA methylation profiling using the Illumina Infinium HumanMethylation27 platform on 59 matched lung adenocarcinoma/non-tumor lung samples, with genome-scale verification on an independent set of tissues. We identified 766 genes showing altered DNA methylation between tumors and non-tumor lung. By integrating DNA methylation and mRNA expression data, we identified 164 hypermethylated genes showing concurrent downregulation, and 57 hypomethylated genes showing increased expression.

Integrated pathways analysis indicates that these genes are involved in cell differentiation, epithelial to mesenchymal transition, RAS and WNT signaling pathways and cell cycle regulation, among others. Comparison of DNA methylation profiles between lung adenocarcinomas of current and never-smokers showed modest differences, identifying only LGALS4 as significantly hypermethylated and downregulated in smokers. LGALS4, encoding a galactoside-binding protein involved in cell-cell and cell-matrix interactions, was recently shown to be a tumor-suppressor in colorectal cancer. Unsupervised analysis of the DNA methylation data identified two tumor subgroups, one of which showed increased DNA methylation and was significantly associated with KRAS mutation and to a lesser extent, with smoking. Our analysis lays the groundwork for further molecular studies of lung adenocarcinoma by providing new candidate DNA methylation biomarkers for early detection, identifying novel molecular alterations potentially involved in lung adenocarcinoma development/progression, and describing an epigenetic subgroup of lung adenocarcinoma associated with KRAS mutation.

A29 Genomic alteration of E2F/Rb activates EZH2 in small cell lung cancer.. Kelsie L. Thu[1], Roland Hubaux[1], Sarit Aviel-Ronen[2], Calum E. MacAulay[1], Ming-Sound Tsao[2], Stephen Lam[1], Wan L. Lam[1], Bradley P. Coe[1]. [1]British Columbia Cancer Research Centre, Vancouver, BC, Canada, [2]Ontario Cancer Institute, Toronto, ON, Canada.

Introduction: Small Cell Lung Cancer (SCLC) is a highly aggressive lung tumor with a 5 year survival rate of only 5% for extensive stage disease, which has only modestly improved over the last few decades. Identification of new molecular diagnostic and therapeutic targets is thus imperative. The purpose of this study was to employ genomic profiling of SCLC tumors to identify novel genomic alterations responsible for driving the aggressive biology of SCLC.

Methods: DNA was extracted from a panel of 14 formalin fixed paraffin embedded SCLC tumors and used to perform high resolution array comparative genomic hybridization (aCGH) on a tiling array to identify recurrent copy number alterations. RNA was harvested from a series of SCLC cell lines and used to perform qRT-PCR on candidate genes. Publicly available SCLC tumor microarray data was also analyzed to interrogate the expression of candidate genes.

Results: Through application of integrated genome and transcriptome microarray profiling, and comparison of SCLC to less aggressive non-small cell lung cancers (NSCLC) we have identified novel patterns of genomic alteration mediated pathway disruption specific to SCLC. This includes activation of the cell cycle through deregulation of downstream pathway components, as opposed to upstream deregulation of receptors such as EGFR which is characteristic of NSCLC.

Strikingly we observed direct genomic activation of E2F transcription factors, in addition to the classically described loss of the Rb tumor suppressor. Analysis of targets of the E2F/Rb pathway identified EZH2, a polycomb repressive complex 2 (PRC2) member involved in epigenetic silencing of genes involved in differentiation. EZH2 has been characterized as a target of genomic amplification in prostate and breast cancers, however, no genomic amplifications were detected in our SCLC samples, thus any overexpression is likely regulated by upstream elements of the E2F/Rb pathway. qRT-PCR confirmed EZH2 as being specifically hyper-activated with a mean 42 fold over-expression (range 10 to 74 fold) in SCLC compared to 13 fold in NSCLC. This pattern was verified from an analysis of an independent array study of SCLC. Moreover, shRNA-mediated knockdown demonstrated a significant reduction in cell viability in SCLC cell lines.

Conclusion: We conclude that EZH2 activation through genomic alteration of E2F/Rb contributes to the malignant phenotype of SCLC and may represent a potential therapeutic target.

A30 Radiation resistance of human lung cancer stem cells (CSCs). Roberto Gomez-Casal, Simul Parikh, Roje Saleet, Per Basse, Michael K. Gibson, Elieser Gorelik, Vera Levina. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Non-small cell lung cancer (NSCLC), the most common form of lung cancer, is treated with tumor resection and chemotherapy, with ionizing radiation (IR) treatment and chemotherapy used for patients with unresectable tumors. Despite improvements in medical management, 5-year survival rates for NSCLC remain low. Evidence suggests that resistance to radio- and chemotherapy is associated with cancer stem cells (CSCs), which are undifferentiated cells with the capacity to self-renew and differentiate, restoring the tumor cell population following treatment. However, the mechanisms through which lung CSCs evade and resist radiation therapy remain poorly studied.

We previously reported on our ability to isolate, culture, and assay human lung CSCs from cultured cell lines. We found the stem cell factor (SCF) and its receptor c-kit functions in a crucial pathway for the self-renewal and proliferation of human lung CSCs. We discovered that a combination of cisplatin (to eradicate bulk tumor cells) and agents targeting SCF/c-kit signaling in CSCs (e.g., antibody neutralizing SCF and anti-c-kit RTK inhibitors) is an effective therapy against NSCLC in vitro.

Here we extend our work to CSCs derived from primary NSCLC surgical tumor specimens. We analyzed lung CSC resistance to ionizing radiation (IR) and molecular mechanisms associated with CSC radioresistance.

We found that human lung CSC radioresistance is mediated by preferential activation of the DNA damage checkpoint response and an increase in DNA repair capacity. Tyrosine kinase inhibitors with anti c-kit activity (e.g., imatinib, dovitinib, sunitinib, and axitinib) were compared for their ability to abrogate lung CSC proliferation and potentiate effect of IR on NSCLC. We found that lung CSCs proliferation was abrogated by all of the four c-kit inhibitors; however, axitinib showed the most profound anti-CSC effect.

The combination of IR treatment with SCF/c-kit pathway inhibition eliminated bulk NSCLC cells and radiation resistant CSCs.

A31 Predictive and prognostic value of KRAS mutations in advanced non-small cell lung cancer (NSCLC) patients treated with chemotherapy. Wouter W. Mellema[1], Anne-Marie C. Dingemans[2], Egbert F. Smit[1], Jules Derks[2], Daniëlle A.M. Heideman[1], Robertjan van Suylen[2], Frederik B. Thunnissen[1]. [1]VU University Medical Center, Amsterdam, The Netherlands, [2]Maastricht University Medical Center, Maastricht, The Netherlands.

Introduction: K-Ras mutation is thought to be related with poor outcome of NSCLC patients with an advanced stage of disease. The role of K-Ras mutation as a predictor of response for these patients treated only with chemotherapy is poorly understood.

Patients and methods: From a retrospective database from 2 university hospitals all patients with advanced stage, nonsquamous, NSCLC treated with palliative platinum containing chemotherapy were selected. Patients were included when archive tumour tissue was available for DNA extraction. High resolution melting followed by PCR sequencing was performed for KRAS exon1 and 2. Response to treatment was assessed by RECIST. The databases were matched by treatment.

Results: A total of 179 patients were included in this study. All patients were treated with chemotherapy and were no candidate for treatment with curative intent. There were 59 patients with a K-Ras mutation (32.9%). There was no significant difference in basic characteristics between the group with a K-Ras mutation and the group with K-Ras wild-type. Mean age 59 years (range 32-83 years), 108 males and 71 females. ECOG performance score 0/1/2/3 73/77/13/2. Thirty patients with stage IIIb disease, 149 patients with stage IV disease. Progression as best response to treatment was reported in 21/59

patients with a K-ras mutation (35.6%) and 34/120 patients (28.3%) with a K-Ras wild type, this was not significant (p=0.611). The median progression free survival (PFS) in the group with a K-Ras mutation was 4.1 months (95% CI 3.12-5.0 months) compared to 5.4 months (95% CI 4.4-6.4 months) in patients with K-Ras wild type (p=0.202). The overall survival (OS) in the group with a K-Ras mutation, was 8.3 months (95% CI 5.2-11.4 months) compared to 12.5 months (95% CI 10.2-14-7 months) in patients with K-Ras wild type (p=0.037).

Conclusion: In our series K-Ras mutation was not predictive for response to platinum containing chemotherapy in patients with advanced NSCLC. K-Ras mutational status was prognostic for overall survival.

A32 Mechanistic dissection of lung cancer promotion by airway inflammation. Maria Miguelina De La Garza, Seon Hee Chang, Cesar E. Ochoa, Amber Cumpian, Seyedeh Golsar Mirabolfathinejad, Burton F. Dickey, Chen Dong, Seyed Javad Moghaddam. The University of Texas MD Anderson Cancer Center, Houston, TX.

Cigarette smoking is the principal cause of lung carcinogenesis, however, smokers with chronic obstructive pulmonary disease (COPD) have an increased risk of lung cancer (3 to 10 fold) compared to smokers with comparable cigarette exposure but without COPD. Histopathologic studies have clearly demonstrated lung inflammation in COPD, and it persists even after cessation of cigarette smoking. These facts suggest a strong link between COPD-related airway inflammation and lung cancer promotion independent of smoking but the precise mechanistic link is not known. We developed a COPD-like mouse model of airway inflammation through repeated aerosol challenge to a lysate of non-typeable *Haemophilus influenzae* (NTHi) which commonly colonizes the airways of COPD patients. Then, we showed that this type of airway inflammation, but not asthma-like airway inflammation, promotes lung cancer in a K-ras mutant mouse model of lung cancer (CC-LR). This was associated with NF-kB pathway activation, and up-regulation of its downstream target genes, including IL-6 and T helper 17 (Th17) response. Therefore, we studied the role of NF-kB in lung cancer promotion by targeting its expression in airway epithelium. CC-LR mice were crossed with IKK-$\beta^{f/f}$ mice to develop a mouse with lack of NF-kB activity in airway secretory cells (CC-LR-IKKβ mice). NF-kB deficiency in the airway epithelium changed the bronchoalveolar lavage fluid (BALF) cellular component (mostly reduction in macrophage number) of the CC-LR mice, and resulted in a ~70% (3.4-fold) reduction in lung surface tumor number compared to age and sex matched control CC-LR mice. It also significantly reduced the number of visible tumors on the lung surface of the CC-LR mice by >60% (2.6-fold) after inducing COPD-like airway inflammation using weekly NTHi exposure for 8 weeks. We then genetically ablated IL-6 in the CC-LR mice. Lack of IL-6 did not change the BALF inflammatory cell component of the CC-LR mice. However, it not only inhibited intrinsic lung cancer development (1.7-fold, 41%), but also inhibited the promoting effect of extrinsic NTHi-induced COPD-like airway inflammation (2.6-fold, 62%) on lung carcinogenesis, suggesting a role for IL-6 mediated epithelial cell signaling in tumor promotion by COPD-like airway inflammation. This is under further investigation by our group. IL-6 is required for differentiation of Th17 cells from naïve T cells. Th17 cells mostly produce IL-17A, and IL-17F. These both bind to the IL-17 receptor (IL-17R), and IL-17R signaling is required for lung CXC chemokine expression and neutrophil recruitment, however, we found that only the IL-17R KO and IL-17A KO but not IL-17F KO mice showed lower levels of neutrophilic influx into the BALF and less inflammatory cell infiltration in lung tissue in response to NTHi. Furthermore, IL-17A induces more production of IL-6 by epithelial cells. So, CC-LR mice were crossed with IL-17A KO mice. Lack of IL-17A resulted in a ~70% (3.4-fold) reduction in lung surface tumor number compared to age and sex matched control CC-LR mice. It also significantly reduced the number of

visible tumors on the lung surface of the CC-LR mice by ~54% (2.2-fold) after inducing COPD-like airway inflammation using weekly NTHi exposure for 4 weeks from the age of 10 weeks. We conclude that airway inflammation through NF-kB mediated activation of the IL-6/Th17 signaling pathway promotes lung carcinogenesis.

A33 Markov model of VeriStrat© selection criteria for EGFR inhibitor therapy predicts improved survival for previously treated patients with NSCLC. Richard E. Nelson, Wallace Akerley. University of Utah, Salt Lake City, UT.

Background: EGFR inhibitor therapy extends survival as 2nd or 3rd line treatment of patients with previously treated metastatic non-small cell lung cancer (NSCLC), but a randomized trial of 2nd line therapy in unselected patients showed similar benefit versus conventional chemotherapy. EGFR mutation analysis is useful in choosing therapy, but it is applicable to only a minority of patients due to low frequency of mutation in a North American population and ignores the majority of patients who may benefit with wild type status. Alternative selection strategies are needed. VeriStrat is a protein expression profile utilizing matrix-assisted laser desorption/ionization time of flight mass spectrometry to analyze serum. The test classifies patients as VeriStrat Good or VeriStrat Poor based on likelihood of benefitting from EGFR inhibitor therapy. The objective of this research was to model the anticipated survival of patients using a Veristrat Selection Strategy for 2nd line therapy of NSCLC to the alternative treatment strategies of chemotherapy for all (Chemo-all), EGFR inhibitor to all (EGFR-all) I, or to performance status selection (PS selection), a common practice, where those with good performance status receive chemotherapy and those with poor PS receive EGFR inhibitor therapy.

Methods: We developed a Markov model to estimate the expected survival of patients for 4 different treatment strategies for NSCLC: (1) VeriStrat Selection, (2) chemotherapy to all patients, (3) EGFR inhibitor to all patients, and (4) PS selection. Model inputs, taken from published literature, for the base-case analysis (and ranges) were the following: PS 0-1, 50% (35%-65%); VeriStrat Good, 60% (45%-75%); VeriStrat Good if PS >1, 40% (25%-55%); 1-year survival on EGFR inhibitor if VeriStrat Good, 47% (43-50); 1-year survival on EGFR inhibitor if VeriStrat Poor, 12% (8-16) and 1-year survival on chemotherapy, 29% (23-35). Probabilistic sensitivity analysis was performed using 10,000 2nd order Monte Carlo simulations.

Results: In the base case analysis, the median survival for EGFR-all was 8.97 months, Chemo-all was 8.48 months, PS selection was 8.20 months and VeriStrat selection was 10.03 months. In the sensitivity analysis, the VeriStrat selection strategy yielded the longest survival in each of the 10,000 simulations.

Conclusion: These results suggest that VeriStrat-based selection of “standard of care” 2nd line therapy of NSCLC improves survival to a clinically significant degree. This strategy, in the absence of new therapies, appears to improve median survivals by 4-6 weeks.

A34 Nitric oxide enhances inflammation, microRNA-21 expression, and KRAS-induced lung carcinogenesis. Hirokazu Okayama[1], Motonobu Saito[1], Oue Naohide[1], Jonathan M. Weiss[2], Jimmy Stauffer[2], Seiichi Takanoshita[3], Robert H. Wiltrout[2], S. Perwez Hussain[1], Curtis C. Harris[1]. [1]National Cancer Institute, Bethesda, MD, [2]National Cancer Institute, Frederick, MD, [3]Fukushima Medical University School of Medicine, Fukushima, Japan.

Mutant KRAS in lung cancers induce molecular pathways that regulate cellular proliferation, survival and inflammation, which enhance tumorigenesis. Inducible nitric oxide synthese (NOS2) up-regulation and sustained nitric oxide (NO•) generation are induced during the inflammatory response and correlate positively with lung tumorigenesis. To explore the mechanistic contribution of NOS2 to KRAS-induced

lung tumorigenesis and inflammation, we used a genetic strategy of crossing NOS2 knockout (NOS2KO) C57BL6 inbred mice with a $KRAS^{G12D}$-driven mouse lung cancer model. $KRAS^{G12D}$/NOS2KO mice exhibited delayed lung tumorigenesis, resulting in a longer overall survival time compared with that of $KRAS^{G12D}$/NOS2WT (wild-type) controls. Correspondingly, tumors in $KRAS^{G12D}$/NOS2KO mice had reduced tumor cell proliferation in adenomas and carcinomas. NOS2-deficiency also led to dramatically suppressed inflammatory response by attenuation of macrophage recruitment into alveoli and within tumor foci. In contrast, FOXP3+ regulatory T cells were increased in tumors from $KRAS^{G12D}$/NOS2KO mice. We further analyzed the expression of microRNA-21 (miR-21), an oncogenic non-coding RNA involved in oncogenic Ras signaling, by quantitative reverse transcription PCR and in situ hybridization. Lung carcinomas dissected from $KRAS^{G12D}$/NOS2KO mice showed a significantly reduced miR-21 expression along with decreased tumor cell proliferation, suggesting that NOS2-deficiency could attenuate RAS signaling pathways that transactivate miR-21 expression. Therefore, deletion of NOS2 decreases lung tumor growth as well as inflammatory responses initiated by oncogenic KRAS, suggesting that both KRAS and NOS2 cooperate in driving lung tumorigenesis and inflammation. Inhibition of NOS2 may have a therapeutic value in lung cancers with oncogenic KRAS mutations.

A35 The role of Mdm2 cleavage in p53 function and chemotherapy response. Trudy G. Oliver. Huntsman Cancer Institute, Salt Lake City, UT.

Chemotherapy resistance is a major obstacle in cancer treatment, yet the mechanisms of resistance to specific therapies have been largely unexplored in vivo. Employing genetic, genomic, and imaging approaches, we have examined the dynamics of response to a mainstay chemotherapeutic, cisplatin, in multiple mouse models of human non-small cell lung cancer (NSCLC). We show that lung tumors initially respond to cisplatin and exhibit a significant reduction in tumor burden. Prolonged cisplatin treatment promotes the emergence of resistant tumors with enhanced repair capacity that are cross-resistant to platinum analogs, exhibit advanced histopathology, and possess an increased frequency of genomic alterations. Cisplatin-resistant tumors express elevated levels of multiple DNA damage repair and cell cycle arrest-related genes, including p53-inducible protein with a death domain (Pidd).

We show that PIDD expression promotes growth arrest and chemotherapy resistance by a mechanism that depends on Caspase-2 and wild-type p53. PIDD-induced Caspase-2 directly cleaves the E3 ubiquitin ligase Mdm2 at Asp 367, leading to loss of the C-terminal RING domain responsible for p53 ubiquitination. As a consequence, N-terminally truncated Mdm2 binds p53 and promotes its stability. Upon DNA damage, p53 induction of the Caspase-2-PIDDosome creates a positive feedback loop that inhibits Mdm2 and reinforces p53 stability and activity, contributing to cell survival and drug resistance. These data establish Mdm2 as a novel cleavage target of Caspase-2 and provide insight into a mechanism of Mdm2 inhibition that impacts p53 dynamics upon genotoxic stress. Our current goals are to: 1) elucidate how cleaved Mdm2 dictates p53 function and 2) create mouse models to manipulate Mdm2 cleavage in vivo in order to interrogate the role of Mdm2 cleavage in lung tumorigenesis and drug response.

A36 Breath biomarkers in the post-NLST era for the discrimination between malignant from benign pulmonary nodules. Nir Peled[1], Fred R. Hirsch[2], Hossam Haick[3], Meggie Hakim[3], Ulrike Tisch[3], Paul A. Bunn, Jr.[2], York E. Miller[4], Timothy C. Kennedy[2], Jane Mattei[2], John D. Mitchell[2], Michael J. Weyant[2]. [1]Sheba Medical Center, Tel Aviv, Israel, [2]University of Colorado Cancer Center, UC Denver, Denver, CO, [3]Technion-Israel, Haifa, Israel, [4]Denver Veterans Affairs Medical Center, Denver, CO.

Background: The recent National Lung Cancer Screening Trial (NLST) has proven that screening for lung cancer by low dose CT (LDCT) scans reduces the related mortality rate by 20%. However, in this program, the false positive rate was extremely high: 96% out of the 24% positive CT findings were non-cancerous. A high rate of false positives leads to unnecessary invasive procedures, which are both costly and associated with significant morbidity and mortality. It is now widely anticipated that LDCT screening programs will be launched in many countries in the near future. This will lead to a dramatically increased detection of small solitary pulmonary nodules (SPNs) for invasive investigation. Consequently, additional non-invasive biomarker approach to distinguish between benign and cancerous conditions is necessary. For that purpose, we evaluated in this study the role of exhale breath analysis as a potential non-invasive biomarker to discriminate between benign and malignant SPNs in the post NLST-era.

Objectives: Developing a signature of volatile organic compounds (VOCs) in the exhaled breath that aids to distinguishes between benign and cancerous SPNs, and therefore also aids in 1) Early detection of lung cancer, 2) Improves the specificity of the NLST screening protocol in high risk cohorts, 3) helps prevent unnecessary invasive procedures, 4) results in the early treatment of lung cancer, and 5) Improves the cost-effectiveness of the NLST screening program. Specifically, in this study we report the VOCs signature that discriminates benign from malignant SPNs, between NSCLC and SCLC and between early and advanced NSCLC.

Methods: Cross-sectional comparative survey from 74 patients with solitary pulmonary nodules (SPNs) attending the University of Colorado Cancer Center or Denver Veterans Affairs Medical Center; USA. Breath samples were taken and the VOC profiles for malignant and benign lung nodules were determined by gas-chromatography/mass-spectrometry (GC-MS), and the corresponding collective VOCs patterns were identified by a nanomaterial-based array of sensors.

Results: Among the 74 high risk patients with SPNs on their LDCTs, 53 were malignant and 21 were benign. Age, smoking history, co-morbidity and medications were similar in both groups. Nodule sizes were 2.7±1.7 cm vs. 1.9±1.1 cm accordingly (NS). Within the malignant group, 47 were NSCLC and 6 were SCLC. Thirty had early disease (stage I-II/limited) and 23 had advanced disease (stage III-IV/extensive).

GC-MS analysis identified two VOCs in the exhaled breath of nodule positive patients that showed statistically significant differences in concentration for benign and malignant lung nodules (N=11, 28 respectively; Benzene, 1-methyl-4-(1-methylethyl) and 1-Octene). The sensor array could distinguish between the corresponding collective VOCs patterns with an accuracy of 89.4%. Among the malignant SPNs we could further distinguish between SCLC and NSCLC (accuracy 93.9%) and between early and advanced disease (89.7%).

Conclusions: An array of nanomaterial-based sensors could discriminate significantly between benign and malignant SPNs in a high-risk cohort with positive LDCTs based on lung cancer related VOCs profiles. Further, it was able to discriminate between NSCLC and SCLC, as well as between early and advanced disease. These results could promote the development of a non-invasive, fast and potentially cost-effective diagnostic tool for the management of nodule-positive patients that could help to eliminate risky invasive procedures in patients with benign nodules in the post NLST-era.

A37 Interim analysis of CALGB 150607: A pilot study of the mutational and expression status of MET, HGF, EGFR, KRAS, p53, c-CBL, and E-cadherin in resected lung adenocarcinoma specimens. Ravi Salgia[1], Vidya Nallasura[1], Robert A. Kratzke[2], Everett E. Vokes[1], Herbert Pang[3], Cleo E. Rolle[1], Rajani Kanteti[1], Rifat Hasina[1], William Richards[4], Lydia Hodgson[3], Qudsia Arif[1], Aliya N. Husain[1]. [1]University of Chicago, Chicago, IL, [2]University of Minnesota, Minneapolis, MN, [3]Duke University School of Medicine, Durham, NC, [4]Harvard Medical School, Boston, MA.

MET is a receptor tyrosine kinase that plays a critical role in proliferation, angiogenesis, invasion, and metastasis. It has been shown to be dysregulated in a number of malignancies, including non-small cell lung cancer (NSCLC). Despite reports of alterations in MET, and its ligand, HGF, being highly associated with advanced pathological stage and worse outcome in patients with NSCLC, the value of MET as a prognostic biomarker remains unclear. The primary objective of this study is to determine the correlation between MET alterations and expression with stage and overall survival in adenocarcinoma (AC) patients in a large cohort of patients. The secondary objectives are to determine the correlation between overall survival and the following: 1) epithelial mesenchymal transition (EMT), 2) EGFR mutations & expression, 3) KRAS mutations, 4) TP53 mutations, and 5) CBL mutations. In addition, sera levels of circulating MET and HGF will be evaluated in order to determine their potential as prognostic factors. MET, EGFR exons 18-21, TP53 exons 4-10, KRAS exon 2, and CBL exons 2-16 were sequenced using standard PCR and sequencing techniques. Standard immunohistochemistry (IHC) techniques were used to evaluate MET, phosphorylated (pMET Y1003 and Y1230/34/35) p53, HGF, EGFR, and E-cadherin expression. A total of 280 patients will be included in this study, and the interim analysis reported herein evaluated 100 patients. The intensity of cytoplasmic or membranous staining was scored on four-point scale: (0, no staining; 1+, weak; 2+, moderate; 3+, strong staining). The extent of staining was scored on a similar scale (0, negative; 1+, 1-10%; 2+, 11-50%; 3+, > 50%). The product of the intensity and extent of staining yielded final scores between 0 and 9. The mean expressions were: MET 3.6 (±0.3); pY1230/34/35 MET 2.0 (±0.2); pY1003MET 4.6 (±0.3); HGF 4.4 (±0.3); EGFR 4.3 (±0.3); TP53 3.7 (±0.3); and E-cadherin 5.5 (±0.3). In ten patient samples, six non-synonymous (NS) mutations were detected in MET (SEMA domain: E168D, M362T, N375S, and Q318K; JM domain: T992I and R970C). In EGFR, the NS mutation L858R was detected in two patients. We detected 12 NS mutations in TP53 (exon 4: E68*, P72R; exon 5: V157F, R175H, I162F, H193Y, Y163D; exon 8: R273L, R273C, V274L, A276F, and G266*). Four NS mutations were detected in exon 2 of KRAS (G12C, G12V, G12D, and G12S). Three mutations were found in the proline-rich region of CBL (L676P, A677S and A678S). ELISAs were utilized to determine soluble MET and HGF levels in pre- and post-operative sera samples. Soluble MET serum levels were significantly increased (p<0.0005) in post-operative samples (1760 ng/ml ± 51.66) compared to pre-operative samples (1585 ng/ml ± 44.61). HGF levels were similar in pre-operative (1008 pg/ml ± 74.8) and post-operative samples (1266 pg/ml ± 175.9). In conclusion, MET and pMET (Y1003) were highly expressed in resected lung AC specimens. NS mutations were detected in all of the oncogenic genes assessed and the functional relevance of these mutations on tumorigenesis remains to be determined. Interestingly, novel MET mutations were detected in key functional domains; the SEMA domain which is critical for dimerization and ligand binding, and the JM domain which is important for MET downregulation via c-CBL. We will correlate these mutational and expression data with clinical outcomes in order to determine the prognostic role of MET.

A38 Whole methylome explorations of paired lung tumor and non-tumor clinical samples. Nandita Mullapudi, Masako Suzuki, Melissa Fazzari, Weiguo Han, Simon D. Spivack. Albert Einstein College of Medicine, Bronx, NY.

Background: Hypermethylation in specific candidate gene promoters has been found during progressive lung carcinogenesis. To explore common methylation events on a genome-wide scale in lung cancer, we analyzed the methylation profiles of paired NSCLC tumor and far adjacent non-tumor samples using the HELP-microarray assay, which yields information on 1.2 million fragments throughout the genome.

Methods: The HELP (HpaII tiny fragment Enriched by Ligation mediated PCR) assay is based on the generation of restriction enzyme libraries generated by methylation sensitive (HpaII) and methylation insensitive (MspI) isoschizomers, in its second generation as a microarray platform. The assay lends itself to low starting amounts of DNA (3 ug) and robust assessment of methylation status by comparing ratios of HpaII- generated-fragments to MspI- generated fragments co-hybridized to a Nimbelgen custom high-density microarrays. The CCGG sites were weighted if neighboring CCGGs were methylated in same direction. Here, 24 pairs of tumor and adjacent non-tumor samples were analyzed using the HELP assay.

Summary of results: At p = 5E-6, we identified 26,138 differentially methylated fragments (corresponding to 2 CpG sites each) in tumor versus non-tumor. The overall trend was consistent with genome-wide hypomethylation and locus specific hypermethylation (localized to CG-island containing promoters). We could identify both known and novel regions of the genome as well as specific gene-promoters that are hypermethylated in tumor versus non-tumor.

Region # loci # signif loci T Hypomethyl T Hypermethyl

Promoter 151,568 576 69% 31%

Gene Body 551,628 9,817 62% 38%

Intergenic 540,473 15,745 97% 3%

Conclusion: An interrogation of methylation status of 1.2 million loci throughout the genome in paired lung tumor/non-tumor specimens reveals many more differential methylation events in gene bodies and intergenic regions than in promoters. That said, many previously unreported differentially-methylated gene promoters were identified. We are able to discover individual methylation events common across different clinical specimens. Based on a set of priors, we have narrowed down promoter-specific hypermethylation events for further validation using tagged Bisulfite Genomic Sequencing. We are also working on the integration of methylome date with other genome-wide epigenetic and expression data from the same clinical samples.

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A39 Prevention of tobacco carcinogen-induced lung cancer in mice using antiestrogens. Laura P. Stabile, Mary E. Rothstein, Diana Lenzner, Stephanie R. Land, Sanja Dacic, Jill M. Siegfried. University of Pittsburgh, Pittsburgh, PA.

There is increasing evidence for steroid hormone effects in lung cancer. Estrogens are involved in lung cancer proliferation and progression, and human lung tumors express estrogen receptor β (ERβ) as well as aromatase, the enzyme which catalyzes the final step in 17-β-estradiol synthesis. We have previously demonstrated that high ERβ expression in lung tumors was an independent negative prognostic predictor of survival, and the contribution of aromatase expression resulted in further effects on survival in both men and women. To determine if the aromatase inhibitor anastrozole prevents the development of lung tumors induced by the tobacco carcinogen, NNK, alone or in combination with the ER antagonist fulvestrant, ovariectomized FVB/N female mice were exposed to NNK along with daily supplementation of 0.1mg androstenedione, the substrate for aromatase. Placebo control, anastrozole (0.1mg/kg/day; oral gavage) and/or fulvestrant

(30mg/kg, 2X/week, s.c.) were administered in both an initiation model and a progression model of lung tumorigenesis. In the initiation model, the combination of fulvestrant and anastrozole was administered during NNK exposure and resulted in significantly fewer NNK-induced lung tumors (mean=0.5; range 0-1) compared to placebo (mean=4.6; range 2-8, P<0.001), fulvestrant alone (mean=3.4; range 1-4, P<0.001) or anastrozole alone (mean=2.8; range 0-5, P=0.002). Although a significant decrease in tumor size was not observed among treatment groups, a significant decrease in the cell proliferation index as assessed by Ki67 immunostaining within the tumors treated with anti-estrogens was observed. Beginning anti-estrogen treatment 9 weeks after NNK exposure in the lung tumor progression model, when preneoplastic areas had already formed, also yielded maximum anti-tumor effects with the combination group. In this model, tumor size was modulated by hormonal treatment with the smallest tumors observed in the combination treated group. These results suggest that targeting the estrogen pathway can inhibit lung cancer development during induction of damage to the airways by tobacco carcinogen exposure as well as after damage has occurred. In both animal models, aromatase expression was found mainly in macrophages infiltrating preneoplastic and tumor cells of the lungs, while ERβ was found in both macrophages and tumor cells. An important source of estrogen synthesis may be inflammatory cells that infiltrate the lungs in response to carcinogens, beginning early in the carcinogenesis process. ERβ expressed by inflammatory and neoplastic epithelial cells in the lung may signal in response to local estrogen production. In summary, the effects of combining two endocrine agents with different modes of actions suggest that complete blockade of estrogen action by inhibition of estrogen synthesis and down-regulation of ER has a greater effect on the prevention of tobacco carcinogen-induced murine lung tumorigenesis, and may be useful for lung cancer prevention in both males and females. Additionally, a greater understanding of the inflammatory state of the lung in relation to estrogen signaling is necessary to understand the early stages of lung tumorigenesis. This work was supported by NIH P50CA090440, SPORE in Lung Cancer to JMS and in part by the Comprehensive Cancer Center award P30CA047904.

A40 The genetic status of the EGFR, K-ras, and EML4-ALK genes in multiple primary noninvasive lung adenocarcinomas. Kenji Sugio, Takashi Seto, Yukito Ichinose, Kenichi Taguchi, Kaname Nosaki, Taro Ohba, Gohshi Toyokawa, Fumihiko Hirai, Masafumi Yamaguchi, Riichiroh Maruyama, Motoharu Hamatake. National Kyushu Cancer Center, Fukuoka, Japan.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR2) of the conference *Proceedings*.

A41 SOX11 transcriptionally regulates a cytokine-based gene signature in lung cancer-associated fibroblasts (LCAF) to promote tumor growth. Silvestre Vicent, Anna K. Gillespie, Dedeepya Vakaa, Purvesh Khatri, Ron Chen, Olivier Gevaert, Nicole Clarke, Sylvia Plevritis, Atul Butte, Alejandro Sweet-Cordero. Stanford University, Palo Alto, CA.

Increasing evidence points to an important role for the tumor microenvironment in cancer progression. However, the role of tumor stroma in the pathogenesis of lung cancer is poorly understood. Desmoplasia is a common pathologic feature of many NSCLC. We noted a similar phenotype in a mouse model of lung cancer driven by expression of oncogenic Kras. Lung cancer-associated fibroblasts (LCAFs) and normal lung fibroblasts (NLFs) from the mouse model of Kras-driven lung cancer were isolated and queried at the functional level using a xenograft model. First, we observed that LCAFs promote tumor growth more favorably than NLFs in vivo when co-injected with mouse tumor cells. Moreover, NLFs passaged in vivo acquire features of LCAFs

as indicated by an increased tumor-promoting capacity on mouse and human NSCLC xenografts. In addition, conditioned media from LCAFs stimulated cell proliferation in vitro more significantly than that of NLFs. Expression profiling of NLFs and LCAFs using microarrays uncovered a gene set that includes multiple secreted proteins and genes related to inflammatory processes. Moreover, the SRY-related HMG transcription factor Sox11 was highly up-regulated in LCAFs compared to NLFs. Over-expression and knock-down experiments have confirmed that Sox11 regulates the expression of many of the secreted proteins expressed in LCAFs. The relevance of these findings for human NSCLC is suggested by the observation that the mouse LCAF gene expression signature was predictive of survival in a large cohort of human NSCLC. Besides, Sox11 and several of the secreted proteins expressed in LCAFs, along with their putative receptors, were up-regulated in human NSCLC samples. In conclusion, we have identified a potentially novel regulator of the transition between NLFs and LCAFs and, thus, uncovered a new signaling hub that could be used for therapeutic intervention.

A42 microRNA-564 is underexpressed in stage I and II lung adenocarcinoma in patients developing brain metastasis. Glen J. Weiss[1], Carlos D. Lorenzo[2], Paul A. Kurywchak[2], Mohsin Malik[3], Guy M. Raz[2], Walter P. Wagner[2], Nhan L. Tran[2], Irene Cherni[2], Kim M. Paquette[2], Raghu Metpally[2], Tim G. Whitsett[2], Jeffrey Allen[3], Seungchan Kim[2], Jianping Hua[2], Waibhav D. Tembe[2]. [1]Virginia G. Piper Cancer Center Clinical Trials at Scottsdale Healthcare/TGen, Scottsdale, AZ, [2]The Translational Genomics Research Institute, Phoenix, AZ, [3]University of Tennessee Cancer Institute, Memphis, TN.

Background: Brain metastasis (BM) is a common complication in non-small cell lung cancer (NSCLC) affecting ~20% of patients in the course of their disease. Once detected, BM is often treated with surgery and/or radiation, however the outcome is often poor. Molecular characterization of tumor that is specifically associated with BM development is lacking. Identifying patients who have a high likelihood of developing BM who could benefit from a more aggressive initial treatment has important clinical implications. MicroRNAs (miRNAs) play an integral role in multiple cellular processes that lead to oncopathogenesis, display high tissue specificity, and are rather resistant to degradation. We propose using miRNA for discovery and validation of predictive biomarkers for the risk of development of BM in stage I and II lung adenocarcinoma.

Methods: Formalin-fixed, paraffin-embedded (FFPE) primary lung adenocarcinoma tumor samples from 8 patients who developed BM (BM+) and 26 patients that did not develop BM with a minimum follow-up of 47 months (BM-) were assessed in the Discovery cohort on 8x15 Agilent microarray printed with 886 miRNAs (Discovery cohort). Target miRNAs were selected based on minimum of 2-fold change in expression and Welch t-test p-value cut-off of <0.05. qPCR assay of these individual miRNAs were measured in the Discovery cohort. The Validation cohort, consisting of the Discovery cohort samples (N=34) and additional stage I/II lung adenocarcinoma patient samples (N=36 BM- and N=2 BM+), using PCR are being analyzed to confirm differential expression of target miRNA. Fold-change values are generated with the ΔΔCt method. Development of a PCR-based miRNA classifier using the Validation cohort PCR results is being developed. This miRNA classifier will assessed for accuracy prediction when applied to measured target miRNAs in the Independent cohort of additional stage I/II lung adenocarcinoma samples from another institution blinded for development of BM (N=25 BM- and N=9 BM+).

Results: SAM analysis of microarray results revealed 7 mature miRNAs to be significantly under-expressed in the BM+ compared to BM- patient sample set. These included miR-378, -513c, -564, -513b, -203, -662, and -99a. One of 7 candidate miRNAs, miR-564, was confirmed to be significantly under-expressed in the Discovery cohort by qPCR

(p<0.05). PCR results in the Validation cohort is ongoing and the miRNA BM classifer is being developed and results applied to the Independent cohort is ongoing.

Conclusion: Tumor-based miRNA signatures are gaining increasing credibility as promising biomarkers for early diagnosis and predicting course of a disease. Here we have described a novel miRNA, miR-564, that has not previously been shown to play a role in lung or brain cancers, yet may be associated with predicting lung-to-brain metastasis.

Support provided by Flinn Foundation.

A43 Identification of key tumorigenic pathways in never-smoker lung adenocarcinoma patients using massively parallel DNA and RNA sequencing. Timothy G. Whitsett, Jr.[1], Shripad Sinari[1], Kelly W. Sheff[1], Phillip Y. Cheung[1], Julianna Ross[1], Brock Armstrong[1], Landon Inge[2], Ross Bremner[2], Cheryl Selinsky[1], Fay Betsou[3], Robert Phillips[3], Irene Cherni[1], Jeff M. Trent[1], Daniel D. Von Hoff[4], John D. Carpten[1], David W. Craig[1], Glen J. Weiss[4], Nhan L. Tran[1], Tyler Izatt[1], Jessica Aldrich[1], Ahmet Kurdoglu[1], Alexis Christoforides[1], Angela S. Baker[1], Lori Phillips[1], Winnie S. Liang[1]. [1]The Translational Genomics Research Institute, Phoenix, AZ, [2]St. Joseph's Hospital and Medical Center, Phoenix, AZ, [3]Integrated Biobank of Luxembourg, Luxembourg City, Luxembourg, [4]Virginia G. Piper Cancer Center Clinical Trials at Scottsdale Healthcare/TGen, Scottsdale, AZ.

Lung cancer remains the leading cause of cancer deaths in the US and throughout the world. Never-smokers with lung cancer constitute an understudied subset of these patients, though recent estimates show that ~10% of men and women with lung cancer in the US are never-smokers. Independently, lung cancer in never-smokers ranks among the ten most common causes of cancer mortality, and thus the causes, driver pathways, and potential therapeutics must be investigated for this clinically relevant subpopulation. We hypothesize that novel mutations and pathways identified by whole genome sequencing (WGS) and whole transcriptome sequencing (WTS) drive tumorigenesis in adenocarcinomas of never-smoker patients, and represent potential therapeutic targets. We have completed WGS and WTS on two lung adenocarcinomas from female, never-smokers, one early-stage and one advanced-stage (metastatic), and one female smoker patient with early-stage lung adenocarcinoma. Approximately 100 short nucleotide variants (SNV) causing non-synonymous DNA sequence changes were discerned from the early- and advanced-stage adenocarcinomas from never-smokers. Of interest, these never-smoker patients lacked alterations in common genes associated with lung cancer such as EGFR, KRAS, and EML/ALK translocations, making them ideal cases for the discovery of new mutations that may drive lung adenocarcinomas in never-smokers. In comparison, the adenocarcinoma from a smoker patient contained 78 SNVs, including a well-characterized KRAS mutation. Mutations in MAGEC1, a tumor marker in melanoma and multiple myeloma, were observed in common between the early-stage, never-smoker tumor and the smoker tumor. The mutations observed in the never-smoker cases included genes suspected to play a role in lung carcinogenesis. The early-stage, never-smoker patient contained a mutation in PIK3C3 and DOCK10, known to play a role in cancer cell migration, and CSNK1E, a casein kinase involved Wnt signaling and in beta catenin-induced cancer cell proliferation. The late-stage never-smoker patient demonstrated a p53 mutation, a mutation in the tumor suppressor LATS2, and a mutation in the DNA damage checkpoint gene, ATM. The mutations discovered in known tumor suppressor genes tended to be in the late-stage, never-smoke patient. Ingenuity Pathway Analysis implicated G-protein coupled receptor signaling for the early-stage never-smoker, Hif1α signaling for the smoker, and Sonic Hedgehog Signaling for the late-stage, never-smoker in both WGS and WTS. Currently, we are validating the identified mutations discovered by WGS using Sanger sequencing and surveying their frequency in ~30 and ~60 additional

cases of lung adenocarcinoma from never-smokers and clinically matched smokers, respectively. Validation of these mutations and pathways will lead to a better understanding of tumorigenesis and tumor progression in never-smokers and identify nodes for therapeutic vulnerability.

A44 K-homology splicing regulatory protein modulates tumor suppressor miRNA 29b through the noncanonical Wnt 7a signaling pathway. Lora A. Wilson, Robert A. Winn. University of Colorado Denver Anschutz Medical Campus, Aurora, CO.

Precedent has established the role of the Wnt/β-catenin pathway in oncogenesis. Several members of the Wnt family are known to be tumor promoting in non-small cell lung cancer, NSCLC. In this pathway β-catenin, a downstream component, accumulates and serves as a T-cell transcription /lymphoid enhancer factor. We previously identified the Wnt ligand in the lung, Wnt 7a, as a member of the non-canonical pathway/β-catenin independent pathway, which inhibits transformed cell growth. K-homology splicing regulatory protein (KSRP) is a member of the family of AU rich element binding proteins. It possesses four K-homology motifs and causes decay of specific mRNA transcripts and regulation of miRNA. Wnt 7a positively regulates the expression of KSRP. As a result, KSRP upregulates the processing of miRNA 29b. The miRNA 29b family is comprised of 29a, 29b, and 29c. KSRP and miRNA 29b expression are elevated in nontransformed cells and alternatively, lower levels of expression are in NSCLC. The role of KSRP regulating miRNA 29b in the non-canonical Wnt pathway in the lung has previously been unknown. Over expression of KSRP through upregulation of miRNA 29b in NSCLC cells decreases cell proliferation in MTS assays, a measure of proliferation, as well as reducing colony formation in clonogenic assays. We reveal a novel association of p53 and miRNA 29b. We identified that KSRP increases PPARγ activation which in turn positively modulates miRNA 29b, resulting in upregulation of p53. The antitumorigenic effect is specific to Wnt 7a activation through Erk 5 dependent kinases.

A45 NSCLC and SCLC mouse models mediated by lentiviral gene delivery. Yifeng Xia, Narayana Yeddula, Mathias Leblanc, Reuben Shaw, Inder Verma. Salk Institute for Biological Studies, La Jolla, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR3) of the conference *Proceedings*.

A46 Identification of ALDH1A3 as an important biomarker and therapeutic target for non-small cell lung cancer (NSCLC) cancer stem cells (CSCs). Chunli Shao[1], James P. Sullivan[2], Luc Girard[1], Laura A. Sullivan[1], Alexander Augustyn[1], Yang Xie[1], Carmen Behrens[3], Ignacio Wistuba[3], John D. Minna[1]. [1]University of Texas Southwestern Medical Center, Dallas, TX, [2]Harvard Medical School, Charlestown, MA, [3]Universit of Texas MD Anderson Cancer Center, Houston, TX.

A considerable amount of evidence reveals the important roles of cancer stem cells (CSCs, also called “cancer initiating cells”) in tumor growth, metastasis, relapse and drug resistance. Our group has identified lung CSCs in both lung cancer cell lines and patient tumor samples by their elevated Aldehyde Dehydrogenase (ALDH) activity and demonstrated that NSCLC patients whose tumors are enriched in ALDH+ cells have inferior survival, and that the ALDH+ subpopulations (ranging from 0.5 to 30% of tumor cells) are self renewing, and significnatly more clonogenic and tumorigencic (in xenograft models) than the ALDH- population. To further define the molecular profile of lung CSCs and to identify key pathways involved in lung CSC function we performed genome wide microarray expression profiling of isolated ALDH+ and ALDH- cell populations from 8 non-small cell lung cancer (NSCLC) lines representing a variety of oncogenotypes to determine genes that are differentially expressed in

these two populations. Strikingly we found that different lung cancers exhibited different ALDH+ cell population expression profiles but that the ALDH1A3 isozyme was one of the few commonly genes whose expression was elevated. (Previously our group and other investigators in lung and other cancers had found the ALDH1A1 and not ALDH1A3 isozyme to be a marker of the ALDH+ population). ALDH1A3 differential expression was confirmed by q RT PCR and elevated ALDH1A3 protein levels which in turn was correlated with the % of ALDH+ tumor cells in each tumor. ALDH1A3 mRNA levels in (N = 182) resected lung adenocarcinoma samples showe that the highest levels of ALDH1A3 were associated with inferior survival.

Of great importance, shRNA mediated knockdown of ALDH1A3 dramatically reduced both NSCLC cell colony formation in vitro and tumor growth in vivo demonstrating the functional importance of ALDH1A3 expression in NSCLCs. However, ectopic expression of ALDH1A3 in NSCLC cells did not enhance lung cancer cell growth and tumor formation, suggesting that ALDH1A3 alone is not sufficient to promote lung cancer progression. We conclude that ALDH1A3 is the predominant ALDH isozyme responsible for ALDH activity in NSCLCs of many oncogenotypes, is of potential prognostic importance, and a new therapeutic target for eliminating NSCLC stem cells.

B1 Targeting KRAS mutant NSCLC with the Hsp90 inhibitor ganetespib. Jaime Acquaviva, Don Smith, Jim Sang, Yumiko Wada, David A. Proia. Synta Pharmaceuticals Corp., Lexington, MA.

Background: Mutant KRAS is detected in 20-25% of non-small cell lung carcinomas (NSCLC) and represents one of the most common oncogenic drivers of this disease. NSCLC tumors with oncogenic KRAS respond poorly to currently available therapies necessitating the pursuit of new treatment strategies. Heat shock protein 90 (Hsp90) is a molecular chaperone required for the maturation and stability of hundreds of client proteins, many of which are known oncogenic drivers or effectors of such proteins. Inactivation of Hsp90 results in the simultaneous inhibition of multiple oncogenic signaling pathways, making Hsp90 a highly attractive therapeutic target. Ganetespib is a second generation, small molecule Hsp90 inhibitor currently being evaluated in multiple clinical trials. Recent results from a Phase 2 trial with ganetespib revealed that >60% of patients with KRAS mutant NSCLC exhibited tumor shrinkage at 8 weeks, indicating that ganetespib has promising potential to benefit this disease.

Aims: To further understand the actions of ganetespib in mutant KRAS NSCLC tumors, preclinical studies were executed in a diverse panel of KRAS mutant NSCLC cell lines to: (1) Investigate whether ganetespib is effective in suppressing critical cell signaling nodes responsible for KRAS-driven NSCLC cell survival and (2) Assess whether ganetespib can synergize with both clinical agents targeted against these signaling nodes and standard of care chemotherapies.

Results: Ganetespib displayed potent anticancer activity across 15 KRAS mutant NSCLC cell lines assayed in vitro, with an average IC50 of 24 nM. Combining ganetespib with anti-mitotics, alkylating agents or topoisomerase inhibitors resulted in an increase in cell death of up to 44, 61 and 26%, respectively, versus monotherapy. At the molecular level, ganetespib induced the destabilization of several KRAS substrates, including BRAF and CRAF, leading to inactivation of their downstream effectors followed by programmed cell death. Ganetespib effectively suppressed the growth of human KRAS mutant NSCLC tumor xenografts in vivo; however, ganetespib did not induce tumor regression. In light of this, we sought to investigate whether inhibitors targeting KRAS driven signaling nodes would confer greater sensitivity to ganetespib. In vitro, combinations of low dose ganetespib with either MEK or PI3K/mTOR inhibitors consistently resulted in greater activity than monotherapy, up to 77% and 42%, respectively. Furthermore, ganetespib suppressed activating feedback loops that occur in response to MEK and PI3K/mTOR inhibition, providing a rationale for the enhanced combinatorial activity. To validate these results, in vivo combinations were performed with ganetespib and a PI3K/mTOR inhibitor in KRAS mutant NSCLC xenografts. While both agents promoted tumor shrinkage on their own, considerable improvement in tumor growth inhibition was observed in the combination arm.

Conclusions: Ganetespib, a potent inhibitor of Hsp90, has shown encouraging evidence of clinical activity, including tumor shrinkage in patients with KRAS mutant NSCLC. In vitro, ganetespib exhibited potent anticancer activity in NSCLC cells harboring a diverse spectrum of KRAS mutations due in part to degradation and inactivation of critical KRAS signaling effectors. Combination with targeted therapies that overlap with these signaling nodes led to enhanced anticancer activity in vitro and in mouse models of KRAS mutant NSCLC. Taken together, these results could have interesting clinical utility in patients with KRAS mutant NSCLC.

B2 Evaluation of TRAIL-resistance mechanisms and apoptosis induction by LBY135, a DR5 agonist, in NSCLC cell lines. Mauricio S. Caetano, Cinthya Sternberg, Carlos G. Ferreira. CPCIT, National Cancer Institute of Brazil (INCA), Rio De Janeiro, Brazil.

Lung cancer accounts for the majority of deaths by cancer in the world. Histologically, lung cancer is classified into two groups: Non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), differing not only in its morphological and clinical characteristics, but also in therapeutic approach and management. NSCLC accounts for 80% of the cases and is further divided into three different histological subtypes: adenocarcinoma, large cell carcinoma and squamous cell carcinoma. Treatment options include complete surgical resection, but most of the patients present at diagnosis already in advanced or metastatic disease stage. Therefore, the therapy of choice in these cases is platinum-based chemotherapy, which presents high general toxicity and stumble into an intrinsic feature of lung cancer, the high rate of chemo-resistance. In spite of the recent development of the field of target-drugs (e.g., tyrosine kinase inhibitors), several sub-groups of lung cancer patients still have unmet needs for therapy and drugs with specific mechanisms emerge as a rational alternative. Aberrations found in the apoptotic pathway are a common feature in lung cancer. TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of cytokines, which can induce apoptotic cell death in a variety of tumor cells by engaging specific death receptors, DR4 and DR5, while having low toxicity towards normal cells. This peculiar expression pattern turns DRs into a potential therapeutic target for lung cancer. In this study, we sought to explore TRAIL pathway activation as a strategy to subvert the resistance to cisplatin presented by NSCLC cell lines. We have used a panel of NSCLC cell lines harboring different mutations and displaying distinct histology profiles (A549 – adenocarcinoma: KRAS mut, TPp53 WT and BRAF WT; LC319 – Adenocarcinoma: KRAS mut, TPp53 mut and BRAF mut; H460 – Large Cell Carcinoma: KRAS mut, TPp53 WT and BRAF WT; Calu-1: Squamous Cell Carcinoma: KRAS mut, TPp53 null and BRAF WT. As expected, these cell lines display differential sensitivity to cisplatin. NSCLC cell lines were treated with suboptimal concentrations of cisplatin (IC30) for 48 hours and expression of TRAIL receptor family was accessed by real-time PCR. Our results demonstrate that suboptimal dose of cisplatin (IC30) induces an increase in the expression of DR4 and DR5 in NSCLC cell lines, and Western blot analysis confirmed the increase in DR5 expression at protein level. Literature suggests that NSCLC tumors are naturally resistant to TRAIL-induced cell death. LBY135 (Novartis) is a monoclonal antibody agonist to DR5, mimicking cell death signaling triggered by TRAIL. LBY135 was capable to decrease metabolic activity measured by MTT assay in NSCLC cell lines. As we consistently observed an increase in DR5 expression after cisplatin treatment, we postulated that it could chemo-sensitize lung cancer cells to DR5-induced cell death. Accordingly, the combination of a suboptimal dose of cisplatin with LBY135 elicited a synergistic effect in cell death induction in NSCLC cell lines. Our results suggest an alternative way to subvert chemoresistance in lung cancer, where the association of cisplatin in low doses with an agonist of DR5 (LBY135) is able to restore the apoptotic machinery and reverse the resistance to cell death.

B3 Relevance of genotyping non-small cell lung cancer patients on response to platinum-basedchemotherapy and tyrosine kinase inhibitors. Alma D. Campos-Parra[1], Graciela Cruz[1], Carlos Zuloaga[1], Alejandro Aviles[1], María E. Vázquez Manríquez[2], José R. Borbolla-Escoboza[3], Andrés Cardona[4], Meneses Abelardo[1], Oscar Arrieta[1]. [1]Instituto Nacional de Cancerologia, México, DF, Mexico, [2]Instituto Nacional de Enfermedades Respiratorias, México, DF, Mexico, [3]Instituto Tecnológico de Monterrey, Monterrey, Nuevo León, Mexico, [4]Grupo de Oncología Clínica y Traslacional, Fundación Santa Fe de Bogotá, Bogotá, Colombia.

Background: Subdividing non-small cell lung cancer (NSCLC) based on molecular alterations such as EGFR, KRAS and ALK genes is important for selecting treatment involving EGFR and EML4-ALK tyrosine kinase inhibitors (TKI). However, little information is available comparing patients' response and progression-free survival in the presence or absence of EGFR, KRAS mutations or the EML4-ALK fusion gene when being treated with chemotherapy.

Methods: NSCLC patients were treated with chemotherapy and/or TKIs. Tests were performed for EGFR and KRAS gene mutation as well as EML4-ALK fusion genes. Progression-free survival and overall survival association with type of treatment and mutational status was analyzed.

Results: The factors associated with a response to chemotherapy were the presence of EGFR and KRAS mutation (p=0.006 and p=0.028, respectively). Factors associated with TKI response were adenocarcinoma (HR 2.7: 1.6-4.6 95%CI; p<0.001), EGFR mutation (HR 0.5: 0.3-0.8 95%CI; p= 0.009) and wild-type KRAS (HR 1.7: 1.1-2.8 95%CI; p= 0.013). Mean progression-free survival in the chemotherapy group was 5.3 months (4.8 - 5.7 95%CI).

Conclusion: EGFR and KRAS mutation status appeared to subdivide NSCLC patients into TKI and chemotherapy response groups.

B4 A "DNA replication stress" gene signature associated with a poor prognosis in early non-small cell lung cancer. Camille Allera-Moreau[1], Marie-Bernadette Delisle[1], Jean-Sebastien Hoffmann[1], Christophe Cazaux[1], Isabelle Rouquette[1], Benoit Lepage[2], Naima Oumouhou[2], Karine Gordien[1], Laurent Brouchet[3], Julien Mazieres[1], Philippe Pasero[4]. [1]Cancer Research Center of Toulouse Inserm University of Toulouse, Toulouse, France, [2]Service of Epidemiology Inserm University of Toulouse, Toulouse, France, [3]Rangueil-Larrey Hospital, University of Toulouse, Toulouse, France, [4]Institute of Human Genetics CNRS, Montpellier, France.

The conventional clinical staging classification is not sufficient to predict the survival of patients who suffer from early lung cancer. Additional prognostic factors are then needed to better forecast their outcome.

Since perturbation of the genome replication i.e. the so-called "replicative stress" is admitted to contribute to neoplasia from its early stages, we hypothesized that genes involved in such process may therefore represent a still under-explored source of such biomarkers.

We specifically assessed in primary tumors and adjacent normal tissues from a series of 93 patients suffering from early- or mid-stage non-small cell lung adenocarcinoma the expression (RT-qPCR) of 77 "DNA replication" genes involved in either initiation/licensing at the 50,000 replication origins dispersed along the genome, elongation of DNA chains onto damaged or undamaged DNA, maintenance of stalled DNA replication forks or intra-S phase DNA damage processing and signaling.

A 4-gene signature separated patients to high-risk and low-risk subgroups with significantly different survival. This prognostic effect was independent on age, sex, treatment, stage classification and expression of proliferation markers.

We propose that a "cancer replisome" signature could be a predictor of the cancer survival and might also help understanding

the molecular mechanisms underlying tumor progression in lung cancer patients.

B5 Ascochlorin inhibits growth factor-induced HIF-1α activation and tumor angiogenesis through the suppression of EGFR/ERK/p70S6K signaling pathway in CaSki cells. Hyun-JI Cho, Yun-Jeong Jeong, Su-Hyun Park, Young-Chae Chang. Catholic University of Daegu School of Medicine, Daegu, Republic of Korea.

Ascochlorin, a non-toxic prenylphenol compound derived from the fungus Ascochyta viciae, has been shown recently to have anti-cancer effects on various human cancer cells. However, the precise molecular mechanism of this anti-cancer activity remains to be elucidated. Angiogenesis was quantified by measuring direct hemoglobin content and matrigel plug assay. The expression of genes and proteins associated with tumor-angiogenesis and its signaling pathway was measured suing RT-PCR and Western blotting anlysis. Here, we investigated the effects of ascochlorin on hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) expression in human epidermoid cervical carcinoma CaSki cells. Ascochlorin inhibited epidermal growth factor (EGF)-induced HIF-1α and VEGF expression through multiple potential mechanisms. First, ascochlorin selectively inhibited HIF-1α expression in response to EGF stimulation, but not in response to hypoxia (1% O2) or treatment with a transition metal (CoCl2). Second, ascochlorin inhibited EGF-induced ERK-1/2 activation but not AKT activation, both of which play essential roles in EGF-induced HIF-1α protein synthesis. Targeted inhibition of epidermal growth factor receptor (EGFR) expression using an EGFR-specific small interfering RNA (siRNA) diminished HIF-1α expression, which suggested that ascochlorin inhibits HIF-1α expression through suppression of EGFR activation. Finally, we showed that ascochlorin functionally abrogates in vivo tumor angiogenesis induced by EGF in a Matrigel plug assay. Our data suggest that ascochlorin inhibits EGF-mediated induction of HIF-1α expression in CaSki cells, providing a potentially new avenue of development of anti-cancer drugs that target tumor angiogenesis.

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B6 Folylpoly-glutamate synthetase and thymidylate synthase are associated with clinical outcome from pemetrexed-based therapy in non-small cell lung cancer (NSCLC). Daniel C. Christoph[1], Heike Loewendick[2], Anja Peglow[2], Martin Schuler[2], Wilfried E. Eberhardt[2], Fred R. Hirsch[1], Bernadette Reyna Asuncion[1], Murry W. Wynes[1], Thomas C. Gauler[3], Jeremias Wohlschlaeger[2], Dirk Theegarten[2], Stefan Welter[3], Cindy Tran[1], Biftu Hassan[1]. [1]University of Colorado, Aurora, Colorado, [2]University Duisburg-Essen, University Hospital Essen, Essen, Germany, [3]University Hospital Essen, Ruhrlandklinik, Essen, Germany.

Background: The antifolate pemetrexed targets multiple enzymes involved in pyrimidine and purine synthesis including thymidylate synthase (TS). After entry into cells, pemetrexed is converted to polyglutamated forms by folylpoly-γ-glutamate synthetase (FPGS), a critical step to achieve full target inhibition. We hypothesized that FPGS and TS protein expression may predict outcome following pemetrexed-treatment of patients with advanced NSCLC, like in malignant pleural mesotheliomas (Christoph et al., J Clin Oncol. 2011: 29 suppl.). This is the largest report on pemetrexed-treatment outcome based on TS and FPGS in Caucasian patients with advanced NSCLC.

Methods: Pretreatment tumor samples from 161 patients (pts) with metastatic NSCLC, treated with pemetrexed combined with platinum (74 pts (46%)) or as single agent (82 pts (51%)) or within other combinations (5 pts (3%)), were retrospectively analyzed. FPGS and TS protein expression levels

were evaluated by IHC using the H-Scoring system (0-300), which relies on the product of intensity (range 0 to 3) and the percentage of positive tumor cells (0-100%). Radiographic evaluation of response was performed according to RECIST criteria (version 1.1).

Results: Median pretreatment H-scores were 180 for FPGS (range: 0-280) and 205 (range: 120-290) for TS. Using the log-rank test and the median H-score as cut-off, we found a significant association between low TS protein expression and improved progression-free survival (PFS) (median PFS of 5.5 months vs. 3.4 months; hazard ratio [HR] 0.66, 95% CI, 0.45 to 0.96; P=0.03) or prolonged overall survival (median OS of 33.9 months vs. 15.0 months; hazard ratio [HR] 0.52, 95% CI, 0.31 to 0.86; P=0.01). Moreover, high FPGS protein expression was only associated with better PFS (median PFS of 5.5 months vs. 3.4 months; hazard ratio [HR] 0.58, 95% CI, 0.37 to 0.89; P=0.03). Considering exclusively patients suffering from adenocarcinomas (110 pts (68%)), TS was associated with objective response to pemetrexed-based treatment (mean H-score 192 for responders vs. 210 for non-responders, P=0.03).

Conclusions: We have investigated FPGS and TS protein expressions in tumor specimens from the largest series of pemetrexed-treated Caucasian NSCLC patients. Baseline determination of TS and FPGS expression by IHC using the H-score system is associated with clinical outcome from pemetrexed-based therapy in advanced NSCLC. Further prospective validation studies are warranted.

B7 Use of ponatinib to restrict growth of non-small cell lung cancer (NSCLC) cells overexpressing FGFR1. Mingqiang Ren, Mei Hong, Suiquan Wang, John Cowell, Zhonglin Hao. Georgia Health Sciences University, Augusta, GA.

For advanced stage NSCLC, targeted molecular therapy based on an understanding of the oncogenic driver mutations is emerging as the new front line care replacing chemotherapy where the oncogenic driver mutation is clearly defined e.g EGFR mutation or EML4-ALK translocation. However, the proportion of patients that fall into this category is small and are mostly non-smokers with adeno histology. Identifying new oncogenic drivers in NSCLC is therefore paramount.

In our initial Oncomine database search, we found that FGFRs are overexpressed in various NSCLC tissue specimens. Quantitative real time PCR and Western blot analysis subsequently confirmed overexpression of FGFR1 in about one fourth of NSCLC cell lines analyzed. Among these, H520, H1299 and A549 had significantly higher levels of FGFR1 expression compared to H3122 which carries an EML4-ALK fusion protein. Real time PCR also demonstrated FGFR1 overexpression compared to normal human bronchial epithelial cells transformed by Herpes Papillary Virus E6/E7 (HBE4). Furthermore, as a sign of epithelial to mesenchymal transition, these cells switched from expressing the FGFR1 3b to the 3c form. In a colonogenic assay the growth of cells carrying FGFR1 overexpression/activation is markedly suppressed by ponatinib, a pan FGFR inhibitor as expected. Similarly, ponatinib treatment of NSCLC cell lines using 1 μM concentrations effectively suppressed the phosphorylation level of FGFR1 and its known down-stream targets including pSrc and pPLCγ without significant impact on the total protein expression. Treatment of these cells harboring FGFR1 activation with ponatinib arrests cells at the G1 phase as shown in the flow cytometry analysis. To determine whether the observed effects are specifically due to the suppression of FGFR1 by ponatinib, an shRNA knock down of FGFR1 was performed. Consistently, shFGFR1 significantly inhibited the growth of H520 and H1299 cells, whereas knock-down did not significantly affect the growth of H3122. Similar to ponatinib treatment, shFGFR1 caused more cells to arrest in G1 phase. In a MTT assay, ponatinib synergistically induced growth suppression/killing of H520 cells with cisplatin and paclitaxel. We are currently determining whether ponatinib is effective in inhibiting NSCLC cells derived directly from freshly

resected lung cancer specimens.

In conclusion, the FGFR1 pathway is constitutively active in a proportion of NSCLC cell lines and ponatinib is a potent inhibitor of constitutive FGFR1 activation. Ponatinib is potentially useful as a molecularly targeted agent against this portion of NSCLC when used alone or in combination with traditional chemotherapy regimens.

B8 Targeting p21-activated kinases in human lung cancer. Christy C. Ong[1], Adrian M. Jubb[1], Peter M. Haverty[1], Wei Zhou[1], Adrian L. Harris[2], Marcia Belvin[1], Lori S. Friedman[1], Hartmut Koeppen[1], Klaus P. Hoeflich[1]. [1]Genentech, South San Francisco, CA, [2]Weatherall Institute of Molecular Medicine, Oxford, United Kingdom.

P21-activated kinases (PAKs) are important mediators of Rac and Cdc42 GTPase function as well as pathways required for Ras-driven tumorigenesis. PAKs have been implicated in signaling by growth factor receptors and morphogenetic processes that control cell polarity, invasion and actin cytoskeleton organization. To better understand the role of PAK1 in lung tumorigenesis, PAK1 genomic copy number and expression were determined for a panel of adenocarcinoma and squamous non-small cell lung cancers (NSCLC) and small cell lung cancers (SCLC). 64% of squamous NSCLC samples were positive for PAK1 expression and 52% of all cases showed staining of moderate (2+) or strong (3+) immunohistochemical intensity in the malignant cells. Nuclear localization of PAK1 was also evident in a significant proportion of squamous NSCLCs. Adjacent normal lung tissue did not express appreciable levels of PAK1. Selective PAK1 inhibition was associated with delayed cell cycle progression in vitro and in vivo. NSCLC cells were profiled using a library of pathway-targeted small molecule inhibitors and several synergistic combination therapies, including combination with antagonists of inhibitor of apoptosis (IAP) proteins, were revealed for PAK1. Dual inhibition of PAK1 and X chromosome-linked IAP (XIAP) efficiently increased effector caspase activation and apoptosis of NSCLC cells. Together, our results provide evidence for dysregulation of PAK1 in NSCLC and a role for PAK1 in cellular survival and proliferation in this indication.

B9 Cost effectiveness of gene expression profiling for tumor site origin. John Hornberger[1], Irina Degtiar[2], Hialy Gutierrez[2], Ashwini Shewade[2], W. David Henner[3], Shawn Becker[3], Stephen Raab[4]. [1]Stanford University School of Medicine, Stanford, CA, [2]Cedar Associates, LLC, Menlo Park, CA, [3]Pathwork Diagnostics, Redwood City, CA, [4]Memorial University of Newfoundland/Eastern Health Authority, St. John's, NL, Canada.

Background: Gene expression profiling (GEP) reliably supplements traditional clinicopathological information on the tissue of origin (TOO) in metastatic or poorly differentiated cancer. A cost-effectiveness analysis of GEP TOO testing versus usual care was conducted from a third-party payer perspective in the United States.

Methods: A retrospective, observational study examined treatment changes in patients whose physicians had received the GEP TOO test results to help diagnose the tissue-site of their patient's malignancy and to guide appropriate therapy. Changes in planned chemotherapy, surgery, radiation therapy, added blood tests, imaging investigations, and referral to hospice care before and after the GEP TOO test results were recorded. The effect of changes in chemotherapy on survival were based on randomized controlled trials informing appropriate use of chemotherapy cited in National Comprehensive Cancer Network (NCCN) and Up-to-Date guidelines. Drug and administration costs were based on average doses reported in NCCN guidelines. Centers for Medicare and Medicaid Services (CMS) fee schedules were used to obtain other unit costs. Quality-of-life weights were obtained from literature sources. Changes in overall survival, costs, and cost per quality-adjusted life year (QALY) gained were estimated using bootstrap methods.

Results: Use of chemotherapy regimens consistent with guidelines for the final tumor-site diagnosis increased significantly from 42% to 65% (net difference 23%; p< 0.001). Overall survival was projected to increase from 15.9 months to 19.5 months (mean difference 3.6 months, 95%CI [2.0, 5.1]). The average increase in survival adjusted for quality of life was 2.7 months (95%CI [1.4, 3.9]), and average third-party payer costs per patient increased by $10,360 (95% CI [$5,668, $15,053]). The cost per QALY gained was $46,858 (95% CI [$17,995, $75,718]).

Conclusions: GEP TOO testing significantly altered clinical practice patterns for treating metastatic cancer of uncertain primary. It is projected to increase overall survival, QALYs, and costs, resulting in an expected cost per QALY of less than $50,000.

B10 Detection of DNA methylation in the serum of lung cancer cases and controls using Digital MethyLight. Candace J.S Johnson[1], Janice S. Galler[1], Paul P. Anglim[1], Jeffery A. Tsou[1], Suhaida A. Selamt[1], William N. Rom[2], Ite A. Laird-Offringa[1]. [1]University of Southern California, Los Angeles, CA, [2]New York University, New York, NY.

Background: Lung cancer is the leading cause of cancer death worldwide. Over 1.3 million people succumbed to lung cancer in 2008 according to the World Health Organization (WHO). The predicted five year survival rate for non-small cell lung cancer (NSCLC) for the year 2010 is 16%. An early diagnosis would significantly increase survival of lung cancer patients. Low dose computed tomography (LDCT) detects tumors as small as T0. However, in a recent study by The National Lung Screening Trial Research Team, the false positive rate was 96%. DNA methylation, which has been shown to be associated with gene inactivation in various cancers, exhibits cancer-specific profiles. We hypothesize that DNA methylation in plasma/serum could be a powerful biomarker to complement LDCT when a lesion is detected.

Results: Using candidate gene analysis in combination with epigenetic profiling we have identified 12 DNA methylation markers based on comparisons between archival lung cancer samples and matched adjacent non-tumor lung: 2C35, GDNF, HOXA1, HOXB4, MT1G, NEUROD1, NID2, OPCML/HNT, SFRP1, TNFRSF25, TRIM58, TWIST1 are all highly significantly and frequently hypermethylated in lung cancer tissues. Because detection of shed DNA in bodily fluids requires a very sensitive method, we have adapted Digital MethyLight to interrogate the 12 markers simultaneously on 384-well plates. Digital MethyLight is a sensitive real time-based limiting dilution method in which free floating DNA extracted from bodily fluids is diluted over a large number of wells (in our case 90) so that the number of detectable methylated DNA molecules can be counted. We used three different colors of probes to generate four sets of multiplexed probes, covering our 12-marker panel. Using this method, we examined the serum of 90 patients with lung cancer and 90 high-risk controls from New York City. We also examined serum from an additional 30 low risk, non-smoker controls. Many of our markers showed a statistically significant difference in methylation when comparing cases to non-smoker controls, but the difference with the high risk smokers was much less pronounced. Several subjects from the high-risk controls showed DNA methylation levels comparable to the cases, and several of these controls were ultimately diagnosed with cancer. Interestingly, we found no correlation between tumor stage and amount of DNA shed, and were able to detect methylated DNA in the serum of several stage 1A patients.

Conclusion: Elevated DNA methylation is observed in lung cancer cases, as well as in a number of high risk control subjects. Serum DNA methylation levels are markedly lower in non-smoking controls. While sensitivity is low, methylated DNA can be detected in stage 1A patients, suggesting that even small tumors can shed sufficient DNA to allow detection. It is not clear why some patients do not show detectable DNA, even with progressed disease. This merits further investigation.

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B11 EGFR polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI. Minkyu Jung[1], Chul Ho Lee[2], Hyung Soon Park[2], Ji Hyun Lee[2], Joo Hang Kim[2], Young Ae Kang[2], Se Kyu Kim[2], Joon Chang[2], Dae Jun Kim[2], Byoung Chul Cho[2]. [1]Gachon University Gil Hospital, Incheon, Republic of Korea, [2]Yonsei University Health System, Seoul, Republic of Korea.

Background: Mutations in the epidermal growth factor receptor (EGFR) have been confirmed as predictors of efficacy for EGFR-tyrosine kinase inhibitors (TKIs). We investigated whether polymorphisms of the EGFR gene were associated with clinical outcome in NSCLC patients treated with EGFR-TKI.

Methods: A polymorphic dinucleotide repeat (CA simple sequence repeat 1 [CA-SSR1]) in intron one and single nucleotide polymorphisms in the promoter region (SNP -216 GG or GT) were evaluated in 71 NSCLC patients by PCR-RFLP and DNA sequencing. Genetic polymorphisms were correlated with clinical outcomes of EGFR-TKIs.

Results: SNP-216G/T polymorphisms were associated with the efficacy of EGFR-TKI. The response rate for the SNP-216G/T was significantly higher than that for the GG (62.5% vs. 27.4%, P=0.044). SNP-216G/T genotype was also associated with longer progression-free survival compared with GG genotype (16.7 months vs. 5.1 months, P=0.005). However, Genotypes for the CA-SSR1 was not associated with the efficacy of EGFR-TKI.

Conclusions: SNP-216G/T polymorphism is potential predictor for clinical outcome in NSCLC patients treated with EGFR-TKI.

B12 Predictibility of response to anti-EGFR treatment with respect to assessment of receptor status in different geographical population with lung cancer (NSCLC). Veerendra Kumar K V. Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, India.

Lung cancer affecting populations in different geographical areas varies with respect to incidence, smoking history, histology and response to treatment with monoclonal antibodies inhibiting EGFR.

Assessment of EGFR status in lung cancer varies with respect to methodology, type of antibody etc, resulting in wide variation in reported incidence of positivity. In addition EGFR receptor is a polyclonal antigen (eg: EGFR1, EGFR2) and standardization of antigen methodology of assessment and interpretation of the test results is not yet uniformly accepted worldwide. Response to treatment with monoclonal antibodies to EGFR in lung cancer patients vis a vis receptor status is varied in different geographical areas. Study in Erlotinib has shown response in selected sample of patients in western population whereas there are no well established clinical trials in Asian population establishing relevance of receptor status with respect to clinical response to EFGR antibodies.

It is well known fact from different clinical trials that Asian especially Southeast Asian populations with lung cancer respond differently in comparison to European population (cacuasions). But receptor status and response to treatment not known.

Hence it is opinioned that Asian and especially Southeast Asians population's treatment with monoclonal antibodies should be given a clinical trial irrespective of receptor status or unknown receptor status. However clinical trials with respect to response v/s receptor status should be conducted. Till then these populations should not be denied treatment with monoclonal antibodies in lung cancer (NSCLC).

B13 FGFR2 as a therapeutic target in squamous cell lung carcinoma. Rachel G. Liao[1], Peter Hammerman[1], Andrey Sivachencko[2], Heidi Greulich[1], Gad Getz[2], Qingsong Liu[1], Nathanael Gray[1], Matthew Meyerson[1]. [1]Dana-Farber Cancer Institute, Boston, MA, [2]The Broad Institute of Harvard and MIT, Cambridge, MA.

Squamous cell lung carcinoma (lung SCC)) is the second most common subtype of non-small cell lung cancer, with 40,000 new cases diagnosed every year in the United States. Unlike lung adenocarcinoma, few targetable genomic events are known drivers of lung SCC, and therefore, therapeutic options are limited for patients with this disease. As part of The Cancer Genome Atlas (TCGA) project, we have analyzed the entire coding sequence of 196 lung SCCs for mutated genes which may be amenable to targeted therapeutics. In the 196 patient samples analyzed to date, we have observed mutations in the Fibroblast Growth Factor Receptor 2 (FGFR2) kinase gene in ten cases, or 5% of samples. The observed FGFR2 mutations are present in both the extracellular domain and the kinase domain of the protein, an observation that is consistent with activating mutations in FGFR2 that are known to drive endometrial cancer. We therefore hypothesized that these mutations are oncogenic and may be driving transformation in a subset of lung SCC patients. Expression of mutated FGFR2 in NIH-3T3 cells led to anchorage-independent colony formation, which was inhibited by treatment with the pan-FGFR inhibitor FIIN-1. A known mechanism of FGFR2 activation is the constitutive dimerization of receptors with mutated extracellular domains via intermolecular disulfide bond formation, and we have observed this phenomenon in two of our ECD mutants as well. These data suggest that both ECD and kinase mutations in the FGFR2 gene are sufficient to transform cells. This finding, along with recently published data suggesting that FGFR1 amplification is also able to drive lung SCC development, demonstrates that the FGFR family may be a promising therapeutic target in lung SCC.

B14 FISH-based differentiation of adenoma carcinoma (AC) from squamous cell carcinoma (SqCC): Optimizing probe selection using in silico modeling. Calum MacAulay[1], Akhila Srinidhi[1], Albertina Wong[1], Will Lockwood[1], Brad Coe[2], Ian Wilson[1], Stephen Lam[1], Wan Lam[1]. [1]BC Cancer Agency, Vancouver, BC, Canada, [2]University of Washington, Seattle, WA.

Introduction: FISH is a powerful technique to detect specific genomic sequences within an individual cell. Differences in the genetic makeup of AC and SqCC of the lung have been documented. A robust genetic based test applicable to sections from FFPE for the classification of AC vs SqCC tissue could have clinical utility as this classification can determine treatment selection. The optimal translation of array based genetic alterations data (in the form of copy number alterations) into a select set of FISH probes is nontrivial. One must consider the type (gain or loss), the extent and fidelity of the alteration (length in base pairs of the alteration common across the data set being considered which will affect FISH probe signal strength). Also one must consider effects the sectioning process will have on the actual FISH measurements; thick sections – more complete nuclei (better genetic loci fidelity) but more nuclear overlap (more difficult recognition of individual nuclei), thin sections – less nuclei overlap between cells but less complete nuclei. We use an in silico simulation to model the effect sectioning will have on the robustness and efficacy of a FISH based classifier for the genetic based differentiation of AC from SqCC tissue.

Methods: High resolution array comparative genomic hybridization (aCGH) tiling array data was used to identify recurrent copy number alterations that could be used to differentiate between 169 AC and 92 SqCC cases. An in silico simulation of the sectioning process on the detection of these alterations was programmed in MATLAB. This simulation modeled the physical distribution of the DNA sequence, chromosome by chromosome within individual modeled nuclei. For each of the 261 cases chromosomal loci gains were randomly inserted into the cell's DNA

irrespective of chromosome and chromosomal loci deletions were assumed to occur in only one of that specific chromosome type. Gains and losses were assumed to linearly affect the size of chromosomes and cell nuclei. Cell nuclei sectioning was simulated 300 times for each case (10 different nuclear rotations for each of ten different chromosomal placement simulations within the nucleus for three different section thicknesses of 4um, 7um and 10 um). The number of spots that would be detected by FISH for each of these 300 simulations for each case for all 24,817 loci measured by the aCGH was saved for later classification accuracy analysis.

Results: The accuracy of the in silico model was checked against expected out comes; 1) such as the average loss of FISH spots as a function of the loss of nuclear volume to sectioning for loci with 1, 2 or 3 expected copies and 2) the concurrent loss of adjacent loci, or the loss of all loci within a chromosome with the loss of that chromosome. These measures were validated across at least 10K simulations. From the simulations we computed graphs of the number of loci retained as a function of the maximum radius of the nuclear material left within the section for each cell (an observable feature under microscopy). Further we calculated what percentage of nuclei within the section would have a maximum radius larger than 50, 60, 70, 80 or 90% of the pre-section nuclear radius for each case. This was done the 3 section thickness. Finally we used the simulation data to see the effect of sectioning on case classification, the number of cells which must be measured per case for optimal classification and the selection of the optimal genomic loci to differentiate the two types of tissue.

B15 Genomic and epigenomic events in arsenic-related lung squamous cell carcinomas from smokers and never smokers. Daiana D. Becker-Santos, Emily A. Vucic, Stephen Lam, Wan L. Lam, Victor D. Martinez. BC Cancer Agency, Vancouver, BC, Canada.

Background: Arsenic is a well-known human carcinogen. An estimated of 160 million people (~2% of total human population) are exposed to levels above the recommended threshold (10 µg/L) in Bangladesh, Taiwan, Mongolia, India, China, Argentina, Mexico, Canada, USA, and Chile, among others countries. Skin, bladder, liver, kidney and lungs are the main targets of arsenic carcinogenicity. Lung cancer (LC) is the most deadly form of neoplasia associated to arsenic ingestion. Additionally, lung squamous cell carcinomas (SqCC) occur at higher rates than other LC subtypes following exposure to this metalloid. Both genetic and epigenetic changes (some of them related to arsenic biotransformation) have been proposed to drive carcinogenesis; however, mechanisms are not fully understood. Here, we have compiled a panel of lung tumors from a population with chronic arsenic exposure, including a rare set of lung SqCC from patients who have never smoked. We analyzed to identify whole genome arsenic associated copy-number alterations (CNAs), copy-number variations (CNVs) and global DNA methylation changes.

Methods: 52 lung SqCC were analyzed by whole-genome tiling path comparative genomic hybridization for CNA. Twenty-two were from arsenic exposed patients from Chile (10 never smokers and 12 smokers), and 30 additional non-exposed cases were from North America. In addition, 22 blood samples from healthy individuals from Northern Chile were examined to identify naturally occurring germline CNVs. Global DNA methylation analyses for 5 arsenic-exposed cases from never smokers were performed using Illumina's Infinium Human Methylation 450K array.

Results: We identified arsenic related CNAs occurring in lung SqCC from patients with

chronic exposure to this. The most recurrent events were represented by DNA losses at chromosomes 1q21.1, 7p22.3, 9q12, and 19q13.31. Also, we observed a single arsenic-associated DNA gain at 19q13.33, which contains genes related to single strand DNA breaks repair and neoplastic processes. Interestingly, alterations in this region have been reported to be more frequent among lung adenocarcinomas from never smokers compared with smokers. Additionally, distinctive DNA methylation patterns were associated to arsenic related lung SqCC from never smokers, indicating these changes can have an impact on carcinogenic mechanisms for this subgroup of lung tumors

Conclusions: Our study provides insights into the molecular mechanisms of arsenic-induced lung SqCC. Moreover, findings specifically associated to cases among never smokers contribute to understand malignant events occurring in this rare subgroup of lung tumors. The unique and recurrent set of arsenic-associated genetic and epigenetic alterations suggests that this group of tumors may be considered as a distinct disease subclass. Finally, elucidation of the mechanisms underlying the initiation and promotion of carcinogenesis related to arsenic biotransformation processes is of foremost importance to the development of early detection protocols and treatment options for affected individuals.

B16 Histology and intervention related miRNA expression changes from baseline to follow-up paired bronchial biopsies of patient in the iloprost chemoprevention trial. Celine Mascaux, Wilbur A. Franklin, Paul A. Bunn, Jr., York E. Miller, Fred R. Hirsch, Robert L. Keith, William J. Feser, Marina T. Lewis, Anna E. Baron, Christopher D. Coldren, Dan T. Merrick, Timothy C. Kennedy, John I. Eckelberger. University of Colorado, Denver, CO.

The iloprost lung cancer chemoprevention trial is the first to meet a primary endpoint of improvement in endobronchial histology, which is currently considered to be the best intermediate endpoint for chemoprevention (Keith et al, Cancer Prev Res, 2011). This placebo-controlled study offers the opportunity for discovering other surrogate endpoints and predictive biomarkers to incorporate into chemoprevention trials.

We compared miRNA expression in follow-up (FU) versus baseline biopsies in relation with histology change or not globally and stratified by treatment arm and smoking status.

In the 152 patients randomized in the iloprost trial, the paired baseline and FU biopsies were available in 125 patients: 40/35 current/former smokers in the iloprost arm and 25/25 current/former smokers in the placebo arm, respectively. We planned to analyze 500 biopsies: four biopsies from each of the 125 patients, 2 biopsies at baseline (worst and best diagnosis) and 2 biopsies at FU at the same site after six months of treatment with iloprost or placebo. We selected and analyzed 14 miRNAs differentially expressed during squamous cell lung carcinogenesis in our previous study (Mascaux et al, Eur Resp J, 2009).

In total, 496/500 biopsies with appropriate tissue were available. For every biopsy, total RNA was extracted from 8 adjacent 4 um cuts of formalin fixed paraffin embedded (FFPE) adjacent to the diagnostic section. A good reproducibility of the previously published data for the expression changes in the 14 miRNA accross lung preneoplasia stages was shown in the current study in baseline samples. This new study identifies significant changes in miR-34c expression between paired samples when histology is up- or downgraded. miR-34c is significantly down-regulated in paired biopsies when histology is up-graded and inversely. This correlation between miR-34c expression and histology changes is shown including all samples (r spearman correlation=0.23 p=0.0003), but also consistently in subgroups (iloprost.arm, current r=0.26, p=0.041 and former smokers r=0.24, p=0.066, placebo arm, current (r=0.23, p=0.046)). In contrast, miR-9 was significantly down-regulated in follow-up as compared with baseline biopsies, but was not correlated with histology changes. The down-regulation of miR-9 in follow-up versus

baseline samples was found in all samples (t test, p<0.0001) and was consistent in all and significant in most of subgroups (iloprost arm, all patients (p=0.0007) and current smokers (p=0.0023) and placebo arm, all patients (p=0.001), current smokers (p=0.047) and former smokers (p=0.0071).

In conclusion, miR-34c, a transcriptional target of p53 which is down-regulated by hypermethylation in lung cancer, is down-regulated when histology changes from the normal bronchial mucosa to high-grade bronchial lesions, and inversely, independently of treatment and smoking status. miR-34c expression is a good reflection of baseline histology and histology changes. Alternatively, miR-9 is systematically down-regulated in follow-up biopsies 6 months later at the same site independently of histology, treatment and tobacco status. This suggests that the down-regulation of miR-9 in the follow-up samples may be related to the biopsy: this miRNA could be involved in the tissue repair.

B17 The small RhoGTPase RhoB as a new potential marker of EGFR-TKI resistance. Claire Marty, Gilles Favre, Julien Mazieres. INSERM UMR1037, Toulouse, France.

Introduction: EGFR tyrosine kinase inhibitors (EGFR-TKI) are widely used in metastatic non small cell lung cancer (NSCLC). Nevertheless, the majority of patients with wild type EGFR do not respond to these treatments and, in the selected group with EGFR mutations, we some primary resistance and all the patients will ultimately develop acquired resistance.

The small RHO GTPase RhoB is involved in cell proliferation, invasion, apoptosis and can act as a tumor suppressor gene in lung carcinogenesis by controlling survival and invasion through AKT pathway. Furthermore, RhoB participates in the regulation of endocytic trafficking of EGFR. Specifically, activated RhoB is able to delay the transport of internalized EGFR to lysosomes, thus modifying its downstream signaling.

The objective of our study is to know if RhoB is a molecular determinant of the response to the EGFR-TKI erlotinib, and if RhoB could be a marker for erlotinib resistance.

Material and Method: We used various lung cancer cell lines harboring either wild-type or mautated EGFR and K-Ras. RhoB was either overexpressed by adenovirus transfection or inhibited by interfering RNA (siRNA) in these cells. Cell proliferation after 72h of treatment with erlotinib was measured by a colorimetric assay. In the same way, we performed invasion assays and western-blot analyses of downtsream signaling pathways.

Results: (1) RhoB overexpression decreases the sensitivity to erlotinib of cells presenting a wild type or mutated EGFR in a dose-dependent manner whereas the sensitivity to erlotinib of cells bearing KRas mutation was not affected by RhoB overexpression. (2) Erlotinib-induced inhibition of cellular invasion is impaired by overexpression of RhoB in wild type or mutated EGFR cell lines. (3) The phosphorylation of Akt (and not of ERK) is inhibited by erlotinib and is restored upon overexpression of RhoB invasion.

Conclusions: Our results suggest that RhoB is involved in the sensitivity to erlotinib in wild-type K-Ras cells harboring either mutated or wild-type EGFR. RhoB is able to block erlotinib-induced inhibition of cell proliferation and invasion mainly through AKT pathway modulation. We plan to extend our in vitro findings in a cohort of patients treated with erlotinib.

B18 A phase II study of sorafenib in patients with stage IV non-small cell lung cancer (NSCLC) with a K-Ras mutation. Anne-Marie C. Dingemans[1], Wouter W. Mellema[2], Harry J.M. Groen[3], Atie van Wijk[2], Sjaak Burgers[4], Peter W.A. Kunst[5], Frederik B. Thunnissen[2], Daniëlle A.M. Heideman[2], Egbert F. Smit[2]. [1]Maastricht University Medical Center, Maastricht, The Netherlands, [2]VU University Medical Center, Amsterdam, The Netherlands, [3]University Medical Center Groningen, Groningen, The Netherlands, [4]Netherlands Cancer Institute, Amsterdam, The Netherlands, [5]Academic Medical Center, Amsterdam, The Netherlands.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR5) of the conference *Proceedings*.

B19 Fetal lung adenocarcinoma: Characterization of primary cell culture and in vitro drug response. Cecilia Menna[1], Mohsen Ibrahim[1], Daniela Peruzzi[2], Luca Pacini[2], Michela Ciancamerla[2], Antonella Calogero[2], Erino Angelo Rendina[1]. [1]La Sapienza University, Sant'Andrea Hospital, Rome, Italy, [2]La Sapienza University, Latina, Italy.

Purpose: Fetal lung adenocarcinoma (FLAC) is an extremely rare subtype of pulmonary adenocarcinoma estimated to be the 0.5% of all lung cancers. Therefore a standardized model of FLAC primary cell culture and evidence-based treatment guidelines are not available. The aim of this study is to characterize FLAC primary cell culture, to evaluate the biological features of this uncommon tumor and the in vitro drug response.

Methods: FLAC specimen was obtained from a 59-years-old female patient, cultured in vitro with DMEM-F12 medium supplemented with 5% FBS and maintained in culture for more than 6 passages. Primary cells from a lung adenocarcinoma specimen were as well cultured and used as control for all the in vitro experiments. Immunohistochemistry for β-catenin, TTF-1, Ki67, cKit, MDM2, p53, S-100, EGFR, E-cadherin, cytokeratin 7 and 8/18 was performed on paraffin-fixed specimen. Immunofluorescence on cells in culture was assessed for MDM2, p53, CD133. Specimen and cells in culture were analyzed by real time PCR for oncogenic mutations (KRAS, EGFR) and for a stem cells gene panel. In vitro tumorigenicity was tested by plating efficency and soft agar colony formation assays. Cell proliferation was determined and a cell viability test was performed after treatment of FLAC cells with tyrosine kinase inhibitors (erlotinib and gefitinib), temozolomide (TMZ), cisplatin, gemcitabine and cysplatin+gemcitabine by MTS assay.

Results: A primary cell culture of human FLAC was characterized and standardized. FLAC histotype was confirmed by immunohistochemistry. Immunofluorescence showed a positivity for MDM2, but not for p53 and CD133. By molecular analysis, DNA from both specimen and cells in culture was found to harbor epidermal growth factor receptor (EGFR) kinase domain non-sense mutation in exon 20 and no mutations in the K-ras gene. Primary FLAC cells showed a colony formation activity and the soft agar assay revealed an anchorage-indipendent growth. At 48 hours FLAC cells proliferation was inhibited of 56% and 41% by erlotinib and gefitinib respectively, with an IC50 value of 10 µM, compared with the control proliferation (lung adenocarcinoma cells) inhibited of 25% and 60% by erlotinib and gefitinib respectively (p=0,17; p=0,005). However, FLAC cell viability at 48 hours after being treated with cisplatin 10 µM was 3%, compared with the control viability that was 16% (p=0,007).

Conclusion: This well-characterized FLAC primary cell culture provide a unique model for future studies in lung cancer biology and drug response. Although chemosensitivity to cisplatin was determined, erlotinib and gefitinib were effective against FLAC cells and appear to represent a novel targeting approach for the treatment of this rare tumor.

B20 Thyroid transcription factor 1 is embedded in a microRNA network. David Mu. Penn State University College of Medicine, Hershey, PA.

Thyroid transcription factor 1 (TTF-1 or NKX2-1) is an essential fetal lung developmental factor which can be recurrently activated by gene amplification in adult lung cancer. These findings imply that TTF-1 is a gain-of-function oncogene selected for gene amplification due to an undefined biological pressure in lung cancer cells. However, recent data in the literature showed that TTF-1 suppressed lung cancer progression in a mouse model system and that the chromosomal area harboring the TTF-1 amplicon also underwent allelic DNA loss in lung cancer. Despite the complexity surrounding TTF-1 in lung cancer, characterization of TTF-1 gene regulatory mechanisms is warranted as lung adenocarcinomas frequently amplified for TTF-1 are the most common histologic type of lung cancer. We have discovered the first microRNA that directly regulates TTF-1 by interacting with its 3'-untranslated region. By gene expression profiling, we identified other putative targets of the microRNA. In line with the microRNA/target relationship, the expression patterns of the microRNA and TTF-1 were in an inverse relationship in human lung cancer. Exploration of human lung cancer genomics data uncovered that TTF-1 gene amplification was significantly associated with DNA copy number loss at one of the two genomic loci encoding the precursor RNA of mature microRNA. This implies the existence of genetic selection pressure to lose the repressive microRNA that would otherwise suppress amplified TTF-1. We detected a signaling loop between transforming growth factor beta (TGFβ and the microRNA and this loop reinforced suppression of TTF-1, consistent with the concept that TTF-1 possesses anti-epithelial mesenchymal transition (EMT) activity. Paradoxically, the microRNA also targets an EMT-promoting gene HMGA2 which is known to be repressed by TTF-1. Our data suggest that there is a group of microRNAs that are directly regulated by TTF-1, reinforcing our hypothesis that TTF-1 in embedded in a microRNA network. In summary, these data connect the lung transcriptional program to the microRNA network.

B21 E7050, a Met kinase inhibitor, reverses three different mechanisms of hepatocyte growth factor-induced resistance to tyrosine kinase inhibitors in EGFR mutant lung cancer cells. Takayuki Nakagawa, Shinji Takeuchi, Tadaaki Yamada, Kenji Kita, Seiji Yano. Kanazawa University, Kanazawa, Ishikawa, Japan.

Purpose: Hepatocyte growth factor (HGF) induces resistance to reversible and irreversible epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) in EGFR mutant lung cancer cells by activating Met and downstream PI3K/Akt pathway. In addition, HGF accelerates expansion of minor clones with Met amplification and facilitates EGFR-TKI resistance. Purpose of this study is to determine whether E7050, a Met kinase inhibitor, could overcome these three resistant mechanisms to EGFR-TKIs.

Experimental design: Effect of E7050 on HGF-induced resistance to reversible (gefitinib), irreversible (BIBW2992), and mutant selective (WZ4002) EGFR-TKIs was determined using EGFR-mutant human lung cancer cell lines, PC-9 and HCC827 with exon 19 deletion, and H1975 with T790M secondary mutation. As an in vivo models, PC-9 cells mixed with HGF producing fibroblasts, MRC-5, were subcutaneously inoculated into SCID mice and therapeutic effect of E7050 combined with gefitinib was examined.

Results: We found that E7050 circumvented resistance to all of reversible, irreversible, and mutant selective EGFR-TKIs induced by exogenous and/or endogenous HGF in EGFR mutant lung cancer cell lines, by blocking Met/Gab1/PI3K/Akt pathway in vitro. E7050 also prevented emergence of gefitinib resistant HCC827 cells induced by continuous exposure to HGF. In vivo model, E7050 combined with gefitinib dramatically regressed the tumor growth, associating with inhibiting

Akt phosphorylation in cancer cells.

Conclusions: E7050, a Met kinase inhibitor, reverses three different mechanisms of gefitinib resistance induced by HGF, suggesting usefulness of E7050 for overcoming HGF-induced resistance to gefitinib and new generation EGFR-TKIs.

B22 A noninvasive system for monitoring EGFR mutation status with plasma DNA. Tomomi Nakamura[1], Eisaburo Sueoka[2], Shinya Kimura[1], Naoko Sueoka-Aragane[1], Kentaro Iwanaga[3], Akemi Sato[1], Kazutoshi Komiya[1], Naomi Kobayashi[1], Shinichiro Hayashi[1], Toshiya Hosomi[4], Mitsuharu Hirai[4]. [1]Saga University, Saga, Japan, [2]Saga University Hospital, Saga, Japan, [3]Saga Prefectural Hospital Koseikan, Saga, Japan, [4]ARKRAY Inc., Kyoto, Japan.

Detection of EGFR mutations is indispensable to determine appropriate lung cancer treatment. Although 70% of lung cancer patients with EGFR activating/sensitive mutations benefit from EGFR tyrosine kinase inhibitor (EGFR-TKI), they eventually acquire resistance. After chemotherapy is undertaken, re-treatment with EGFR-TKI is often effective. Considering that clinical trials for irreversible EGFR-TKI and MET inhibitors have already been performed, these treatment strategies should become available in the near future. We have sought a non-invasive monitoring system for EGFR mutation status using circulating plasma DNA and have established novel methods to identify EGFR mutations: wild inhibiting PCR and quenched probe system (WIP-QP) for exon 19 deletions, and mutation-biased PCR and quenched probe system (MBP-QP) for L858R and T790M. These detection systems include fully automated genotyping based on analysis of the probe DNA melting curve, which binds the target mutated site using a fluorescent guanine quenched probe (QP-system) combined with MBP or WIP to achieve higher sensitivity. Detection limit was 0.005-0.2 ng in genomic DNA and 0.1-0.3% with mutant plasmids. DNA isolated from lung cancer specimens and circulating plasma DNA samples of 39 adenocarcinoma patients whose primary tumors harbored EGFR exon 19 deletions or L858R were examined. Results in cancer tissue specimens were identical to those based on existing systems (nucleic acid-locked nucleic acid PCR clamp (PCR clamp) or cycleave PCR), except that two samples evidenced both exon 19 deletions and L858R. Using plasma DNA, exon 19 deletions and L858R were detected in 44.7% and 8.7% of patients, respectively, among those who carried the identical mutations in primary tumors; all of those cases evidenced pathological stage IV except for one patient, suggesting that EGFR mutations might be preferentially detected in plasma DNA obtained from patients in advanced stages. Sixty-seven plasma DNA samples from forty-nine lung adenocarcinoma patients and thirty healthy volunteers evaluated for investigation of T790M revealed that T790M mutation was detected in plasma DNA from ten of nineteen patients (53%) who acquired resistance, but not in non-responders, patients responding to treatment, or those not treated with EGFR-TKI. Other mutation detection systems, such as PCR clamp, the cycleave PCR technique, and allele-specific oligonucleotide PCR, detected T790M in three, four, and six patients, respectively, among ten in which T790M was detected by the MBP-QP method. Serial monitoring of exon19 deletion, L858R, and T790M demonstrated correlations with disease state. These monitoring systems are simple, sensitive, and—intriguingly—reflective of clinical course, which can be applicable for deciding lung cancer treatment.

B23 Cost-effectiveness analysis of VeriStrat® selection criteria for EGFR inhibitor therapy for previously treated patients with NSCLC. Richard E. Nelson, Wallace Akerley. University of Utah, Salt Lake City, UT.

Background: EGFR inhibitor therapy extends survival as 2nd or 3rd line treatment of patients with previously treated metastatic non-small cell lung cancer (NSCLC), but a randomized trial of 2nd line therapy in unselected patients showed similar benefit

versus conventional chemotherapy. EGFR mutation analysis is useful in choosing therapy, but it is applicable to only a minority of patients due to low frequency of mutation in North America and ignores the majority of patients who may benefit with wild type status. Alternative selection strategies are needed. VeriStrat is a protein expression profile utilizing matrix-assisted laser desorption/ionization time of flight mass spectrometry to analyze serum. The test classifies patients as either VeriStrat Good or VeriStrat Poor based on likelihood of benefitting from EGFR inhibitor therapy. The objective of this research was to model the anticipated survival and cost analysis of patients using a Veristrat Selection Strategy for 2nd line therapy of NSCLC to the alternative treatment strategies of chemotherapy for all (Chemo-all), EGFR inhibitor to all (EGFR-all) , or to performance status selection (PS selection), a common practice, where those with good PS receive chemotherapy and those with poor PS receive EGFR inhibitor therapy.

Methods: We developed a Markov model using US health care system perspective. Model inputs, taken from published literature, for the base-case analysis (and ranges) were the following: PS good, 50% (35%-65%); VeriStrat Good, 60% (45%-75%); VeriStrat Good if PS bad, 40% (25%-55%); survival on EGFR inhibitor if VeriStrat Good, 10.5 months (8-12); 1-year survival on EGFR inhibitor if VeriStrat Good, 47% (43-50); 1-year survival on EGFR inhibitor if VeriStrat Poor, 12% (8-16) and 1-year survival on chemotherapy, 29% (23-35); cost of EGFR inhibitor, $3,125/month ($2,813-$3,438); cost of chemotherapy, $1,324/vial ($1,192-$1,457); cost of VeriStrat test, $2,860 ($1,000-$5,000). Effectiveness outcome was quality-adjusted life years (QALYs). Probabilistic sensitivity analysis was performed using 10,000 2nd order Monte Carlo simulations.

Results: In the base case analysis, the PS selection and E-all strategies were eliminated due to extended dominance (i.e. both more costly and less effective, on average, than a linear combination of other strategies in model) and the VeriStrat selection strategy had the highest average effectiveness (.886 QALYs). The incremental cost-effectiveness ratio (ICER) for VeriStrat selection compared to the C-all strategy was $66,381/QALY. Probabilistic sensitivity analysis revealed that VeriStrat selection was most often the most cost-effective strategy for willingness to pay levels > $76,000.

Conclusion: These results quantify the trade-off between increased cost and improved effectiveness of a new method for selecting treatment for patients with metastatic NSCLC and provide valuable information to help providers decide whether or not to adopt this new technology. Historically, a threshold of $50,000/QALY has been cited as the cutoff for cost-effectiveness. Recently, however, health economics experts have advocated increasing this threshold to $100,000/QALY. Based on this $100,000/QALY cutoff, VeriStrat selection appears to be a cost-effective treatment strategy for 2nd or 3rd line treatment of patients with previously treated metastatic NSCLC.

B24 Untangling the interplay of lung cancer biomarkers and preanalytic variability with SOMAmer proteomic technology. Rachel Ostroff[1], Michael Mehan[1], Stephen Williams[1], Ed Brody[1], Alex Stewart[1], Harvey Pass[2], William Rom[3], William Bigbee[4], Jill Siegfried[4], Joel Weissfeld[4]. [1]SomaLogic, Inc., Boulder, CO, [2]NYU Langone Medical Center, New York, NY, [3]New York University School of Medicine, New York, NY, [4]University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Biomarker discovery studies may fail to validate because the clinical population does not represent the intended clinical use or because hidden preanalytic variability in the discovery samples contaminates the apparent disease specific information in the biomarkers. This preanalytic variability can arise from differences in blood sample processing between study sites, or worse, introduce case/control bias in samples collected differently at the same study site. To better understand the effect of different blood sample processing procedures, we evaluated protein measurement bias in a

large multi-center lung cancer study. These analyses revealed that perturbations in serum protocols result in changes to many proteins in a coordinated fashion. We subsequently developed protein biomarker signatures of processes such as cell lysis, platelet activation and complement activation and assembled these preanalytic signatures into quantitative multidimensional Sample Mapping Vectors (SMV) scores. The SMV score provides critical evaluation of both the quality of every blood sample used in discovery, and also enables the evaluation of candidate protein biomarkers for resistance to preanalytic variability.

The underlying platform technology uses SOMAmers (Slow Off-rate Modified Aptamers) as affinity reagents to quantify proteins. Following analytic validation of the assay, which simultaneously measured approximately 850 proteins with sub-pM limits of detection and intra and inter-assay CV of <5%, we initiated a lung cancer discovery program. The intended use was defined as early detection of lung cancer in individuals with indeterminate pulmonary nodules and in high-risk smokers. This case/control study included almost 1000 samples from 4 different centers, with blinded verification in ~400 samples. Although the AUC of ~0.9 in both training and blinded verification was promising, we had to eliminate several markers due to preanalytic bias leading to site-to-site differences. Even worse, when the SMV scores of preanalytic effects were applied retrospectively, we found there were substantial case-control biases within centers, and that a number of cancer markers were influenced by these biases. As a result, our initial diagnostic performance was partly dependent on markers that not only related to cancer biology, but that were also contaminated by preanalytic case/control bias.

To eliminate this effect, we used the SMV score to define an unbiased fraction of the original sample set, including samples from the Pittsburgh Lung Screening Study (PLuSS, supported by NCI SPORE in Lung Cancer grant P50 090440), and re-assayed the unaffected samples using a new version of the assay with 1033 analytes. The new 7-marker classifier had an AUC of 0.86 and contained several new markers that were not available in the prior version of the assay, which in turn enabled the elimination of some markers that were partly contaminated with case/control bias. The modest loss in performance was an acceptable price for avoiding dependence on hidden case/control information.

Applying quantitative measures of preanalytic variability, we identified preanalytic sample bias across 4 large study centers, revealing unintentional differences inherent in how biological samples are obtained, processed and stored for different study populations. This study highlights the consistency of cohort studies and provides a tool for evaluating bias in case/control studies before proceeding to biomarker discovery. Choosing biomarkers with not just the best case/control discrimination but that are also resistant to sample processing bias increases the likelihood that a robust biomarker panel will perform well in the clinic.

B25 The tumor suppressor role of Wnt/β-Catenin pathway in development of small cell lung cancer. Kwon-Sik Park, Jing Shan Lim, Andrew Singh, Julien Sage. Stanford University, Palo Alto, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR4) of the conference *Proceedings*.

B26 NSCLC personalized medicine via integration into biomathematical model individual clinical data and prognostic/ predictive biomarkers. Assaf Zohar[1], Nir Peled[2], Orna Amir[1], Michal Alumot-Yehoshua[2], Marina Kleiman[1]. [1]Optimata, Ramat Gan, Israel, [2]Thoracic Cancer Research and Detection Center, Sheba Medical Center, Ramat Gan, Israel.

Introduction: Over the last decades many new chemo and targeted therapies were developed and approved to treat patients

with non-small-cell lung cancer (NSCLC). Nevertheless, little progress has been achieved in improving the survival of these patients. Considering the heterogeneity of human cancer, it is well accepted that the 'one-size-fits-all' approach is suboptimal, and the need of personalized cancer therapy has been recognized.

In light of these needs, PrediCare™ is medical software that provides personalized predictions of efficacy and drug induced toxicity for NSCLC patients. Accordingly, PrediCare™ is a supportive system for Oncologists in choosing the optimal tailored treatment for a patient considering the balance between efficacy, toxicity, and quality of life, given the approved treatment options. PrediCare™ is based on Optimata Virtual Patient® (OVP™) technology, a set of mechanistic and statistical bio-mathematical models that simulate the progression of solid tumor and effect of treatment. Integrating individual patient data (imaging, histopathology, blood analysis, urine analysis & genotype) into PrediCare™ will generate personal set of parameters that will be utilized to simulate tumor progression and effect of treatment.

Methods: PrediCare™ is composed of the following main components:

• Drug Pharmacokinetics/Pharmacodynamics Module: models of drugs, as mono or combination therapies.

• Efficacy Module: simulates the predicted effect of different drugs on tumor size and subsequently on response and/or time to progression (TTP).

• Toxicity module: simulates the predicted likelihood for adverse events following administration of different drugs.

• Database: storage of PK/PD, Efficacy and Toxicity module background data as well as personal patient data.

• Engine: handles, executes, and integrate the different processes (models) and connect them with the database.

• Results Analyzer: Interpret the simulation results into clinical standards based on RECIST criteria and Common Toxicity Criteria.

Personalizing the biomathematical models will be achieved in collaboration with Sheba Medical Center. Specifically, retrospective clinical data from 100 of NSCLC patients will be collected. The models will be fitted to the individual clinical outcomes and correlations between the fitted parameters and the individual data will be analyzed. Cross validation, predictions accuracy, bias and relative error will be performed to guarantee rigorous and quantitative personalized predictions.

Summary: Developing PrediCare™ will address the major unmet need in personalized oncology, particularly: 1/ Predicting optimal treatment for individual patients, 2/ Developing individual screening programs based on best practices, technologies and research, and 3/ Facilitating and accelerating oncology drug development through patient stratification and recommended treatment strategies.

Conclusions: Implementing PrediCare™ in Medical Centers offers integration of a broad range of medical examinations and treatment options with unique predictive technology. This will assist health care providers in treating patients on an individual basis, insurance companies for efficient resources allocation and pharmaceutical companies for targeted clinical trials and informed Go/No-Go decision, ultimately leading to significant improvements of the treatment provided to the patients.

B27 Integrative analysis identifies GAS41 as a novel oncogene in NSCLC, localizing to the 12q15 amplicon. Larissa Pikor[1], William W. Lockwood[2], Emily Vucic[1], Raj Chari[3], Stephen Lam[1], Wan L. Lam[1]. [1]BC Cancer Research Center, Vancouver, BC, Canada, [2]National Institutes of Health, Bethesda, MD, [3]Harvard Medical School Department of Genetics, Boston, MA.

Background: The application of a multidimensional integrated analysis of recurring genomic and expression alterations

can identify new insights into the molecular mechanisms involved in the pathogenesis of NSCLC. Distinguishing the key mechanisms and causal events driving tumorigenesis will lead not only to a better understanding of lung cancer phenotypes and biology, but also new molecular markers and therapeutic targets. Using this approach, we identified the chromosomal region at 12q13-15, and more specifically *Glioma amplified sequence 41* (*GAS41*) to be frequently amplified and overexpressed in NSCLC. A putative transcription factor, amplification of *GAS41* has been reported in dedifferentiated liposarcomas and in the earliest stages of glioma and astrocytoma. While the oncogene *MDM2* has long been believed to be the driver of this amplicon, we hypothesize *GAS41* is an oncogene capable of driving selection of the 12q15 amplicon, and not merely a passenger event.

Methods: An integrative genomics approach, examining 261 NSCLC tumors (169 adenocarcinomas (AC) and 92 squamous cell carcinomas (SqCC)) profiled for copy number and gene expression alterations was used to identify novel candidate oncogenes in NSCLC. Recurrent DNA amplifications were identified using the GISTIC algorithm and integrated with gene expression data to identify genes frequently amplified and overexpressed. Genes were classified as overexpressed if the fold change between tumor and matched non-malignant tissues was greater than 2 fold. The functional significance of GAS41 was assessed by lentiviral knockdown and ectopic overexpression in lung cancer cell lines with and without *GAS41* amplification, and human bronchial epithelial cells respectively. In vitro assays measuring proliferation, anchorage independent growth, senescence and apoptosis were used to assess the phenotypic effect of gene dosage manipulation. Survival analysis was performed using the Cox regression model for multiple independent cohorts.

Results: *GAS41* is gained or amplified in over 20% of NSCLC tumors, with similar frequencies of amplification in both AC (26%) and SqCC (24%). Although frequently co-amplified with *MDM2*, amplification of *GAS41* was observed to occur in the absence of *MDM2* amplification. Overexpression of GAS41 in human bronchial epithelial cells abrogated senescence, whereas knockdown reduced cell proliferation, impaired colony formation and induced cellular senescence only in lung cancer cell lines with amplification. Western blotting revealed increased p21, cleaved PARP and reduced levels of phospho-p53 in knockdown lines as compared to empty vector controls, suggesting GAS41 is implicated in the regulation of the p21-p53 pathway. Consistent with in vitro results, patients expressing high levels of GAS41 displayed poorer outcomes compared to those with lower levels of GAS41.

Conclusions: Our findings reveal *GAS41* as a candidate oncogene frequently amplified and overexpressed in NSCLC, both in the presence and absence of *MDM2* amplification. Gene dosage manipulation resulted in distinct phenotypic changes characteristic of oncogenes, and thus implicate amplification of *GAS41* as a novel mechanism of NSCLC tumorigenesis.

B28 Variability of biomarker expression among spheres derived from human NSCLC cell lines under clonal conditions. Susan F. Radka, Marty R. Jacobson. Saccomanno Research Institute, St Mary's Hospital and Regional Cancer Center, Grand Junction, CO.

The cancer stem cell (CSC) hypothesis proposes that a small population of cells with self-renewing tumorigenic potential exists within a tumor, and may be responsible for disease relapse after therapeutic intervention. In lung cancer, the search for CSCs and biomarkers defining them is complicated by the morphological and functional heterogeneity of the tumor tissue, and the site of carcinogenesis. Biomarkers for lung CSCs are not clearly defined. To aid in understanding the nature of human lung CSCs, we are developing in vitro culture sphere-forming conditions in an attempt to

isolate and propagate cells with proposed functional properties of CSCs from early stage lung tumors resected from patients, without bias toward biomarker expression. We have used human NSCLC cell lines to optimize sphere-forming conditions which allow for propogation of cells under clonogenic, yet gentle, in vitro culture conditions. Utilizing defined "stem cell" media in soft agarose, anchorage independent conditions, we have cultured and isolated clonal spheres. These spheres were expanded in vitro, cryopreserved, and recultured in vitro for further analyses. To date, we have generated clonal sphere populations from six different NSCLC lines: one adenocarcinoma (A549), one squamous cell carcinoma (SW900), one NSCLC of neuroendocrine origin (H1299), and three large cell carcinomas (H460, H661, and H1581). Efficiency of sphere formation varied among the 6 lines, with H460, H1581, and SW900 having about 50% sphere formation under clonal conditions, approximately 10% for H1299, less than 3% for A549, and less than 1% for H661. Cell surface biomarkers were evaluated by western blot analysis of detergent solubilized membranes from paired NSCLC cell lines and their respective spheres. CD133, a proposed biomarker for lung CSCs, was detected albiet at different levels on membranes of each of the six sphere cultures. In contrast, CD133 was absent from the membranes of five of the six parent NSCLC cell lines grown under adherent conditions. The single parent cell line with expression of CD133 is H1581. To determine whether there are changes in epithelial-to-mesenchymal transition (EMT) markers between the parent cell lines and their corresponding spheres, we examined E-cadherin and fibronectin expression by western blot. No relationship between loss of the epithelial marker E-cadherin and sphere formation was observed. In fact, both A549 and its corresponding spheres expressed very high levels of E-cadherin, while most of the other parent cell lines and their corresponding spheres expressed low levels of this marker. Expression of fibronectin, which is upregulated during EMT, showed no consistent pattern between cell lines and spheres, suggesting that there is no relationship between in vitro sphere formation and EMT in these NSCLC cell lines. Expression of ALDH1, a marker whose expression in NSCLC tumors has been associated with poor prognosis, along with nuclear expression of OCT4, a transcription factor important in maintenance of normal stem cell states, and cKit, the receptor for Stem Cell Factor, are under examination.

We expect that the development and analysis of spheres generated from human NSCLC cell lines under clonal conditions will assist us in developing conditions that will promote the in vitro isolation and expansion of sphere-forming cells from early stage lung cancer patients.

B29 Oncogenic K-ras addiction in NSCLC switches PKCδ from a pro-apoptotic to a pro-survival signal. Angela M. Ohm, Aria Vaishnavi, Jennifer Symonds, Mary E. Reyland. University of Colorado Denver, Aurora, CO.

Oncogenic mutations in K-ras occur in about 25% of lung cancers, however only a subset of lung tumors with KRAS mutations are functionally dependent upon oncogenic K-ras for survival. Our previous studies show that the function of Protein Kinase C-δ (PKCδ), a serine/threonine kinase that regulates apoptosis in non-transformed cells, is altered in non-small cell lung cancers (NSCLC) that are dependent on oncogenic K-ras, such that these cells now require PKCδ for survival and transformed growth (Symonds et al, Cancer Research, 2011). The purpose of the current studies was to determine if this switch in PKCδ function alters the response of lung cancer cells to chemotherapeutic drugs. We analyzed apoptosis and PKCδ function in five NSCLC cells lines previously characterized in our laboratory as dependent on oncogenic K-ras for survival, and five NSCLC cell lines characterized as K-ras independent. Our results show that NSCLC cell lines that are functionally dependent on K-ras are highly resistant to apoptosis induced by DNA damaging agents such as etoposide. In contrast, K-ras independent NSCLC cell lines

are highly sensitive to apoptosis. Our previous studies have shown that PKCδ translocates to the nucleus in response to apoptotic agents and that nuclear localization of PKCδ is essential for apoptosis. To determine the mechanism underlying the switch in PKCδ function from pro-apoptotic to pro-survival, we analyzed PKCδ expression and cytoplasmic/nuclear localization in apoptosis sensitive (K-ras independent) and apoptosis resistant (K-ras dependent) NCSCL cells. Relative to insensitive NSCLC cells, apoptosis sensitive cells showed increased expression of PKCδ by qrtPCR, a reduced cytoplasmic:nuclear ratio of PKCδ under basal conditions, and increased nuclear import of PKCδ in response to etoposide. This suggests that PKCδ may be a pro-apoptotic signal in K-ras independent cells, similar to what we have shown for non-transformed cells. In contrast, nuclear import of PKCδ in response to etoposide was suppressed in K-ras dependent/apoptosis resistant NSCLC cells as was phosphorylation of PKCδ on tyrosines Y64 and Y155, which we have previously shown is required for importin –α binding and nuclear import. Exclusion of PKCδ from the nucleus may explain the resistance of K-ras dependent cells to apoptotic agents. To probe the contribution of PKCδ to these two phenotypes more directly, we depleted PKCδ from K-ras independent/apoptosis sensitive A549 and K-ras dependent/apoptosis resistant H2009 cells using an lentivirus delivered shRNA to PKCδ or a scrambled shRNA control. Depletion of PKCδ in A549 cells resulted in suppression of etoposide-induced apoptosis, similar to what we have previously reported in non-transformed cells. Suppression of apoptosis was accompanied by increased activation of the Akt and MEK/ERK cell survival pathways in A549 cells. In contrast, depletion of PKCδ in H2009 cells resulted in increased apoptosis in response to etoposide, and suppression of the Akt and MEK/ERK cell survival pathways. Our studies suggest NSCLC cells functionally dependent on oncogenic K-ras may be resistant to chemotherapeutic agents in part due to loss of the pro-apoptotic function of PKCδ.

B30 Src tyrosine kinase inhibitors activate protective, ULK1-dependent autophagy by downregulating oncomir-106-393-cluster expression in non-small cell lung cancer cells. Sacha I. Rothschild[1], Oliver Gautschi[2], Jasmin Batliner[1], Melanie Jost[1], Martin F. Fey[3], Mathias Gugger[4], Mario P. Tschan[1]. [1]University of Bern, Department of Clinical Research, Bern, Switzerland, [2]Cantonal Hospital, Medical Oncology, Luzern, Switzerland, [3]Inselspital, Bern University Hospital, Medical Oncology, Bern, Switzerland, [4]University of Bern, Institute of Pathology, Bern, Switzerland.

Autophagy has been proposed to play a role as cytoprotective mechanism for tumor cell survival under unfavorable conditions and upon anticancer treatment. In lung cancer autophagy is mostly studied as possible resistance mechanism to therapy. There is increasing evidence that protective autophagy occurs upon various types of anti-cancer treatments, including chemo- and radiation therapy. Src tyrosine kinase inhibitors (TKIs) have been shown to inhibit cell migration and invasion in non-small cell lung cancer (NSCLC) cell lines. In clinical trials, however, they show modest activity in combination with chemotherapeutic agents. In the current study we show a marked induction of autophagic activity upon incubation of NSCLC cell lines with Src TKIs as measured by an increased LC3-I to -II conversion, a significant increase in GFP-LC3 dot formation, and a decrease in p62/SQSTM1 protein expression. Increased autophagic activity was found in three NSCLC cell lines (A549, H460, H1299) with two different Src TKIs (saracatinib, dasatinib). Interestingly, the addition of pharmacological autophagy inhibitors such as chloroquine or bafilomycin resulted in cell death in combination with Src TKI treatment. Moreover, we found that Src TKI-induced autophagy is associated with ULK1 expression in all three cell lines investigated. This effect was Src-specific since knocking down endogenous Src using RNAi resulted in a similar induction of ULK1. Using shRNA targeting ULK1 we showed that Src inhibitor induced autophagy is ULK1-

dependent. Furthermore, ULK1 is a novel target of microRNA (miR)-106a as shown by ULK1 3'-UTR luciferase experiments and ectopic expression of miR-106a and anti-miR-106a in NSCLC cell lines resulting in decreased and increased ULK1 expression upon Src inhibition, respectively. Lastly, in human lung adenocarcinoma compared to matched normal lung tissue (n=23) miR-106a levels were significantly increased (p<0.0001) whereas ULK1 mRNA expression levels were significantly lower (p<0.0002) in tumor tissue. In conclusion, Src inhibitor-induced protective autophagy might explain their low success in clinical trials. Autophagy induced by Src TKIs depends on ULK1 and combining Src with autophagy inhibitors results in massive cell death as compared to single treatments. Furthermore, downregulation of the ULK1 targeting miR-106a upon Src inhibition allows for the induction of protective autophagy. Combining Src and autophagy inhibitors or Src inhibitors and miR-106a expression may represent attractive treatment options for NSCLC.

B31 Single-agent pemetrexed in patients with ALK-positive NSCLC: A retrospective analysis of investigator-reported outcomes. R Salgia[1], S I. Ou[2], G Riely[3], D W. Kim[4], A Shaw[5], K Wilner[6], Y Tang[6], A Polli[7], G V. Scagliotti[8]. [1]University of Chicago, Chicago, IL, [2]University of California at Irvine, Irvine, CA, [3]Memorial Sloan-Kettering Cancer Center, New York, NY, [4]Seoul National University Hospital, Seoul, Republic of Korea, [5]Massachusetts General Hospital Cancer Center, Boston, MA, [6]Pfizer Oncology, La Jolla, CA, [7]Pfizer Inc., Milan, Italy, [8]University of Turin, Turin, Italy.

Recent retrospective data in small patient cohorts have suggested that *ALK*-positive NSCLC may be particularly sensitive to pemetrexed therapy. Based on investigator-documented tumor response data, we assessed objective response rate (ORR) and time to progression (TTP; based on the Kaplan–Meier method) for patients with *ALK*-positive NSCLC treated with pemetrexed prior to enrolment in the PROFILE 1005 (NCT00932451; Pfizer) phase 2 multi-center single-arm study of crizotinib.

As of June 1st 2011, 439 *ALK*-positive patients had been enrolled into PROFILE 1005. All patients had progressed on at least one prior chemotherapy regimen for advanced/metastatic NSCLC. In total, 369 patients (84.1%) had received treatment with pemetrexed prior to enrolment with an ORR of 18.7%. In patients who received second-line single-agent pemetrexed (n=80), ORR was 12.5% and TTP was 5.3 months (95% CI: 3.0–6.6 months). In those who received pemetrexed as third-line or later therapy (n=138), ORR was 16.7%.

Retrospective ORR, TTP and progression-free survival (PFS) data in small *ALK*-positive patient cohorts have been reported. For example, Camidge et al (J Thoracic Oncol 2011;6:774–780) reported an ORR of 42%, with median PFS of 9 months (95% CI: 3–12 months) in 19 patients who received any-line pemetrexed for *ALK*-positive NSCLC (including first line combination and maintenance therapy), and Lee et al (J Thoracic Oncol 2011;6:1474–1480) found an ORR of 47%, and median TTP of 9.2 months (95% CI: 4.7–13.7 months) in 15 patients with *ALK*-positive NSCLC who received pemetrexed as second-line or later therapy.

These figures exceed those reported for larger populations in *ALK*-untested NSCLC. Scagliotti et al (The Oncologist 2009;14:253–263) reported an ORR of 12.8% and PFS of 3.5 months in 158 patients with adenocarcinoma NSCLC who received second-line single-agent pemetrexed (n=158). Hanna et al (J Clin Oncol 2004;22:1589–1597) reported similar values for second-line single-agent pemetrexed in *ALK*-untested all-histology NSCLC: ORR 9.1% and median TTP 3.4 months (n=283). Retrospective outcomes data in NSCLC from *ALK*-positive patients in PROFILE 1005 are therefore more in agreement with data from large unselected populations than from small *ALK*-positive patient cohorts.

There are limitations of the PROFILE 1005 analysis besides the retrospective nature of the data. These include the availability

of investigator-documented rather than independently reviewed tumor response data; strict adherence to RECIST criteria for response data cannot therefore be assured. However, these limitations are countered by the large patient sample size available and narrow confidence intervals reported, supporting the credibility of conclusions drawn from these data. Preliminary efficacy data from PROFILE 1005 for crizotinib in 136 patients with *ALK*-positive advanced NSCLC found an ORR of 51.1% (95% CI: 42.3–59.9%), with 68.4% of patients still on-treatment at the time of analysis; median duration of response was 41.9 weeks (range 6.1–42.1 weeks; Kim et al, 2011 poster at the European Multidisciplinary Cancer Congress; abstract 9084).

Prospective randomized data are required for a definitive comparison and such a study is currently ongoing. Until these data are available, retrospective data from PROFILE 1005 suggest that the extent of benefit with pemetrexed in terms of ORR and TTP in patients with *ALK*-positive NSCLC is considerably less than that reported in much smaller retrospective cohorts, and is more consistent with that reported in unselected populations.

B32 The FOXO1/KLF6 transcriptional network modulates response to anti-EGFR based therapy.. Jaya Sangodkar[1], Rachel Okrent[1], David Burstein[1], Michael Ohlmeyer[1], Katerina Politi[2], Analisa DiFeo[1], Goutham Narla[1], Neil Dhawan[1], Heather Melville[1], Varan J. Singh[1], Caroline Farrington[1], Eric Yuan[1], Huma Rana[1], Blake Smith[1], Vikram Gidwani[1]. [1]The Mount Sinai School of Medicine, New York, NY, [2]Yale University, New Haven, CT.

Epidermal growth factor receptor (EGFR) activation is both a key molecular driver of disease progression and the target of a broad class of molecular agents designed to treat advanced cancer. Numerous studies have implicated a causal role for epidermal growth factor signaling in lung cancer. Genotype-guided stratification of metastatic lung adenocarcinoma patients has heralded in a new era of personalized medicine. Specifically, targeted molecular therapies directed against EGFR have become a mainstay for the treatment of metastatic lung adenocarcinoma. Unfortunately, the clinical utility of anti-EGFR-based strategies is limited by primary or acquired drug resistance. Though much is known about the specific molecular lesions conferring resistance to anti-EGFR-based therapies, additional molecular characterization of the downstream mediators of EGFR signaling may lead to the development of new classes of targeted molecular therapies to treat resistant disease.

Here we identify a transcriptional network involving the KLF6 and FOXO1 tumor suppressor genes that negatively regulate activated EGFR signaling in both cell culture and in vivo models of lung cancer. In a murine model driven by the EGFRL858R allele, a commonly mutated residue in human lung cancers results in EGFR activation and is associated with decreased KLF6 expression. Furthermore, we show that in a Kras driven mouse model, KLF6 expression is not significantly changed whereas the Pten/Mmac1+/- heterozygous mouse model, which results in activation of AKT, is associated with FOXO1 mislocalization and decreased KLF6 expression. Consistent with these findings, inhibition of AKT signaling promotes increase in nuclear FOXO1 resulting in transactivation of the KLF6 tumor suppressor gene in lung adenocarcinoma cell lines.

Given our data supporting the hypothesis that EGFR activation results in KLF6 downregulation through activated AKT signaling, we sought to inhibit this pathway and assess effects on KLF6 expression. The EGFRL858R mouse model demonstrates spontaneous tumor regression when treated with anti-EGFR based therapy, erlotinib, an FDA-approved small-molecule inhibitor of EGFR signaling. We analyzed L858R mouse tumors samples treated with erlotinib and found increased KLF6 expression following EGFR inhibition. Conversely, targeted reduction of KLF6 resulted in decreased erlotinib response in both cell culture and in vivo models of disease suggesting that KLF6

upregulation is necessary for the induction of apoptosis by anti-EGFR based therapy in metastatic lung cancer cell lines. Based upon these findings, we hypothesized that acquired resistance to anti-EGFR based therapies could be overcome by restoring downstream function of the FOXO1/KLF6 transcriptional network. Inactivation of the FOXO1 transcription factor in cancer predominantly occurs through alterations in its subcellular localization. We therefore sought a clinically viable approach to activate FOXO1 by retaining nuclear localization. Trifluoperazine hydrochloride (TFP), a FDA-approved antipsychotic and antiemetic, was identified in a chemical genetic screen to be an effective nuclear export inhibitor of FOXO1. Here we demonstrate that TFP restores sensitivity to AKT-driven erlotinib-resistance through modulation of the KLF6/FOXO1 signaling cascade in both cell culture and xenograft models. We chose TFP for several reasons including 1) it is already FDA approved and has been used in patients for over 20 years with a well defined toxicity and safety profile, 2) the path to clinical translation would be most evident and accessible given that both drugs are FDA approved, and 3) at the molecular level, TFP potentially regulates the most downstream portion of AKT signaling axis, mainly the FOXO-KLF6 transcriptional network, which allows for the least number of potential mechanisms for the development of resistance. Combined, these studies define a novel transcriptional network regulating oncogenic EGFR signaling and identify a class of FDA-approved drugs to restore chemosensitivity to anti-EGFR-based therapy for the treatment of metastatic lung adenocarcinoma.

B33 Identification of the early molecular cancerization features in the airway epithelium of smokers without cancer. Renat Shaykhiev, Tomoya Fukui, Ronald G. Crystal. Weill Cornell Medical College, New York, NY.

Purpose: The field cancerization concept proposes that molecular alterations caused by oncogenic stressors are present throughout the airway epithelium. Cigarette smoking, the major risk factor for the development of lung cancer, induces molecular changes in the airway epithelium prior to the development of disease; these molecular changes may indicate the risk for lung cancer development. Given that gene expression changes reflect genomic and epigenomic alterations preceding the development of pathologic lesions, we hypothesized that the earliest features of molecular cancerization can be detected in the airway epithelium of a subset of healthy smokers based on the expression of genes associated with the progression toward lung cancer. We also asked whether chronic obstructive pulmonary disease (COPD), a smoking-induced lung disease representing an independent risk factor for lung cancer, is associated with further acquisition of the molecular cancerization features in the airway epithelium.

Methods: Large airway epithelium (LAE; 3rd-4th order bronchi) of healthy nonsmokers (LAE-NS, n=21) and healthy smokers (LAE-S, n=31) and small airway epithelium (SAE; 10th-12th order bronchi) of healthy nonsmokers (SAE-NS, n=63), healthy smokers (SAE-S, n=73) and smokers with COPD (SAE-COPD, n=37) were obtained by bronchoscopic brushings and analyzed using Affymetrix HG-U133 Plus 2.0 arrays. LAE/SAE common airway epithelium-expressed genes ("AE genes") were defined as a set of genes expressed in at least 20% of any of the LAE/SAE groups. Lung cancer samples (adenocarcinoma, adenoCa, n=45; squamous cell carcinoma, SqCa, n=27; large cell carcinoma, LCC, n=19) and adjacent nonmalignant lung tissue (n=65) were from the published data set of Hou et al (PLoS ONE 2010; 5:e10312). "Cancerization" genes were identified by comparison of expression of the AE genes in lung cancer samples vs nonmalignant adjacent tissue (fold-increase >2, p<0.01 with Benjamini-Hochberg correction). A "cancerization index" (CI) was calculated for each LAE/SAE sample as a number of cancerization genes having expression level > mean ± 2 standard deviations of healthy nonsmokers.

Results: Gene expression analysis identified 641 AE genes up-regulated in lung cancer vs nonmalignant adjacent tissue. This "cancerization" gene set was enriched in biological processes related to cell cycle (M phase, $p<10^{-30}$) and p53 pathway ($p<10^{-5}$). Progressive up-regulation of these genes in the tumor tissue was confirmed using 50 lung cancer samples of the original cohort having matched adjacent nonmalignant tissue samples ($p<0.001$). The top 10 cancerization genes were MMP12, SPP1, GREM1, KRT6A, TOP2A, ANLN, MMP1, AKR1B10, DLGAP5, and GPX2 (p values 10^{-12}-10^{-34}). Notably, 20% of the identified cancerization genes overlapped with our previously described signature of human airway basal progenitor cells (Hackett et al, PLoS ONE 2011;6:e18378). In the LAE-S, there was significantly higher overall expression of the cancerization genes vs LAE-NS ($p<0.001$), whereas in the SAE, only SAE-COPD, but not SAE-S, demonstrated significantly higher overall expression of these genes ($p<0.003$ vs SAE-NS, $p<0.03$ vs SAE-S). Among 23 healthy smokers with paired LAE/SAE samples, 14 had high CI in the LAE only, 1 in the SAE only, and 2 in both regions.

Conclusion: Smoking is associated with acquisition of the molecular features of cancerization in the LAE, which likely precedes that in the SAE, where the molecular cancerization has a stronger association with the development of COPD. These molecular cancerization features in the airway epithelium of subjects without cancer are relevant to understanding the early pathogenesis of lung cancer and for the development of strategies to prevent lung cancer in susceptible individuals.

B34 Overcoming resistance to EGFR-tyrosine kinase inhibitor therapy in non-small cell lung cancer. Rafael Sierra[1], Anderson Chang[1], Jason Moffat[2], Benjamin G. Neel[1], Ming-Sound Tsao[1]. [1]Ontario Cancer Institute, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada.

Lung cancer is the leading cause of cancer death in developed countries both for men and women; and the second most common type of cancer diagnosed, with less than 15% of patients surviving beyond 5 years. Since more than 60% of lung adenocarcinomas and more than 90% of lung squamous cell carcinomas express high levels of EGFR mRNA and protein, it has been the focus of efforts to develop new agents that target the EGFR pathway. Clinical trials have demonstrated that patient selection plays a major role for patient response. Patients that present amplification or activating mutations (L878R or exon 19 deletions) of EGFR, have higher response rates. Selection improved response rates from less than 10% to over 60-80%. Despite the promising results, all patients that receive TKI therapy develop resistance to treatment in less than one year. The mechanisms of resistance to EGFR TKi's described so far are those dependant on the overactivation/amplification of other receptor tyrosine kinases (RTK) capable to sustain anti-apoptotic signaling pathways . Our study proposes the use of genome-wide screenings using an 80,000 shRNA library on EGFR resistant cells can identify new genes that mediate the resistance to EGFR targeted therapy and provide the basis for designing new therapeutic approaches to avoid the emergence of resistance. So far, we have generated a shortlist of gene candidates that confer synergistic synthetic lethal interactions with EGFR targeted therapies and that are also highly expressed in resistant cells. We are currently overexpressing candidate genes in sensitive cells searching for those that can confer resistance to EGFR targeted therapies. Our study proposes target genes that can synergize with the current treatments to create new therapeutical strategies that can avoid the acquisition of resistance to targeted therapies. Overall, the information gained

from this type of study can be applicable to tumors where ERBB receptor(s) play an important role and targeted therapies are currently used.

B35 Activation of putative compensatory pathways upon deletion of Erbb3 in mutant EGFR-driven lung cancer. Xiaoling Song[1], Pang-Dian Fan[2], Udayan Guha[3], David Threadgill[4], Harold Varmus[5], Katerina Politi[1]. [1]Yale Cancer Center, Yale University School of Medicine, New Haven, CT, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, [3]Medical Oncology Branch, National Cancer Institute, Bethesda, MD, [4]North Carolina State University, Raleigh, NC, [5]National Human Genome Research Institute, Bethesda, MD.

Lung cancer is the leading cause of cancer-related death in the USA and worldwide. Mutant epidermal growth factor receptor (EGFR)-driven non-small cell lung cancer represents 15% of lung adenocarcinomas. Tumors bearing *EGFR* mutations are sensitive to treatment with specific tyrosine kinase inhibitors (TKIs) and show radiographic responses in about 70% of cases. However, it is not known why the remaining 30% of the tumors do not respond to these drugs, and patients who initially respond to TKI treatment eventually develop drug resistance on average within a year. These facts underscore the need for a better understanding of the molecular mechanisms that underlie transformation of the lung epithelium by mutant *EGFR*.

Mutant *EGFR*-induced signaling is initiated by the formation of EGFR homodimers or heterodimers with other members of the EGFR family (ERBB2, ERBB3 or ERBB4). To investigate the role of EGFR family members in the tumorigenic process driven by mutant *EGFR*, we used a tetracycline-inducible transgenic model to test the requirement for Erbb3 in mutant *EGFR*-induced lung tumorigenesis. In this model, deletion of *Erbb3* had no effect on tumorigenesis induced by mutant *EGFR*, suggesting that it is not required to initiate tumorigenesis. Tumors that develop in the absence of *Erbb3* remain sensitive to TKIs. Analysis of the biochemical consequences of *Erbb3* deletion revealed increased levels of phosphorylation of EGFR and Erbb2 in tumors arising in the absence of Erbb3. Moreover, these tumors show increased activation of the mitogen-activated protein kinase (MAPK) signaling pathway. Together these data suggest that recruitment of compensatory pathways may overcome the need for Erbb3 in tumorigenesis driven by mutant *EGFR*. Further experiments to test this are ongoing. Knowledge of the role EGFR heterodimerization partners play in mutant *EGFR*-driven lung cancer can help guide the selection of which EGFR family members to target in the treatment of this disease.

B36 Micronome-wide explorations of NSCLC: microRNA-seq discovery. Saurabh Gombar[1], Miao Shi[1], Joseph Locker[1], Changcheng Zhu[2], Steven Keller[2], Yousin Suh[1], Simon Spivack[1]. [1]Albert Einstein College of Medicine, Bronx, NY, [2]Montefiore Medical Center, Bronx, NY.

Background: Recent studies have revealed that microRNAs play important regulatory roles in carcinogenesis. Some key miRNAs may regulate lung carcinogenesis and be useful for lung cancer early detection. For discovery, we used high-throughput next-gen sequencing technology to investigate miRNA expression profiles in a study of 25 lung adeno- and squamous cell carcinomas, paired with far-adjacent noncancerous lung tissues from the same donors.

Method: Total RNA was isolated by Trizol from 14 pairs of lung adenocarcinomas, 11 squamous cell cancers, and paired noncancerous lung tissues, from the same patients. Small RNA library of each total RNA was constructed with Illumina's Small RNA Sample Prep Kit, followed by high-throughput sequencing using the Illumina IIa platform. In parallel, transcriptome microarrays (Affymetrix GA 1.0) were run on these same paired samples. Statistical differences in microRNA expression between groups were analyzed by GenePattern software, logistic regression, and other methods.

Results: For adeno, 128 micros were differentially expressed and 63 were two-fold differentially expressed between the tumors and non-tumor tissue; for squamous, the numbers of altered microRNAs were 42 overall, and 25 >two-fold. Among adenocarcinoma, the top 10 upregulated were miRs-147b, 577, 877, 556, 449c, and others; and downregulated were miRs 516b, 1251, 486, 139, 516a, and others. Among squamous cell carcinomas, the top up-regulated miRs were 708, 1259, 494, 200a, 216b, and others; and down-regulated miRs were 184, 1251, 144, 516a, 584, and others. MiRs 1259, 135b, 200a, 21, 494, 708 were upregulated in both tumor histologies. When the highest expressed microRNAs were correlated with the most altered mRNA transcripts, putative mRNA targets were more often anti-correlated to the individual microRNAs (Chi Square, p=4.6 x10-6), and this anticorrelation generally held true for the top differentially expressed microRNAs and their putative mRNA targets, and within histology strata.

Conclusion: The lung cancer microRNA complement differs from non-tumor tissue, adeno- differs from squamous, and many of the most dysregulated miRs appear to be anticorrelated with putative mRNA targets. Which of the putative mRNA targets are actual biological targets awaits experimental functional work. Once constructed, the combined microRNA:mRNA signature of lung cancer is likely to be more informative for biologic potential than either signature alone.

[Funding source: NCI 1RC1 CA145422-01; 1K24-CA139054-01]

B37 Investigating the potential of miR-203 as a therapeutic candidate and its role in the pathobiology of malignant pleural mesothelioma (MPM). Milind Suraokar, Kevin Coombes, Anne Tsao, Ignacio Wistuba, Yi Zhang, Chi-wan Chow, David Kim, Lixia Diao, Junya Fujimoto, Reza Mehran, Jin Wang, Carmen Behrens. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: MPM is a lethal neoplasm of the pleural layer of cells surrounding lungs and is in dire need of newer therapeutic treatments. microRNA's (miRs) play a critical role in the pathobiology of many cancers but there are few reports investigating their function in MPM. Recently many studies have highlighted the potential of miRs as therapeutic agents in cancer. We completed a microarray profiling strategy to discover miRs of therapeutic and biological importance in MPM. Methods: We extracted total RNA from 53 frozen resected tumor tissue specimens, comprised of 39 epitheloid, 7 sarcomatoid and 7 biphasic histotypes, along with paired normal tissue. The RNA was labeled and hybridized to Agilent v3 Human miR microarrays. These were scanned and the data was processed using the AgiMicroRna "R" package involving background correction, quantile normalization and summarization. The microarray results were validated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) using Taqman assays on the ABI 7300 platform. Also qRT-PCR was employed to determine the levels of miR-203 expression in a panel of 26 mesothelioma cell lines including Met-5A, an SV-40 immortalized pleural mesothelial control cell line. For all qRT-PCR experiments the miR-203 levels were determined relative to endogenous miR-U6 as control using ΔΔCT calculation. Moreover in a previous study RNA from these same tissue specimens were hybridized on Affymetrix U133 plus 2.0 microarray to obtain transcriptomic profiles.

Results: The bioinformatic analysis of miR microarray data showed that a number of miR's, including miR-203, are differentially expressed between the tumor and normal

samples. A paired T-test conducted on a miRNA-by-miRNA basis and at a highly significant FDR value of 1e-06 showed miR-203 to be down regulated, more than 2 fold, in tumors compared to paired normal tissue. We decided to explore the role of miR-203 in the pathogenesis of MPM since it has been postulated to play a tumor suppressor role in skin and prostate cancer by inhibiting proliferation, metastasis and acting antagonistic to stem cells (1, 2). Using qRT-PCR, we compared levels of expression in 40 pairs of tumor vs. paired normal tissues and demonstrated that miR-203 was down regulated to more than 10 fold in MPM tumors. Also qRT-PCR showed that 70 % of 25 MPM cancer cell lines had lower expression of miR-203 compared to Met-5A cell line. Our previous transcriptomic profiling study had shown differentially expressed transcripts between these same tumors and paired normal specimens. *Survivin* (*BIRC5*) message level, which codes for an apoptosis inhibitor protein and reported to be regulated by miR-203 in prostate cancer cell lines (3), was found to be 2.7 fold higher in tumors ($p = 2.00e-15$) in an expected anti-correlation direction to miR-203 expression levels (i.e. low miR = high target mRNA).

Conclusion: Our findings suggest that miR-203 may play a tumor suppressor role in pleural mesothelioma and regulate levels of *survivin* message. Supported by Grants: DoD W81XWH-07-1-0306 (IW and AT), Fleming Foundation, IASLC Young Investigator Award 2011-2013 (MS).

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B38 Phase 1b/2 trial of HER3 inhibitor U3-1287 in combination with erlotinib in advanced NSCLC patients (pts): HERALD study. Joachim von Pawel[1], Barbara Lueps[1], Jennifer Tseng[2], Catherine Copigneaux[3], Robert Beckmann[3]. [1]Asklepios Fachkliniken Munich-Gauting, Gauting, Bavaria, Germany, [2]MD Anderson Cancer Center Orlando, Orlando, FL, [3]Daiichi Sankyo Pharma Development, Edison, NJ.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR6) of the conference *Proceedings*.

B39 Genome structure-based screening identified epigenetically silenced microRNA associated with invasiveness in non-small cell lung cancer. Kousuke Watanabe[1], Akiteru Goto[2], Masashi Fukayama[1], Takahide Nagase[1], Yutaka Yatomi[1], Nobuya Ohishi[1], Daiya Takai[1], Noriko Emoto[1], Emi Hamano[1], Yosuke Amano[1], Rie Ishikawa[1], Mitsuhiro Sunohara[1], Masanori Kawakami[1], Kentaro Kitano[1], Jun Nakajima[1]. [1]The University of Tokyo Hospital, Tokyo, Japan, [2]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

microRNA expression is frequently altered in human cancers. Epigenetic silencing, especially DNA methylation, is one of many mechanisms of microRNA suppression in cancer. To search for epigenetically silenced microRNAs in non-small-cell lung cancer (NSCLC), we mapped human microRNAs on autosomal chromosomes and selected in silico 55 microRNAs that met one of the following criteria: (i) microRNAs within CpG islands, (ii) microRNAs within 1 kbp downstream of CpG islands, and (iii) microRNAs within gene introns whose promoters have CpG islands. We treated six NSCLC cell lines with the DNA methylation inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR) and determined the expressions of the 55 microRNAs. Fourteen microRNAs (mir-375, mir-196b, mir-126, mir-34b, mir-127, mir-203, mir-148a, mir-181c, mir-30e, mir-449a, mir-340, mir-486, mir-483, mir-139) were

decreased in the cancer cell lines and were induced after 5-aza-CdR treatment. Among the 14 microRNAs, seven (mir-126, mir-34b, mir-203, mir-30e, mir-449a, mir-486, mir-139) were frequently suppressed in primary NSCLCs. After a detailed DNA methylation analysis, we found that mir-34b and mir-126 were silenced by DNA methylation. Mir-34b was silenced by the DNA methylation of its own promoter, while mir-126 was silenced by the DNA methylation of its host gene, *EGFL7*. A chromatin immunoprecipitation assay revealed H3K9me2 and H3K9me3 in mir-34b and *EGFL7*, and H3K27me3 in *EGFL7*. A significant enrichment of H3K27me3 was observed in lung cancer cell lines with decreased mir-126 expression, suggesting the involvement of a polycomb complex in the regulation of *EGFL7* and mir-126. The overexpression of mir-34b and mir-126 by plasmid vectors decreased the expression of c-Met and Crk, respectively. The 5-aza-CdR treatment of lung cancer cell line resulted in increased mir-34b expression and decreased c-Met protein. We next analyzed the DNA methylation status of these microRNAs using 99 primary NSCLCs. Mir-34b and mir-126 were methylated in 41% and 7% of all the cases, respectively. The DNA methylation of mir-34b was not associated with c-Met expression determined by immunohistochemistry, but both mir-34b methylation ($p = 0.007$) and c-Met expression ($p = 0.005$) were significantly associated with lymphatic invasion in a multivariate analysis. The DNA methylation of mir-34b can be used as a biomarker for an invasive phenotype of lung cancer.

B40 microRNA biomarkers prognostic for disease-free and overall survival in stage I and II lung adenocarcinoma. Glen J. Weiss[1], Carlos D. Lorenzo[2], Paul A. Kurywchak[2], Mohsin Malik[3], Guy M. Raz[2], Walter P. Wagner[2], Nhan L. Tran[2], Irene Cherni[2], Kim A. Paquette[2], Raghu Metpally[2], Tim G. Whitsett[2], Allen Jeffrey[3], Seungchan Kim[2], Jianping Hua[2], Waibhav D. Tembe[2]. [1]Virginia G. Piper Cancer Center Clinical Trials at Scottsdale Healthcare/TGen, Scottsdale, AZ, [2]The Translational Genomics Research Institute, Phoenix, AZ, [3]University of Tennessee Cancer Institute, Memphis, TN.

Background: Lung adenocarcinoma is the most common histologic subtype of non-small cell lung cancer (NSCLC), and accounts for ~50% of all NSCLC cases in the USA. Lung resection offers a chance for cure for stage I and II NSCLC. However, up to 57% of stage I and II NSCLC patients undergoing a resection for curative intent will have disease relapse or metastatic disease within 5 years, with the majority occurring within the first 2 years. Thus, pathologic staging alone does not stratify patients for prognosis sufficiently. Identifying patients with aggressive vs. indolent tumors would have critical implications for directing treatment and designing future clinical trials. We propose using microRNA (miRNA) for discovery and validation of prognostic biomarkers for stage I/II lung adenocarcinoma.

Methods: Total RNA was extracted from treatment-naïve AJCC 7th edition stage I and II lung adenocarcinoma formalin-fixed, paraffin-embedded (FFPE) samples. Differential miRNA array expression profiling between 26 disease-free survivors (DFS) and overall survival (OS) at least 47 months compared to 14 patients with DFS and overall survival less than 23 months was assessed in the Discovery cohort on 8x15 Agilent microarray printed with 886 miRNAs. Target miRNAs were selected based on minimum of 2-fold change in expression and Welch t-test p-value cut-off of <0.05. qPCR assay of these individual miRNAs were measured in the Discovery cohort. The Validation cohort, consisting of the Discovery

cohort samples (N=40) and additional stage I/II lung adenocarcinoma patient samples (N=63 with DFS and OS>47 months and N=26 with DFS and OS<23 months), using PCR were analyzed to confirm differential expression of target miRNAs. Fold-change values were generated with the ΔΔCt method. Development of a PCR-based miRNA classifier using the Validation cohort PCR results is being developed. This miRNA classifier will be assessed for accuracy prediction when applied to measured target miRNAs in the Independent cohort of additional stage I/II lung adenocarcinoma samples from another institution blinded for DFS/OS comparisons (N=21 DFS/OS >47 months and N=4 DFS/OS <23 months).

Results: Microarray analysis of the Discovery Cohort revealed 7 miRNAs that were significantly under-expressed in patients with DFS and OS <23 months. They included let-7a/b/c, miR-130a, -451, -29c, and -99a. PCR results in the Validation cohort remained statistically significant for differential expression for all target miRNAs (p<0.05) except miR-99a for those samples with DFS and OS >47 months compared to those samples with DFS and OS <23 months. The miRNA DFS and OS classifier for stage I and II lung adenocarcinoma is being developed and results applied to the Independent cohort is ongoing.

Conclusion: We have validated miRNAs prognostic for DFS and OS in stage I and II lung adenocarcinoma in at least one cohort of 129 patients so far. A miRNA classifier is currently under development and we anticipate results for accuracy prediction on the Independent cohort at the time of the meeting.

Funding supported by the Flinn Foundation.

B41 Consider the source: Normal lung expression classifies cancer histologies, subtypes, and mutations. Matthew D. Wilkerson, Scott H. Randell, D. Neil Hayes. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Lung cancer's pathogenesis from normal cell to metastatic disease involves vast and complex genetic alterations. However, a patient's genetic alterations do not completely explain the pathogenic process, for tumors with the same alterations have different features and therapy responses. We hypothesized that differences in ancestral anatomical regions affect pathogenesis and as such would correlate with differences in cancer histology, subtype, and mutations. Anatomical distal and proximal region specimens from normal lungs were collected and assayed by Agilent 44K gene expression microarrays and immunohistochemistry (IHC). 271 surgically extracted lung cancers were assayed by gene expression, IHC, and gene sequencing for EGFR, KRAS, STK11, and TP53. Using the normal lung specimens, a novel classifier was created to assign cancers with an anatomical region using gene expression alone. Cancers classified as distal by gene expression were 84% adenocarcinoma, while 73% of those classified as proximal were squamous cell carcinoma (P<0.001). While this trend of histology and region is consistent with conventional wisdom, our results also suggest that lung cancers retain substantial gene expression from their ancestral anatomical region. Interestingly, the remainder of the cancers that did not follow this trend was explained by our previously described molecular subtypes (1, 2). Of adenocarcinomas classified proximal, 92% were in the Magnoid molecular subtype. Of squamous cell carcinomas classified distal, 60% were in the Secretory molecular subtype. Complementing microarray gene expression, TTF1 IHC protein expression was greater in distal normal specimens compared to proximal. Consistent with distal classification, adenocarcinomas had the greatest TTF1 expression. Of the squamous molecular subtypes, the Secretory subtype exhibited the

greatest TTF1 expression, consistent with its distal classification. This Secretory subtype may explain previously reported TTF1-positive squamous cell carcinomas. Finally, gene mutation rates were significantly different between cancers classified as proximal and as distal. EGFR and KRAS mutation rates were greater in distal tumors compared to proximal tumors. In contrast, TP53 mutation rates were greater in proximally classified tumors. These results suggest that ancestral anatomical regions may affect lung cancer pathogenesis by modifying susceptibility to and dependency on different gene mutations. In summary, normal lung anatomical gene expression classifies lung cancers with different histologies, molecular subtypes, and gene mutation rates.

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B42 The lineage-specific transcription factor ASCL1 defines a subtype of neuroendocrine non-small cell lung cancers with poor prognosis and points to potential therapeutic targets. Alexander Augustyn[1], John Minna[1], Christopher Tan[1], James Sullivan[2], Mark Borromeo[3], Luc Girard[1], Jane Johnson[3], Carmen Behrens[3], Ignacio Wistuba[3], Yang Xie[1]. [1]University of Texas Southwestern Medical Center, Dallas, TX, [2]Harvard Medical School, Charlestoen, MA, [3]University of Texas MD Anderson Cancer Center, Houston, TX.

The future of cancer therapy relies on identifying certain patient populations that will respond to one treatment but not another based on the properties of the tumor and additionally creating specific drugs that target genetic pathways required for a particular tumor's formation, growth, and maintenance. We have developed a pre-clinical model for a genetically identifiable clade of non-small cell lung cancer that exhibits neuroendocrine features. We utilized whole-genome mRNA expression array data from 119 NSCLC cell lines to identify a class of NSCLC lines that fit the neuroendocrine phenotype (NE-NSCLC). We identified 11 NSCLC cell lines (9.2%) that display a distinct neuroendocrine gene signature. A gene expressed in all putative NE-NSCLC cell lines is the potent neural-specific transcription factor ASCL1. We hypothesize that ASCL1 acts as a "lineage dependent oncogene" for NE-NSCLC and that associated with ASCL1's function in the molecular pathogenesis of these lung cancers will be a gene expression profile that contributes to the malignant phenotype, which will provide insight towards therapeutic targeting of this subset of NSCLC. In this regard, ASCL1 ChIP-Seq data combined with genome wide mRNA expression data identified a subset of genes whose expression appears to be regulated by ASCL1. In NSCLC patient populations, tumors expressing ASCL1 and the neuroendocrine gene signature demonstrated impaired prognosis compared to the other NSCLCs. Knockdown of ASCL1 in representative NSCLC cell lines reduced target gene expression, caused significant cell cycle defects, and induced marked apoptosis. NSCLC cell lines expressing ASCL1 demonstrate a cancer stem cell marker phenotype similar to that of small cell lung cancer, providing clues to the pathogenesis of the NE-NSCLC disease subset. Our results suggest that neuroendocrine gene expression in NSCLC is of clinical relevance, that ASCL1 is required for survival of the NE-NSCLC disease subset, while the integrated ASCL1 ChIP-Seq and mRNA expression data provide a roadmap for systematically searching for therapeutic targets for this phenotype and their mechanistic role in a cancer stem cell (initiating cell) subpopulation within these tumors.

B43 SNP Q787Q of EGFR gene and efficacy of EGFR-TKI in patients with non-small cell lung cancer. Kim Young-Chul, Kim Kyu-Sik, Oh In-Jae, Ban Hee-Jung, Na Kook-Joo, Ahn Sung-Ja, Song Sang-Yun, Choi Song, Choi Yoo-Duk. Chonnam National University Hwasun Hospital, Jeonnam, Republic of Korea.

Background: Many activating mutations in epidermal growth factor receptor (EGFR) gene have been correlated with sensitivity to EGFR tyrosine kinase inhibitors (TKIs). Single nucleotide polymorphism (SNP) in exon 20 of EGFR gene (2361G>A transition) does not alter the amino acids of glutamine at codon 787 (Q787Q). We reviewed the relationship between this SNP and EGFR-TKI sensitivity.

Patients and Methods: Twenty-five patients (male 19, female 6) with only SNP Q787Q in EGFR exon 20 were analyzed. Tissue or cytologic specimens were used for analysis of mutations in exon 18 to 21 of EGFR gene by direct sequencing. Treatments with EGFR-TKIs were performed in 16 patients and response evaluations were eligible in 15 cases.

Results: In 15 response evaluable patients, there were 2 partial responses (PR) and 5 stable diseases (SD) [response rate: 13.3%, disease control rate: 46.7%]. Median time to progression (TTP) was 392 days (range: 105~511). Five patients showed longer than 3 months of TTP (PR 2 and SD 3, adenocarcinoma 4 and squamous cell carcinoma 1, gefitinib 4 and erlotinib 1).

Conclusion: Thirteen percent of NSCLC with 2361G>A transition without other activating mutations responded to EGFR-TKIs.

B44 Development of a serum test for the detection of lung cancer based on oncogenic BARD1 isoform expression. Irmgard Irminger-Finger[1], Pierre-Alain André[1], Maxim Pilyugin[1], Balazs Hegedus[2], Geoffrey J. Laurent[3], Andrea Bianco[4]. [1]Geneva University Hospitals, Geneva, Switzerland, [2]Universitatsklinik Wien, Vienna, Austria, [3]University College London, London, United Kingdom, [4]Universita di Molise, Campobasso, Italy.

The BARD1 protein was originally identified as binding partner of the breast cancer gene product, BRCA1. Highly upregulated expression of aberrant isoforms of BARD1, derived from differential splicing, was correlated with poor prognostic factors in breast and ovarian cancer (Wu et al. Int J Can, 2006; Li et al. Can Res, 2007) and decreased patient survival in lung cancer (Zhang et al. Int J Can, 2011).

Previous and ongoing studies have shown that BARD1 isoforms act antagonistically to the functions of BARD1 and BRCA1 as ubiquitin ligase. In particular, BARD1beta is promoting cell proliferation by stabilizing the Aurora kinases (Ryser et al. Can Res 2009; Bosse et al. submitted). Isoforms BARD1-beta and BARD1-pi are specifically upregulated in lung cancer and correlated with poor prognosis (Zhang et al., Int J Can, 2011). Thus, BARD1 isoforms might be drivers of tumorigenesis and potential markers of lung cancer progression, if detectable in the patients' sera.

To this goal, we performed ELISA tests with antibodies against different regions of BARD1 for the detection of BARD1 isoforms in the blood of lung cancer patients. We also generated a peptide library representing 40 epitopes mimicking BARD1 isoforms, for the detection of autoimmune antibodies recognizing epitopes expressed by BARD1 isoforms.

BARD1 protein isoforms could be detected by ELISA in various serum samples, however this detection was always reproducible due to the BARD1 protein instability. We then performed inverse ELISA assays, using peptides for capturing autoimmune antibodies

directed against BARD1 isoforms. Using serum samples from 60 non-small cell lung cancer (NSCLC) from time of diagnosis, and 40 control sera from phenotypically healthy volunteers, we could distinguish NSCLC cancer patients and controls. When applying a combination of seven peptides lung cancer was detected with 87 percent sensitivity and 68 percent specificity.

Thus, our data show convincingly that antibodies against BARD1 isoforms are telltales of lung cancer and their detection can be further developed towards a blood test for the early detection of lung cancer. Experiments including larger patients and control group numbers, sera from patients with different types of lung cancer, as well as the comparison of this BARD1 isoform test with the standard test for lung cancer detection (CT scan), are currently ongoing, and should lead to optimized test conditions and a definition of the target patient set.

B45 Upfront genomic testing in non-small cell lung cancer (NSCLC) patients: Preliminary result of the MSN study. David Planchard[1], Ludovic Lacroix[1], Thierry Le Chevalier[1], Jean-Charles Soria[1], Benjamin Besse[1], Aljosa Celebic[1], Maud Ngocamus[1], Nathalie Auger[1], Beranger Lueza[1], Patrick Saulnier[1], Arslane Rahal[1], Khadja Kalai[1], Peter Dorfmuller[2]. [1]Institut Gustave-Roussy, Villejuif, France, [2]Marie Lannelongue Surgical Center, Plessis Robinson, France.

Background: Recent advances in lung cancer have identified potential driver mutations that may be targeted. To identify new predictors of response as well as novel targets for therapy, we have initiated a comprehensive large-scale sequencing analysis of genes potentially mutated in NSCLC.

Methods: Genomic DNA was extracted prospectively from untreated advanced NSCLCs. All tumors were obtained on IRB-approved protocols and after patients' consent (MSN trial "Melanoma – Small-cell lung cancer – Non-small cell lung cancer"). Pathology specimens were macrodissected in order to obtain more than 30% of tumor cells and, after DNA extraction, 96 selected exons from 30 genes were analyzed by Sanger sequencing. ALK rearrangements were detected by fluorescence in-situ hybridization. All results were discussed monthly in a molecular thoracic multidisciplinary staff. Second or third lines of treatment were adapted to mutation profiling.

Results: Thus far (between 09/01/2010 and 06/01/2011), 82 tumors have been analyzed for AKT, ALK, APC, BRAF, CTNNB1, EGFR, ERBB2, FBXW7, FGFR2-3, GNAQ, GNAS, HRAS, KIT, KRAS, MAP2K1-2, MET, NOTCH1, NRAS, PDGFRA, PI3KCA, PTEN, RET, STK11, TP53, and VHL mutations. The median age was 60 years (range 26-76), 29 (35%) were female, 58 (70%) had adenocarcinoma, 72 (67%) were former/current smokers. Nine patients had incomplete genomic analysis due mostly to insufficient tumor cells in the specimen or poor quality DNA. Median tumor cells ratio was 50%. Mutations were identified in 43/82 (52%) patients (EGFR: 9; KRAS: 10; STK 11: 8; BRAF: 4; MET: 2; PTEN: 2; NRAS: 1; ERBB2: 1; PIK3CA: 1…) of whom 11 had concurrent mutations. EGFR and KRAS mutations were mutually exclusive. No mutations were identified among 5 never smokers analyzed. The median time to complete testing for this initial phase was 30 days. Half of patients with genomic alterations were treated with molecularly targeted therapy based on their genetic alteration.

Conclusions: Mutational profiling of NSCLC is feasible, can distinguish relevant molecular subsets of lung cancer, and may present an impact on treatment at our cancer institute. Furthers sequencing are in progress and updated results will be presented in January 2012.

B46 Clinicopathologic implications of ALK rearrangement in surgically resected NSCLC: A proposal of molecular algorithm with a small lung biopsy of adenocarcinoma. Jin Ho Paik[1,2], Chang-Min Choi[3], Hyojin Kim[1], Se Jin Jang[3], Sook-Whan Sung[3], Dong Kwan Kim[4], Hwa Jung Kim[4], Hoil Yoon[1], Choon-Taek Lee[1], Sanghoon Jheon[1], Ji-Young Choea[1,2], Jin-Haeng Chung[1,2]. [1]Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea, [2]Tumor Immunity Medical Research Center, Seoul National University College of Medicine, Seoul, Korea, [3] Catholic University, Seoul, Korea, [4]Asan Cancer Center, Asan Medical Center, Seoul, Korea.

Background: To characterize the clinicopathologic features of ALK-rearranged lung cancer, and suggest a molecular test protocol for lung adenocarcinoma in the small biopsy specimen.

Methods: In 735 NSCLC surgical specimens, clinicopathologic features, ALK protein over-expression by immunohistochemistry (IHC), and ALK rearrangement by fluorescence in situ hybridization (FISH) as well as EGFR and KRAS mutation studies were analyzed.

Results: Of the 735 NSCLC cases, 28 (3.8%) were ALK FISH-positive. ALK rearrangement, EGFR and KRAS mutation were mutually exclusive. ALK rearrangement was significantly higher in adenocarcinomas (6.8%, $p<0.001$), younger age ($p<0.0007$), women (7.6%, $p<0.001$), and never-smokers (8.9%, $p<0.001$) with no gender difference in the adenocarcinoma or never-smoker subgroup. ALK FISH-positivity was not associated with disease recurrence (HR, 0.79; 95% CI, 0.42-1.49) or overall survival (HR, 0.61; 95% CI, 0.24-1.55). However, ALK-rearranged lung cancer tended to show more frequent lymph node metastasis despite its lower T stage. Similar to EGFR-mutated lung cancer, ALK-rearranged lung cancer was enriched in adenocarcinoma, women, and never-smokers. The results of ALK IHC and FISH obtained from tissue microarray (TMA)/ biopsy specimens and whole sections after resection were concordant.

Conclusion: ALK rearrangement was not a significant prognostic factor in surgically resectable NSCLC. The clinical profiles of ALK-rearranged lung cancer patients overlapped with those of EGFR-mutated patients. Therefore, we suggest that simultaneous tests for ALK IHC and EGFR mutation (Chung's SNUBH molecular test protocol), which has important implications for the storage and use of small biopsy or cytology samples for genetic analysis.

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