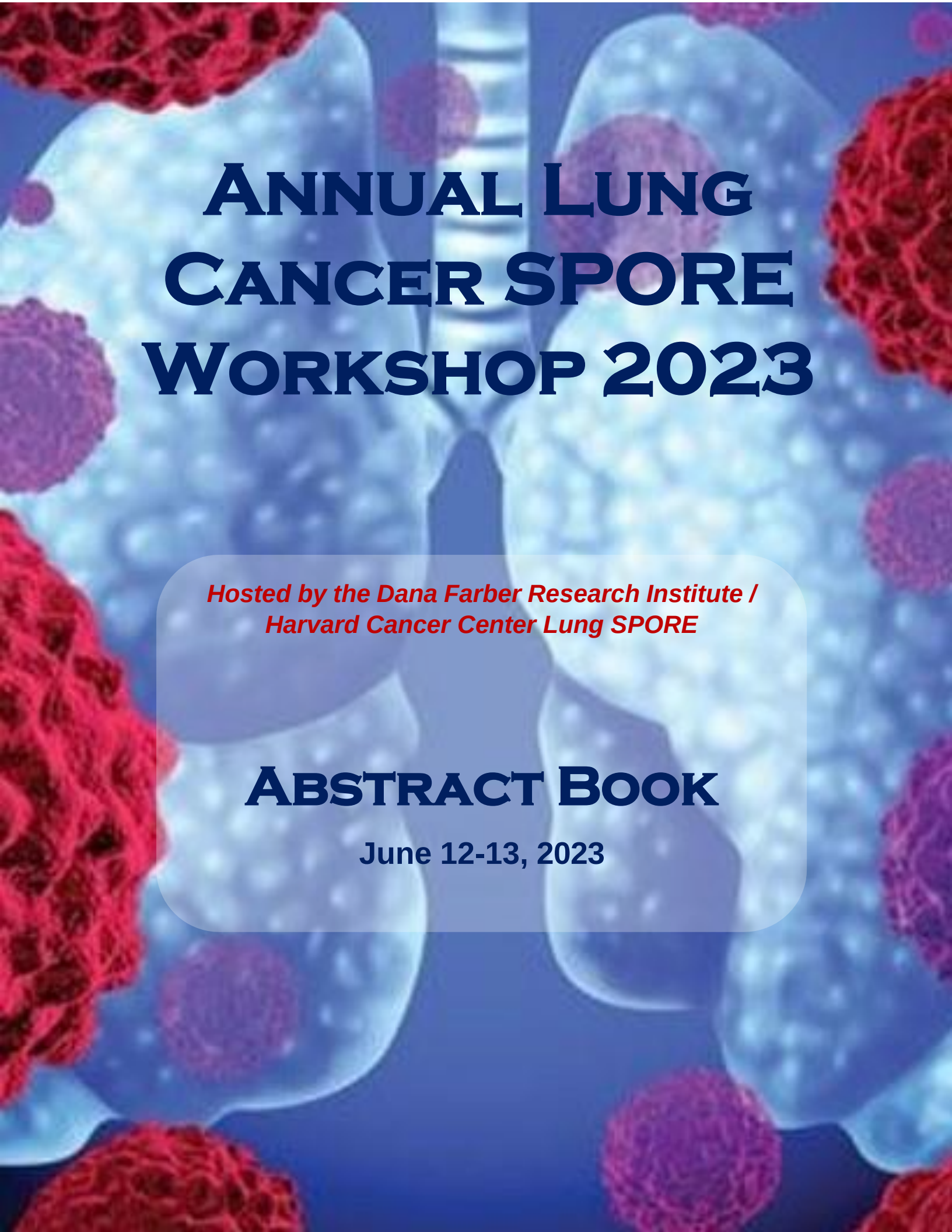


Annual Lung Cancer SPORE Workshop 2023

Hosted by the Dana Farber Research Institute / Harvard Cancer Center Lung SPORE

Abstract Book

June 12-13, 2023

DF/HCC
DANA-FARBER / HARVARD CANCER CENTER

DF/HCC Lung SPORE Workshop
June 12-13, 2023

Mass General Brigham, Assembly Row
Markell Room ABC
399 Revolution Drive
Somerville, MA 02145

To Join Remotely:
2023 SPORE Workshop Zoom Webinar - Monday
https://partners.zoom.us/s/84979576551

Monday June 12th (8:00-6:00)

7:30	Breakfast	
8:00	Opening/Welcome	Lecia Sequist, MD, MPH and David Barbie, MD
8:15	Keynote Address Day 1 Title TBA	Alex A. Adjei, MD, PhD, FACP Taussig Cancer Institute Cleveland Clinic

Session 1: Driver Oncogene Addiction

Time	Speaker	Title
9:00	Jessica Lin, MD Jaclyn LoPiccolo, PhD, MD	Introduction
9:02	Alice Berger, PhD Fred Hutchinson Cancer Center	Update on the function of the RIT1 oncogene in lung cancer
9:14	Aaron Hata, MD, PhD Massachusetts General Hospital	Mechanisms Driving Evolution of TKI-Resistance in Non-Small Cell Lung Cancer
9:26	Paul Stockhammer, MD, PhD Yale University	Alterations in Multiple Tumor Suppressor Genes are Associated with Poor Outcomes in Patients with EGFR-mutant Lung Cancer
9:38	Ken Westover, MD, PhD UT Southwestern Medical Center	Identification and Description of Molecular Mechanisms of On-Target Resistance to Targeted Lung Cancer Therapies with Selpercatinib, Crizotinib, Neratinib, Pralseltinib, Poziotinib and MRTX1133
9:50	All	Q&A

Session 2: Health Disparities in Lung Cancer

Time	Speaker	Title
10:00	Christopher Lathan, MD, MS, MPH Efren Flores, MD	Introduction
10:02	Brittany Dowe, MPH Karmanos Cancer Institute /Wayne State University	Increasing Community Understanding of Precision Oncology and Genetic Literacy in the African American Community: Preliminary Results from an Educational Session
10:14	Sofia Yi UT Southwestern Medical Center	Access and adherence to low-dose CT-based lung cancer screening in an urban, safety-net hospital system
10:26	Jeff Yang, MD Massachusetts General Hospital	Reconsidering the Use of Pack-year Smoking History in Determining Lung Cancer Screening Eligibility
10:38	Felicity Harper, PhD Karmanos Cancer Institute/Wayne State University	Using mHealth to Understand Racial Disparities in Side Effects for Lung Cancer Patients Receiving Immunotherapy Treatment
10:50	All	Q&A

11:00 Break

Session 3: Novel Immunotherapeutic Clinical Approaches

Time	Speaker	Title
11:15	Alissa Cooper, MD Meghan Mooradian, MD	Introduction
11:17	McGarry Houghton, MD Fred Hutchinson Cancer Center	Immune Response Subtype Classifier for NSCLC
11:29	Qiankun Niu, PhD Emory University	Targeting the STING deficiency to augment immune responsiveness in LKB1-mutant lung cancer
11:41	David Barbie, MD Dana-Farber Cancer Institute	MPS1 inhibition primes immunogenicity of KRAS-LKB1 mutant Lung cancer
11:53	B. Leticia Rodriguez, PhD UT MD Anderson Cancer Center	Anti-tumor activity of a novel Lair1 antagonist with anti-PD1 to treat collagen-rich solid tumors
12:05	Scott Gettinger, MD Yale University	NC318, an Anti-Siglec-15 Humanized Monoclonal Antibody, Alone and in Combination with Pembrolizumab in Immunotherapy Pretreated Non Small Cell Lung Cancer

12:17	Justin Gainor, MD Massachusetts General Hospital	Genomic and Transcriptomic Analysis of Checkpoint Blockade Response in Advanced Non-Small Cell Lung Cancer
12:29	All	Q&A

12:45 Lunch

Session 4: Tumor Immune Interactions

Time	Speaker	Title
2:00	James Heather, PhD David Kwiatkowski, MD	Introduction
2:02	Wei Zhou, PhD Emory University	Sex-based differences in the formation of LKB1-mutant lung adenocarcinoma
2:14	Kerryan Ashley, MHS Yale University	Spatial mapping of the CD39/CD73 adenosine pathway as a candidate immunotherapy target in non-small cell lung cancer
2:26	Esra Akbay, PhD UT Southwestern Medical Center	Development of Immunogenic Autochthonous Mouse Lung Cancer Models
2:38	Xin Sun, PhD UT MD Anderson Cancer Center	Characterization of T and B cell antigen specific-engineered NSCLC tumors to study the function of B cells and TLS in murine models of neoadjuvant immunotherapy
2:50	Heather Gibson, PhD Karmanos Cancer Institute, Wayne State University	Evaluation of the tumor immune microenvironment as a contributor to lung cancer disparities
3:02	All	Q&A

3:15 Coffee Break

Session 5: Cancer Biology

Time	Speaker	Title
3:30	Carla Kim, PhD Jaime Schneider, MD, PhD	Introduction
3:32	Maria Fernanda Trovero, PhD Boston Children's Hospital	Replication stress in BRG1 deficient lung adenocarcinoma as a vulnerability for intervention

3:44	**Jerry Shay, PhD** **UT Southwestern Medical Center**	**6-thio-dG represents a new systemic chemotherapy approach for lung cancer with tumor cell selectivity including cancers resistant to current therapies facilitating immune checkpoint blockade anti-tumor immune responses**
3:56	Anna Arnal-Estape, PhD Yale University	Brain metastatic outgrowth and osimertinib resistance are potentiated by RhoA in EGFR-mutant lung cancer
4:08	Chang-Yi Chen, PhD Virginia Commonwealth University, Massey Cancer Center	Cooperation Between PRMT1 and PRMT6 Drives Lung Cancer Health Disparities Among Black/African American Men
4:20	Haian Fu, PhD Emory University	Mutation-mediated neo-protein-protein interactions to inform oncogenic rewiring and therapeutic vulnerability
4:32	All	Q&A

4:45	Poser Session
6:30	Adjourn
7:00	Dinner, Row Hotel

To Join Remotely:
2023 SPORE Workshop Zoom Webinar - Tuesday
https://partners.zoom.us/s/87435310257

Tuesday June 13th (8:30-2:30)

8:00 Breakfast

Session 6: Lung Cancer Risk and Early Detection

Time	Speaker	Title
8:30	Suzanne Dahlberg, PhD Jacob Sands, MD	Introduction
8:32	Wei Wu, MD University of Washington	Comparison of prediction models to diagnose indeterminate pulmonary nodules using clinical variables, semantic imaging features and radiomics
8:54	Florian Fintelman, MD Massachusetts General Hospital	Sex differences in the prediction of future lung cancer risk based on a single low-dose chest computed tomography scan
8:56	Ray Osarogiagbon, MD Baptist Cancer Center	Lung cancer diagnosis hazard in screening and lung nodule programs
9:88	Erica Warner, ScD, MPH Massachusetts General Hospital	Investigating Low Dose CT Findings in Firefighters: Results from the Fire Health Study
9:20	All	Q&A

9:30	Introduction	Lecia Sequist, MD, MPH and David Barbie, MD
9:32	Keynote Address Day 2 Blood-based biomarkers of immunotherapy response: The emerging role of ctDNA dynamics in precision immuno-oncology	Valsamo Anagnostou, MD, PhD Sidney Kimmel Comprehensive Cancer CenterJohns Hopkins University School of Medicine

Session 7: Potpourri

Time	Speaker	Title
10:17	Rebecca Heist, MD, MPH Benjamin Kann, MD	Introduction
10:19	David Gerber, MD UT Southwestern Medical Center	What next? Navigating the dynamic, unpredictable, and challenging realm of cancer clinical trials during and after a global pandemic
10:31	Narjust Florez, MD Dana-Farber Cancer Institute, Harvard Medical School	Sexual Dysfunction in Women with Lung Cancer: Updates from the SHAWL Study
10:43	Xiancheng Wu, MD University of Pittsburgh	A radiomic based predictive model of lung adenocarcinoma brain metastases
10:55	Madhusmita Behera, PhD Emory University	Lung Cancer Information System: enabling precision oncology through informatics
11:07	Ken Kehl, MD, MPH Dana-Farber Cancer Institute	Artificial intelligence-aided clinical annotation of a large multi-cancer genomic dataset
11:19	All	Q&A

11:30	Special Session Patient Advocates and Research: Promoting involvment and inclusion throughout the research process	Lecia Sequist, MD, MPH Upal Basu Roy, PhD, MPH Hildy Grossman Diane Legg Laura Petrillo, MD Kim Norris
12:00	Lunch	

Session 8: SCLC

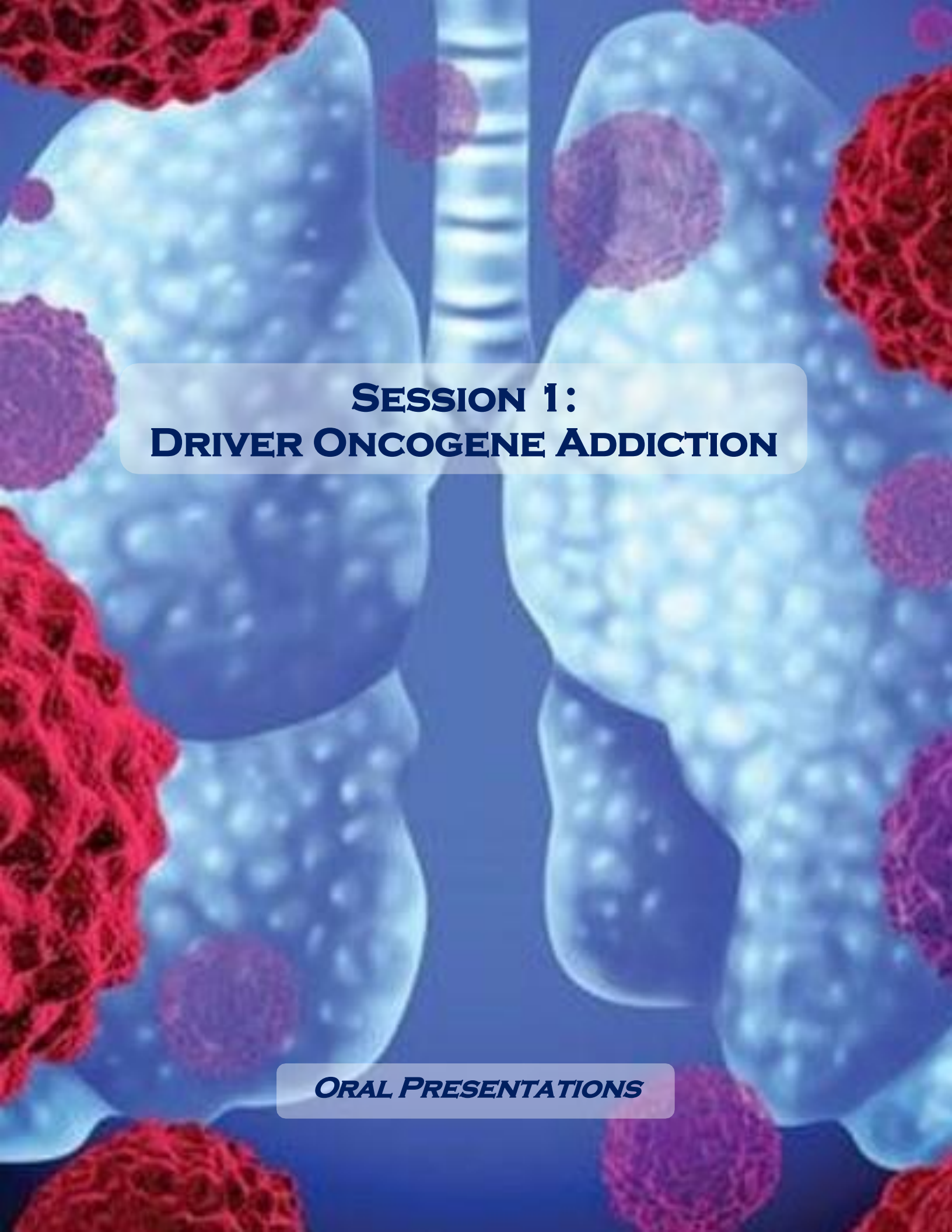
Time	Speaker	Title
1:00	Jia Luo, MD Matt Oser, MD, PhD	Introduction
1:02	Benjamin Drapkin, MD, PhD UT Southwestern Medical Center	Extrachromosomal DNAs Harboring Myc Family Proto-oncogenes Drive Acquired Cross-resistance in Small Cell Lung Cancer
1:14	Catherine Meador, MD, PhD Massachusetts General Hospital	Spatiotemporal Heterogeneity of Transcription Factor-Based Subtype Assignment in Small Cell Lung Carcinoma
1:26	Kristin Lastwika, PhD Fred Hutchinson Cancer Center	Post-translational modifications induce autoantibodies with risk-prediction capability in patients with small cell lung cancer

1:38	Simon Heeke, PhD UT MD Anderson Cancer Center	Tumor and Liquid Biopsy-Based Subtyping of Small Cell Lung Cancer using Transcriptomic and DNA Methylation Classifiers
1:50	Anne Chiang, MD, PhD Yale University	Molecular predictors and immunomodulatory role of dual checkpoint inhibitor blockade using ipilimumab/nivolumab in patients with advanced stage small cell lung cancer
2:02	David MacPherson, PhD Fred Hutchinson Cancer Center	Inference of gene expression and transcription factor activity in small cell lung cancer using targeted sequencing of plasma cfDNA
2:14	All	Q&A

2:30 Wrap-up/Adjourn

Table Contents

Oral Presentations



Session 1:
Driver Oncogene Addiction

Oral Presentations

Update On the Function of the RIT1 Oncogene in Lung Cancer

Fred Hutchinson Cancer Center

Presented by: Alice Berger

Mutation of the Ras pathway is a prevalent feature of lung adenocarcinoma, occurring in approximately 75% of tumors. Activation of Ras signaling can occur via diverse mutational mechanisms, including mutation of *KRAS* itself or via upstream mutation of receptor tyrosine kinases such as *EGFR*. We and others discovered somatic mutation of RIT1, a non-canonical Ras-family GTPase, in ~2% of lung adenocarcinomas. Mutations in *RIT1* are mutually exclusive with other Ras driver oncogenes, suggesting *RIT1* may act as a driver oncogene in its own right.

To test if RIT1 can promote lung cancer in vivo, we generated a inducible genetically engineered mouse model to study expression of $RIT1^{M90I}$, the most recurrent *RIT1* mutation in cancer. Despite the ability of $RIT1^{M90I}$ to transform cells in vitro, delivery of Cre to the murine lung to activate $RIT1^{M90I}$ did not induce tumor formation in mice observed up to 600 days post Cre delivery. Surprisingly, loss of *Trp53*, encoding the p53 tumor suppressor, also did not enhance tumor growth in the $RIT1^{M90I}$+ mice.

To identify factors that synergize with $RIT1^{M90I}$, we performed a genome-wide CRISPR knockout screen in human lung cancer cells whose survival/proliferation is exquisitely dependent on $RIT1^{M90I}$ function. This screen identified genetic inactivation of Hippo signaling as a major synergy with oncogenic *RIT1*. Specifically, loss of the *NF2* tumor suppressor synergized with $RIT1^{M90I}$ to promote *RIT1*-driven drug resistance or in vivo xenograft tumor formation. To test whether loss of *Nf2* might sensitize cells in vivo to oncogenic RIT1, we crossed the $RIT1^{M90I}/Trp53^{fl/fl}$ mice with $Nf2^{fl/fl}$ animals to generate $RIT1^{M90I}+/p53^{null}/Nf2^{null}$ mice ("RPN"). $RIT1^{M90I}$+ animals, but not RIT1-, succumb to aggressive lung tumors within 8-12 weeks of Cre induction ($p < 0.0001$ by log rank test). These data, together with evidence of YAP activation in human lung cancer biopsies from *RIT1*-mutant tumors, suggest that Hippo pathway inactivation may be required to promote *RIT1*-driven lung cancer. Targeting YAP/TEAD activity should therefore be explored as a therapeutic approach in human *RIT1*-mutant lung tumors.

Mechanisms Driving Evolution of TKI-Resistance in Non-Small Cell Lung Cancer

Massachusetts General Hospital

Presented by: Aaron Hata

Molecular targeted therapies have transformed the care of patients with oncogene-driven non-small cell lung cancers, however acquired drug resistance remains an unsolved clinical problem. Although many drivers of acquired drug resistance have been identified, the underlying molecular mechanisms that influence tumor evolution during treatment are incompletely understood. We previously demonstrated that mechanisms of clinical acquired drug resistance can evolve within drug tolerant cells that persist during therapy, suggesting that suppressing mechanisms of tumor evolution may prevent or delay the development of resistance. We find that lung cancer targeted therapies commonly used in the clinic can induce cytidine deaminase APOBEC3A (A3A), leading to sustained mutagenesis in drug-tolerant cancer cells persisting during therapy. Therapy-induced A3A promotes the formation of double-strand DNA breaks, increases genomic instability and leads to acquired mutations and structural variations. Deletion of A3A suppresses the formation of drug tolerant persisters and delays the emergence of drug resistant clones. These results implicate A3A as a driver of tumor evolution leading to acquired drug resistance and suggest that suppressing A3A may represent a potential therapeutic strategy to prevent or delay acquired resistance to lung cancer targeted therapy.

Alterations in Multiple Tumor Suppressor Genes are Associated with Poor Outcomes in Patients with *EGFR*-mutant Lung Cancer

Paul Stockhammer, Michael Grant, Anna Wurtz, Giorgia Foggetti, Francisco Expósito, Jianlei Gu, Sangyun Chung, Fangyong Li, Zenta Walther, Scott Gettinger, Katerina Politi, Sarah B. Goldberg

Yale University

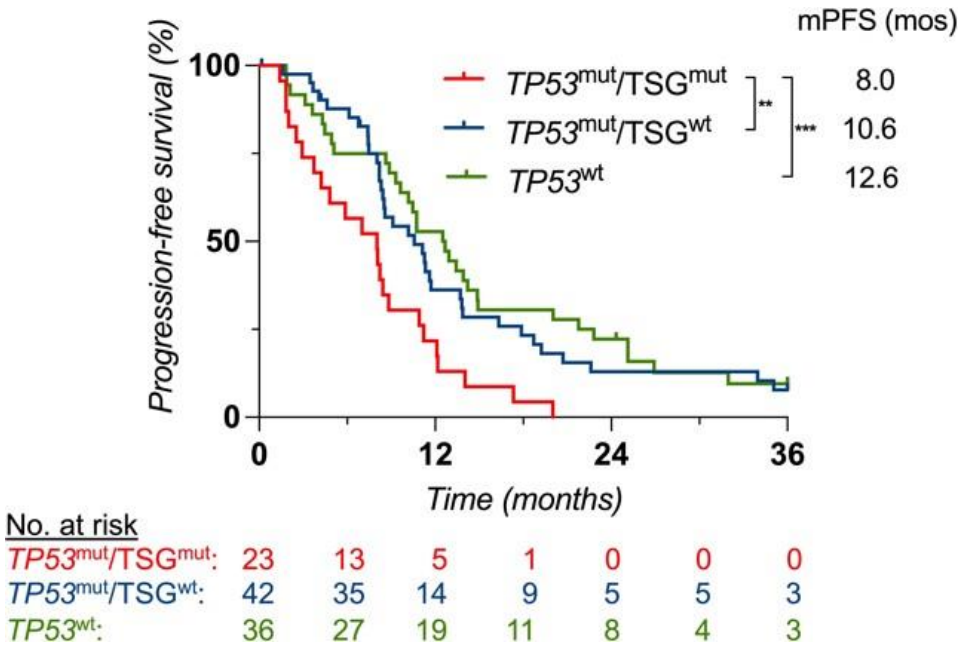
Presented by: Paul Stockhammer

Background: Co-occurring alterations in tumor suppressor genes (TSG) have been described as determinants of disease heterogeneity in *EGFR*-mutant NSCLC, however, detailed analyses of their impact on patient outcomes are limited.

Methods: Patients with *EGFR*-mutant NSCLC treated with EGFR TKIs at the Yale Cancer Center who had tumor genomic profiling before EGFR-TKI therapy (pre-TKI) or at disease progression (post-TKI) were included. Whole exome sequencing (WES) of paired pre- and post-TKI tumors was performed for a subset of patients. The cohort was divided into three subgroups based on alterations in *TP53* and five additional TSGs (*RB1*, *NF1*, *ARID1A*, *BRCA1* and *PTEN*): patients with tumors harboring a *TP53* mutation plus a mutation in at least 1 additional TSG (*TP53*^{mut}/TSG^{mut}), a *TP53* mutation without an additional TSG mutation (*TP53*^{mut}/TSG^{wt}), and *TP53*^{wt}. Clinical characteristics including PFS and OS were assessed.

Results: One-hundred-one patients were included in this study. *TP53* mutations were identified in 65 (64%) tumors, of which 23 (35%) were classified as *TP53*^{mut}/TSG^{mut} and 42 (65%) as *TP53*^{mut}/TSG^{wt}. Among cases with paired WES available (n=34), frequencies of alterations in the included TSGs did not significantly differ between pre- and post-TKI tumors. In the full study cohort, the presence of a *TP53* mutation was associated with worse PFS (HR 1.46, p=0.09) and OS (HR 1.68, p=0.04) on first-line EGFR-TKI. Strikingly, after dividing the *TP53*^{mut} cohort into *TP53*^{mut}/TSG^{mut} and *TP53*^{mut}/TSG^{wt} cases, alterations in additional TSG were found to drive the poor outcomes: *TP53*^{mut}/TSG^{mut} cases had significantly worse PFS (Figure) and OS on first-line EGFR TKI than *TP53*^{mut}/TSG^{wt} (mPFS 8.0 vs 10.6 months, p=0.006; mOS 30.0 vs 33.3 months, p=0.12) or *TP53*^{wt} cases (mPFS 8.0 vs 12.6 months, p<0.0001; mOS 30.0 vs 48.8 months, p=0.001). There was no significant difference in PFS or OS between patients with *TP53*^{mut}/TSG^{wt} and *TP53*^{wt} tumors. Similar outcome differences between the three groups were found in patients who received osimertinib as second-line therapy.

Conclusions: The inferior outcomes associated with *EGFR/TP53*-mutant NSCLC tumors may be due to additional TSG alterations rather than *TP53* mutational status alone. Our findings may have implications for understanding the biologic underpinnings of differential outcomes to EGFR-TKIs.


mPFS (mos)
Progression-free survival (%)
100
50
0
TP53mut/TSGmut
TP53mut/TSGwt
TP53wt
**
***
8.0
10.6
12.6
0
12
24
36
Time (months)
No. at risk
TP53mut/TSGmut: 23 13 5 1 0 0 0
TP53mut/TSGwt: 42 35 14 9 5 5 3
TP53wt: 36 27 19 11 8 4 3

Identification and Description of Molecular Mechanisms of On-Target Resistance to Targeted Lung Cancer Therapies with Selpercatinib, Crizotinib, Neratinib, Pralseltinib, Poziotinib and MRTX1133

Ken Westover, Dhiraj Sinha, Michael Peyton, Ralf Kittler, John Minna

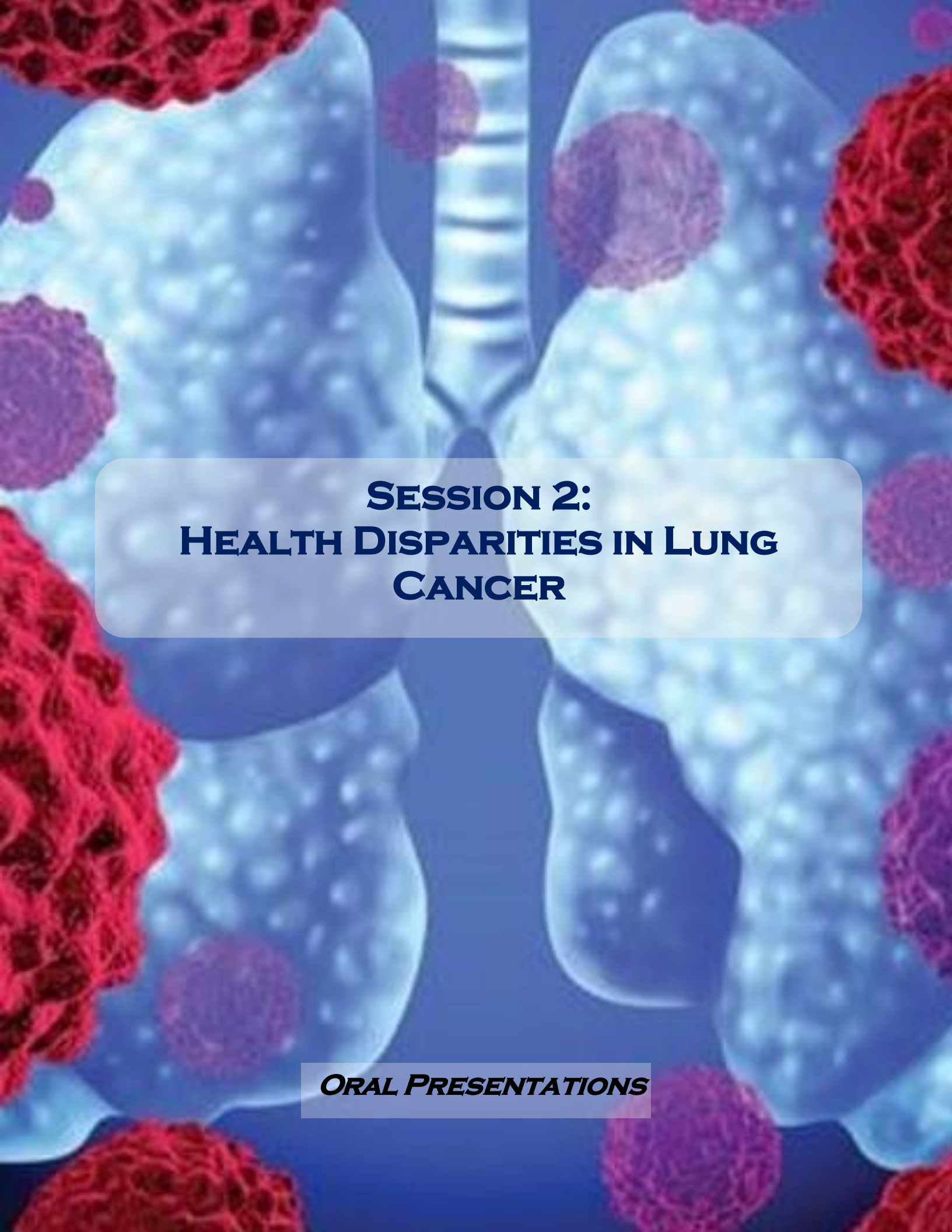
UT Southwestern Medical Center

Presented by: Ken Westover

Targeted therapies have significantly improved medical outcomes for lung cancer patients whose tumors harbor an oncogenic driver mutant protein, such as mutant EGFR, ALK, ROS, or KRAS, that can be selectively therapeutically targeted. Much of this success can be attributed to oncogene-directed small molecule therapies. Currently, over 100 kinase inhibitors and two KRAS inhibitors have been approved worldwide for treating various diseases. However, the development of on-target resistance is common for targeted inhibitors. Resistance mutations not only present a therapeutic challenge but also serve as powerful scientific tools for studying on-target mechanisms of drug action. Therefore, innovative approaches for generating and studying on-target drug resistance mutations are critical for advancing targeted therapy programs.

We have developed and integrated several components for a new approach, including: 1) a panel of tumor cell lines with defined mutations that are very sensitive to specific targeted therapies; 2) a fast, inexpensive assay for identifying specific oncogenic proteins that have acquired an on-target drug-resistant mutation but are still oncogenically active by transfecting cDNAs encoding a wildtype protein into the drug-sensitive tumor cell line and selecting for drug-resistant clones, which are then pooled for sequencing - "LentiMutate" (PMC8448967); and 3) structural and molecular dynamic simulation studies, allowing us to model the impact of each on-target mutation.

Through Lentimutate, we were able to detect on-target mutations conferring lung cancer resistance to clinical-stage inhibitors selpercatinib, crizotinib, neratinib, pralseltinib, poziotinib, and MRTX1133 (~4-5 impactful resistance mutations identified per drug). Subsequent molecular dynamic simulation analyses identified already known drug resistance mutations ("positive controls"), but importantly, also identified and structurally analyzed multiple previously unknown drug resistance conferring mutations. These analyses provide novel functional and structural insights (including allosteric, steric, and water-mediated effects) of the drugs and their targets, which will facilitate analyses of patient specimens and the development of additional targeted therapies.



Session 2: Health Disparities in Lung Cancer

Oral Presentations

Increasing Community Understanding of Precision Oncology and Genetic Literacy in the African American Community: Preliminary Results from an Educational Session

Brittany Dowe, Hayley S. Thompson, Rochelle Chapman, Bertram Marks, Ann G. Schwartz

Karmanos Cancer Institute, Wayne State University

Presented by: Brittany Dowe

Purpose: Immunotherapy with immune checkpoint inhibitors represents an important area of precision oncology. However, little is known about the benefits of precision oncology among African Americans due to a lack of representation in genetic research that informs immunotherapy discoveries. At Karmanos Cancer Institute (KCI) in Detroit, MI, a P20 Cancer Action Council (CAC) was created as part of the Patient Community Engagement Core (PCEC) of the P20 Health Disparities SPORE (P20CA262735) to bridge SPORE activities to the local community. The P20 CAC is composed of 10 African American members recruited from KCI's existing statewide community stakeholder network and a university co-sponsored Faith Community Research Network.

Methods: The P20 CAC collaborated with other community partners to develop and host six educational sessions in November 2022 focused on increasing awareness and knowledge of genetic research and precision oncology. In total, 174 people participated in the sessions, with 106 fully completing the assessments (pre and post-test) including a four-item from the "Genetic Literacy Fast Test (GeneLiFT)" (Milo Rasouly et al., 2021) and an eleven-item measure to assess clinical trials knowledge from the NCI National Outreach Network. The mean age of participants was 46.5 years old, 25.5% identified as male and 89.6% identified as African American.

Results: Results showed that a greater proportion of participants answered the following questions correctly from pre-test to post-test: "Genetic testing may find genetic variants that a person can pass on to his/her children" (pre: 61.3%; post: 76.4%); "Healthy parents can have a child with a genetic condition" (pre: 49.1%; post: 72.6%); "Genetic testing may find genetic mutations that increase a person's chance of developing a genetic condition" (pre: 44.3%; post: 73.6%); "Some people with a genetic mutation may not develop the genetic condition" (pre: 38.7%; post: 56.6%). There was also an increase in clinical trials knowledge.

Conclusion: The current findings of these educational sessions demonstrate the value of creating opportunities for the African American community to learn more about precision oncology. Findings also showcase the importance of working with community stakeholders to disseminate programming in ways that are accessible to local communities.

Access And Adherence to Low-Dose CT-Based Lung Cancer Screening in an Urban, Safety-Net Hospital System

Sofia Yi, Rutu A. Rathod, Vijaya Subbu Natchimuthu, Sheena Bhalla, Jessica Lee, Travis Browning, Joyce O. Adesina, Minh Do, David Balis, Juana Gamarra de Willams, Ellen Kitchell, Noel O. Santini, David H. Johnson, Heidi A. Hamann, Simon J. Craddock Lee, Amy E. Hughes, David E. Gerber

UT Southwestern Medical Center, Parkland Health, University of Arizona, University of Kansas

Presented by: Sofia Yi

Background: Recent modifications to eligibility for low-dose computed tomography (LDCT)-based lung cancer screening have increased the number of qualifying individuals, particularly among racial and ethnic minorities. Because these populations disproportionately live in metropolitan areas, we analyzed the association between location, transportation, and adherence to LDCT within an integrated, urban safety-net healthcare system.

Patients and Methods: Using ESRI's StreetMap Premium, OpenStreetMap, and r5r package in R, we determined the projected travel time between patient home, primary care clinic, and LDCT site for LDCT ordered between March 2017 and December 2022 at Parkland Health in Dallas County, Texas. Travel times were determined for both personal vehicle and public transportation. We used univariable and multilevel multivariable analysis to characterize the association between projected travel time and LDCT completion.

Results: A total of 2,287 patients were included in the analysis, of whom 2,267 (99.1%) lived in Dallas County. Mean age was 63.4 years, and 72.8% were under-represented minorities (URM, 74.1% Black, 22.3% Hispanic). Median distance between patient home and LDCT was 10.72 miles (interquartile range [IQR] 13.50-7.29 = 6.21 miles). Median travel time between patient home and LDCT site was 16.64 minutes (IQR 20.43-13.33 = 7.10 minutes) by personal vehicle and 67 minutes (IQR 80-51 = 29 minutes) by public transportation. Among all LDCT ordered, 1553 (67.9%) were completed. There was a small difference in travel time between completed and not completed LDCT for public transportation (66.6 versus YY 64.6 minutes, P = 0.04) but not for personal vehicle (personal vehicle 17.1 versus 16.9 minutes, P = 0.66). In multivariable analysis, LDCT completion was not associated with projected travel time (personal vehicle HR = 1.001; P = 0.90; public transportation HR = 1.003; P = 0.15).

Conclusion: In a predominantly URM urban population undergoing lung cancer screening, projected travel time was not associated with LDCT completion at a single, centralized LDCT facility after accounting for patient and clinical characteristics.

Using mHealth to Understand Racial Disparities in Side Effects for Lung Cancer Patients Receiving Immunotherapy Treatment

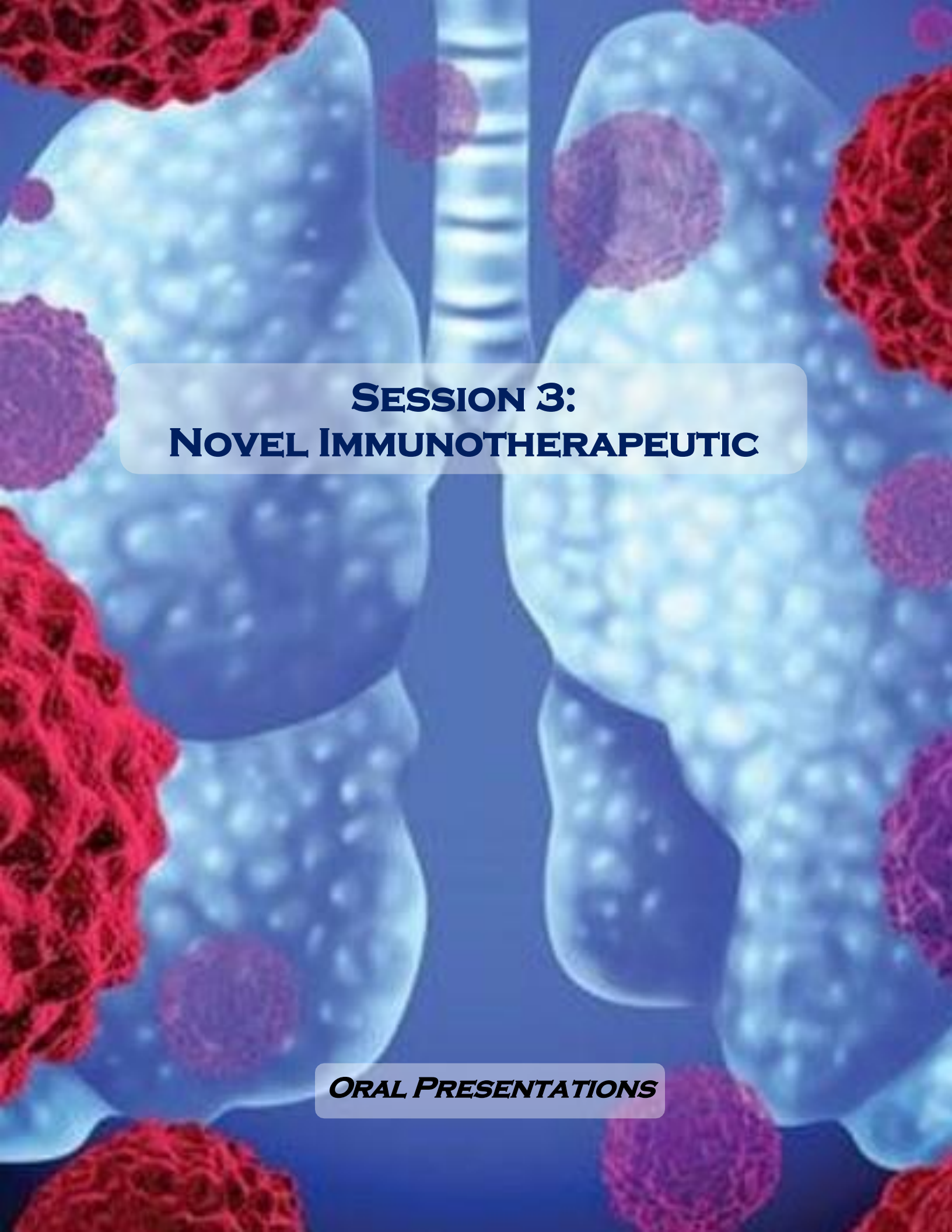
Felicity WK Harper, Jasminder Phalore, Viktoria Carr, Ann Schwartz, Elisabeth Heath, and Hirva Mamdani

Karmanos Cancer Institute, Wayne State University

Presented by: Felicity Harper

Black patients with lung cancer continue to have worse outcomes than white patients, including 5-year overall survival rates of less than 20%. Previous research has demonstrated differences between Black and white patients with respect to symptom burden and treatment of side effects related to chemotherapy. Immune checkpoint inhibitors (ICIs) are a breakthrough in treatment for lung cancer, however, the current standards of care are based largely on samples of white patients. Little is known about potential differences by race with respect to ICI-related side effects due to a lack of inclusion of Black patients in clinical trials leading to FDA approvals. Thus, there is a critical need to explore whether Black and white lung cancer patients experience different patterns of ICI-related side effects.

This study used MyPatientPal, an evidence-based smartphone app developed in collaboration with patient and provider stakeholders (https://www.crosscomm.com/portfolio/mypatientpal), to document differences in patient-reported side effects while on ICI treatment for lung cancer. The app allows patients to easily record the daily frequency and intensity of common CTCAE side effects (e.g., fatigue, nausea, vomiting, diarrhea, cough, shortness of breath, skin rash). A charting feature allows patients to view trends in their symptoms over time (e.g., past week, past month) as well as email reports to their healthcare team. The daily diary data can also be downloaded on the back end for aggregate analysis of trajectories of side effects over time. While participants (N=100; 50% Black, 50% white) continue to be accrued as part of the larger P20 SPORE grant (#P20CA262735), pilot data about the use of MyPatientPal to collect patient-reported outcome data (i.e., usability, feasibility, and implementation challenges) will be presented. The documentation of racial differences in patient-reported side effects is a critical first step towards an equity-focused approach to the treatment of lung cancer patients. Understanding potential differences in response to treatment can better identify those at high risk for side effects and inform the development of interventions tailored to the specific and culturally relevant needs of Black patients, thereby contributing to better patient quality of life and reduced racial disparities in outcomes among lung cancer patients.



Session 3: Novel Immunotherapeutic

Oral Presentations

Immune Response Subtype Classifier for NSCLC

Xiaodong Zhu, Jeff Kwak, Diane Tseng, Isaac Jenkins, Ningxin Ma, Timothy Randolph, McGarry Houghton

Fred Hutchinson Cancer Center

Presented by: McGarry Houghton

Immune checkpoint inhibitor (ICI) therapy has been a tremendous success for non-small cell lung cancer (NSCLC) patients. However, just 20% respond to ICI monotherapy. Responding patients typically feature robust infiltration of $CD8^+$ T cells into the malignant portions of tumor and display the IFNG signature. We previously used flow cytometry, multiplexed-immunohistochemistry (M-IHC), and selected gene expression profiles to identify four fundamental immune response subtypes present in NSCLC. ICI responders fit into the "Active" group, which harbors robust $CD8^+$ cellular content by flow, $CD8^+$ T cell infiltration by M-IHC, and the IFNG signature. The three other immune response subtypes ("Myeloid," "Inert," "Indeterminate") did not display the IFNG signature and did not display T cell infiltration into malignant tumor. Thus, these groups represent three different mechanistic means of immune escape. Our group is developing a novel immune response subtype classifier with which to personalize immune based therapeutic choices for NSCLC patients. We are combining RNA-seq data, three panels of M-IHC data, and clinical features to develop our classifier. We are using a Fred Hutch cohort (N=73) for discovery and an external cohort (N=85) for validation. Using Lasso regression and Random forest, we have identified signatures (100 genes each) that reliably distinguish the immune groups. The performance can be enhanced by the addition of spatial immune data provided by M-IHC. The strongest features in this regard are: $CD8^+$ cellular infiltration in malignant tumor ("Active"); $CD66b^+$ neutrophil content within stroma ("Myeloid"); IL23 barrier signal ("Indeterminate"); and the lack of any of those distinguishing features ("Inert"). Ultimately, we plan to develop a limited Nanostring panel (20 genes) and one 7-color M-IHC panel to classify immune responses in a clinically feasible assay.

Targeting the STING Deficiency to Augment Immune Responsiveness in LKB1-Mutant Lung Cancer

Qiankun Niu, Changfa Shu, Yuhong Du, Andrey A. Ivanov, Wei Zhou, Madhav V Dhodapkar, Suresh Ramalingam, Xiulei Mo, Haian Fu

Emory University

Presented by: Qiankun Niu

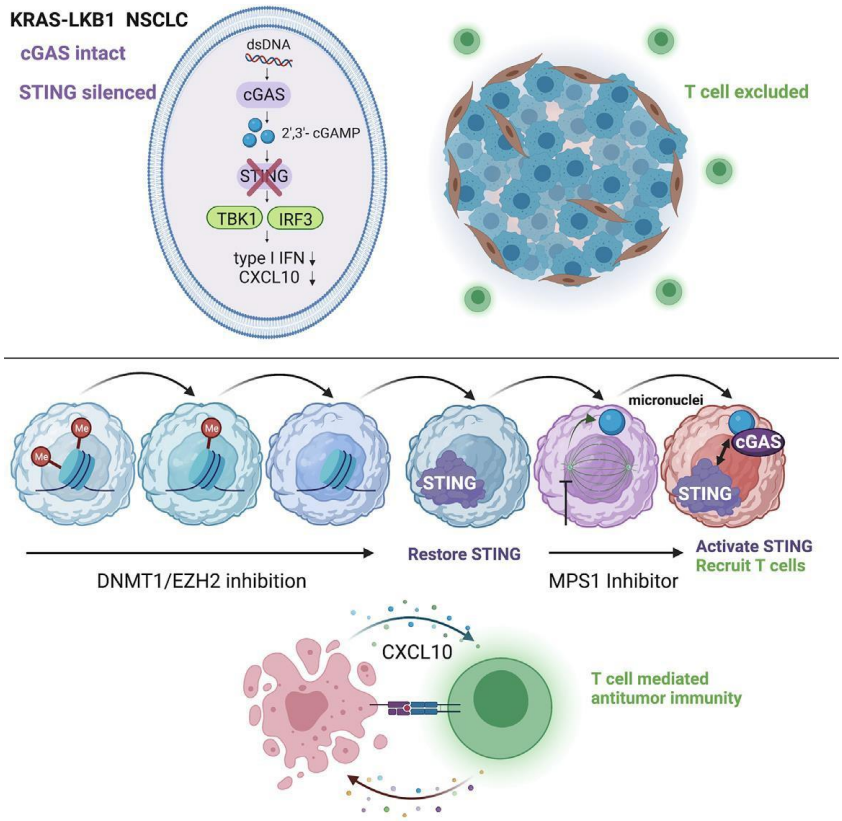
Harnessing the immune system's power to treat cancer has become a core clinical approach. However, genomic alterations can cause tumor cells to escape immune surveillance, leading to therapeutic failure. Patients with LKB1-mutant (mut) lung adenocarcinoma are a major subgroup with primary unresponsiveness and an "immune-cold" phenotype. Such a cold immune phenotype is associated with LKB1-mut tumor intrinsic expression deficiency of STimulator of INterferon Gene (STING), which regulates the tumor-immunity cycle. Thus, strategies to treat the STING deficiency may augment immune responsiveness in LKB1-mut tumors. We have reported that birinapant, a small molecule IAP inhibitor, can restore STING expression, activate STING signaling, and enhance immune cell killing of LKB1-mut cells. These results have led to our central hypothesis that STING deficiency is a tumor-intrinsic vulnerability created by LKB1-mut, and restoring STING using small molecules may reverse the LKB1-mut tumor "immune-cold" phenotype. To rapidly identify STING regulators, we have developed a STING-HiBiT reporter cell line to monitor endogenous STING expression by leveraging the cutting-edge CRISPR/Cas9-HiBiT tagging technology. We utilized this reporter cell line to screen a bioactive compound library and identified hits with cellular activities in inducing the STING expression. The hit compounds may inform regulatory pathways for STING expression. Identification of STING activators may lead to a promising therapeutic approach to restore the responsiveness of "immunologically-cold" LKB1-mutant tumors to immune checkpoint inhibitors or STING-directed therapies.

MPS1 inhibition primes immunogenicity of KRAS mutant lung cancer

Dana-Farber Cancer Institute

Presented by: David Barbie, MD

In prior work, we discovered that KRAS-LKB1 (KL) mutant lung cancers escape cytosolic DNA sensing by epigenetically silencing STING, to avoid mtDNA that accumulates due to their intrinsic mitochondrial dysfunction. Elimination of this viral sensing pathway by KL cancer cells directly contributes to T cell exclusion and resistance to programmed cell death (ligand) 1 (PD-[L]1) blockade. For the most part, KL cancer cells still retain intact upstream expression of cGAS, the enzyme that directly recognizes dsDNA in the cytoplasm. However, they minimize intracellular accumulation of 2'3'-cyclic GMP-AMP (2'3'-cGAMP) to further avoid downstream STING and STAT1 activation. We therefore conducted an unbiased screen using a panel of DNA damaging agents in a pulse-dosed fashion, to identify whether unique drugs could engage cGAS and amplify 2'3'-cGAMP levels in KL cells. Inhibition of MPS1 (monopolar spindle kinase 1), also known as TTK, was an outlier, restoring 2'3'-cGAMP and boosting baseline low levels of STING signaling in a cGAS dependent fashion. MPS1 regulates spindle attachment, and its transient perturbation loads cells with abundant micronuclei when cells re-enter G1 phase of the cell cycle after drug washout. Since micronuclei lack a nuclear lamina, they become decorated by cGAS and are potent sources of its activity. Moreover, epigenetic de-repression of STING using DNMT1 and/or EZH2 inhibitors, followed by pulse MPS1 inhibition (MPS1i), resulted in marked re-activation of STING signaling. A single course of decitabine treatment followed by pulse MPS1i therapy restored T cell infiltration in vivo, enhanced anti-PD-1 efficacy, and resulted in a durable response without evidence of significant toxicity. Since MPS1/TTK1 inhibitors have entered the clinic, these findings reveal their substantial translational potential for KL lung cancer, especially in combination with agents that restore STING expression.



Anti-Tumor Activity of a Novel Lair1 Antagonist with Anti-PD1 To Treat Collagen-Rich Solid Tumors

B. Leticia Rodriguez, Laura Gibson, Jared Fradette, Jiawei Huang, Sisi He, Czrina Cortez, Betty Li, Carmence Ho, Amir Ashique, Kalyani Mondal, Vicky Lin,
Julie M. Roda, Hui Tian, Yan Wang, Bin Fan, Igor Mikaelian, James Sissons, Jonathan Sitrin, Daniel D. Kaplan, Lee B. Rivera, Don L. Gibbons

UT MD Anderson Cancer Center, NGM Biopharmaceuticals Inc.

Presented by: B. Leticia Rodriguez

The extracellular matrix (ECM) consists of a variety of secreted proteins, of which collagen is the most prevalent. Importantly, ECM abundance in tumors has been linked to poor prognosis and resistance to systemic therapy. We reported that the immunosuppressive KP murine lung cancer model resistant to immunotherapy was found to have increased collagen deposition and the collagen-binding inhibitory receptor, leukocyte associated immunoglobulin like receptor 1 (LAIR1). We hypothesized that LAIR1 and collagen interact to suppress therapeutic response. LAIR1 is highly expressed on intratumoral myeloid cells in both primary human tumors and mouse models of cancer. ScRNA seq analysis of melanoma tumors from patients treated with checkpoint inhibitors revealed LAIR1 expression was greater on macrophages from non-responders. In addition, LAIR1 immune cells were distributed in collagen-rich tumor stroma in HNSCC, PDAC, LUAD, and RCC. Here, we propose using a novel anti-LAIR1 antagonist, NGM438 to augment anti-PD1 therapy. Inhibition of collagen/LAIR1 engagement with NGM438, a novel human LAIR1 antagonist monoclonal antibody, enhanced anti-PD-1 response in a mixed lymphocyte reaction. An NGM438 surrogate anti-mouse antibody sensitized refractory KP mouse syngeneic tumors to anti-PD-1 therapy and resulted in increased intratumoral CD8+ T cells and an inflammatory gene expression. The Lair-1/PD-1 blockade treatment group had significantly smaller tumor burden and lower number of metastatic lung nodules. Our data indicates that blockade of the LAIR1/collagen interaction may represent a promising approach to unlock response in patients that fail to respond to existing checkpoint inhibitor therapy.

NC318, an Anti-Siglec-15 Humanized Monoclonal Antibody, Alone and in Combination with Pembrolizumab in Immunotherapy Pretreated Non Small Cell Lung Cancer

Scott Gettinger, Sarah B. Goldberg, Anne C. Chiang, Frederick H. Wilson, So Yeon Kim, Elin Rowen, Heather Gerrish, Emily Duffield, Marianne Davies, Vanna Dest, Roliya Jackson, Jennifer Pope, Han Mynt, Solomon Langermann, Wei Cheng, David L. Rimm, Lieping Chen, Roy S. Herbst

Yale University and NextCure, Inc.

Presented by: Scott Gettinger

NC318 is a humanized IgG1 mAb against the immune checkpoint siglec-15 that blocks interactions between siglec-15 and its receptor on myeloid and T cells within the tumor microenvironment, relieving immune inhibitory signaling.

We conducted a phase II study of NC318 (NCT04699123) given alone (arm 1a) or in combination with pembrolizumab (P) (arm 1b) in pts with progression of advanced PDL1 unselected NSCLC after treatment with PD1 axis inhibitor therapy. NC318 was administered IV weekly as monotherapy (800 mg) or every 2 weeks (400 mg) in combination with P (200 mg IV every 3 weeks), and continued for up to 70 weeks. The primary endpoints were safety and objective response rate (ORR) using RECIST v1.1 in each arm. Secondary endpoints included progression free survival (PFS) and overall survival (OS).

To date, 7 pts have been treated in arm 1a and 18 patients in arm 1b. Best response in arm 1a was partial response (PR) in 1 pt, confirmed stable disease (SD) in 2 pts (lasting 4 and 4.7 months respectively) and progressive disease (PD) in 4 pts. Of 17 response evaluable pts treated on arm 1b, ORR was 6%; best response included, 1 pt with PR, 9 pts with PD and 7 pts with SD, including 4 confirmed SD (decrease in target lesions (TL) of 26%, 27%, 17% and 6% respectively) with 3 ongoing SD at 15.6 months, 8.5 months and 3.6 months, respectively. Additionally, 1 pt with PD by RECIST v1.1 but pseudo-progression continued therapy with PR by immune related response criteria (irRC) and decrease in TL of 58%/ 92% per RECIST v1.1/ irRC. Two pts in arm 1a experienced grade 3 (G3)+ treatment related adverse events (TRAEs), including 1 with G3 transverse myelitis and 1 with G3 NC318 infusion reaction (rxn). 3 pts in cohort 1b experienced G3+ TRAEs (1 G3 pneumonitis and 2 G3 NC318 infusion rxn). With median follow up of 15.4 months, median PFS/ OS in arm 1b were 1.7/ 6.1 months.

NC318 alone and in combination with pembrolizumab is well tolerated and has activity in pts previously treated with PD1 axis inhibitor therapy.

Genomic and Transcriptomic Analysis of Checkpoint Blockade Response in Advanced Non-Small Cell Lung Cancer (NSCLC)

Justin Gainor, Arvind Ravi, Matthew Hellmann, Monica Arniella, Mark Holton, Samuel Freeman, Vivek Naranbhai, Chip Stewart, Ignaty Leschniner, Jaegil Kim, Yo Akiyama, Aaron Griffin, Natalie Vokes, Mustafa Sakhi, Vashine Kamesan, Hira Rizvi, Biagio Ricciuti, Patrick Forde, Valsamo Anagnostou, Jonathan Riess, Don Gibbons, Nathan Pennell, Vamsidhar Velcheti, Subba Digumarthy, Mari Mino-Kenudson, Andrea Califano, John Heymach, Roy Herbst, Julie Brahmer, Kurt Schalper, Victor Velculescu, Brian Henick, Naiyer Rizvi, Pasi Janne, Mark Awad, Andrew Chow, Benjamin Greenbaum, Marta Luksza, Alice Shaw, Jedd Wolchok, Nir Hacohen, Gad Getz

Massachusetts General Hospital, Dana Farber Cancer Institute, AstraZeneca, Broad Institute, GlaxoSmithKline, Columbia University, UT MD Anderson Cancer Center, Johns Hopkins School of Medicine, UC Davis, Cleveland Clinic, New York University, Yale University, Memorial Sloan Kettering Cancer Institute, Icahn School of Medicine at Mount Sinai, Weill Cornell

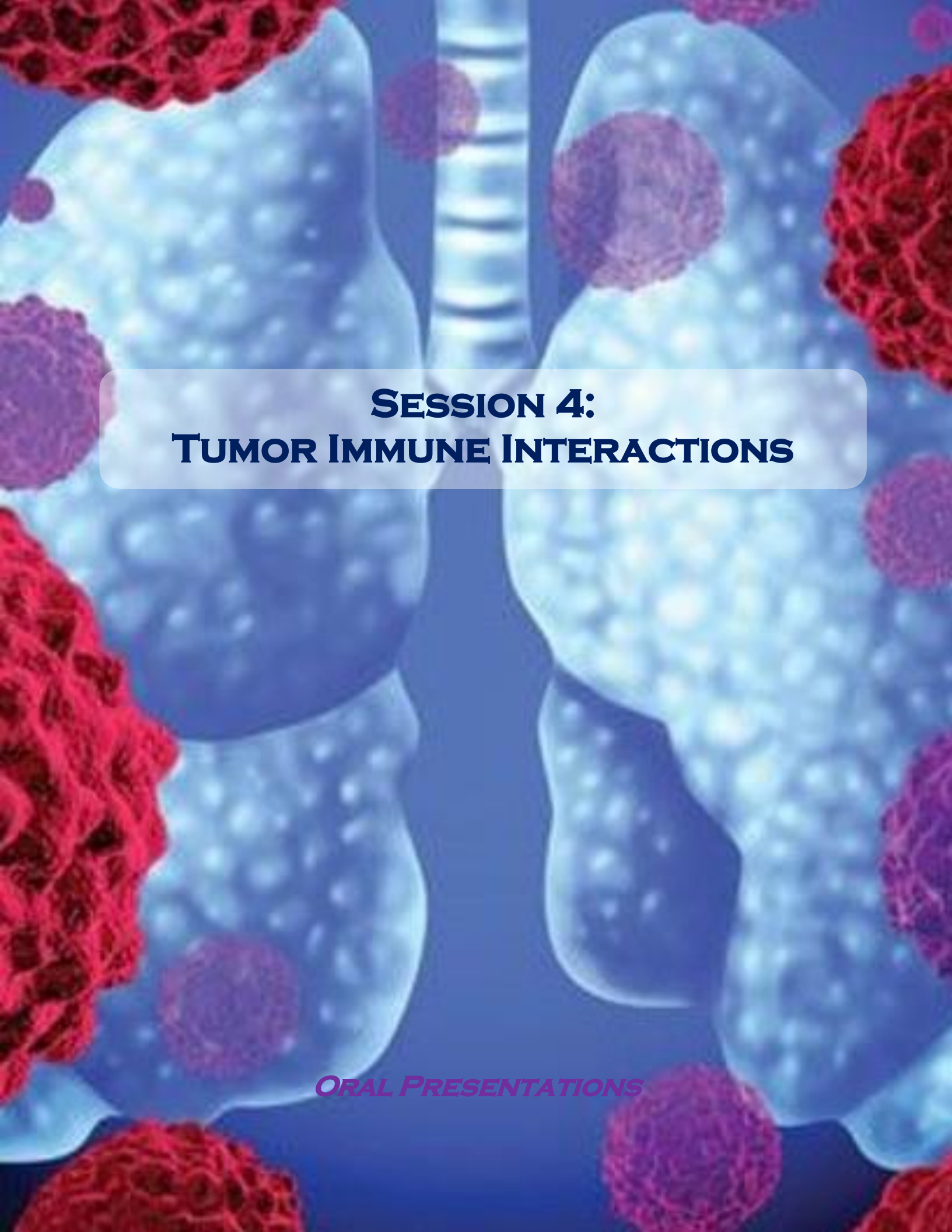
Presented by: Justin Gainor

Background: Immune checkpoint inhibitors (ICIs) are cornerstones of therapy for advanced NSCLC. Despite dramatic and sometimes durable responses to therapy, most patients do not respond to therapy (intrinsic resistance) or subsequently progress after initial clinical benefit (acquired resistance). Currently, insights into the molecular determinants of response and mechanisms of resistance to ICIs in NSCLC are lacking.

Methods: To investigate clinical and molecular features of response to ICIs, we obtained baseline tissue specimens from patients with advanced NSCLC treated with PD-1 pathway inhibitors from nine institutions (Stand Up To Cancer – Mark Foundation [SU2C-MARK] cohort). Samples underwent whole-exome sequencing (WES) and/or whole transcriptome sequencing (RNA-seq), and findings were correlated with clinical response outcomes. A subset of patients had paired pre- and post-ICI biopsies, which were analyzed using the same bioinformatics pipelines.

Results: Between 2016 and 2022, we obtained baseline tissue specimens from 393 patients treated with anti-PD-(L)1 therapy. Most patients received single-agent anti-PD-(L)1 (81%); 17% and 1% received PD-1 blockade in combination with anti-CTLA4 or chemotherapy, respectively. After stringent quality control, a total of 309 WES and 152 RNA-seq specimens were included in the analysis. We identified a number of associations between molecular features and outcome, including: 1) favorable (e.g., *ATM* altered), and unfavorable (e.g., *TERT* amplified) genomic subgroups, 2) a prominent association between expression of inducible components of the immunoproteasome and response, and 3) a de-differentiated tumor-intrinsic subtype with enhanced response to checkpoint blockade. Within the SU2C-MARK cohort, we also identified 41 patients with post-ICI biopsies. Among patients with acquired resistance to ICIs, there was no significant difference in tumor mutation burden in pre- versus post-ICI biopsies. One patient with a complete response ultimately developed a nonsense mutation in B2M at relapse, and one patient with a partial response acquired a nonsense mutation in JAK1 at the time of disease progression.

Conclusions: Findings from this cohort demonstrate the complexity of biological determinants underlying immunotherapy outcomes and reinforce the discovery potential of integrative analysis within large, well-curated, cancer specific cohorts.



Session 4:
Tumor Immune Interactions

Oral Presentations

Sex-Based Differences In The Formation Of LKB1-Mutant Lung Adenocarcinoma

Yijian Fan, Rui Jin, Lenore Monterroza, Chunzi Huang, Xiulei Mo, Yuan Liu, Frank Schneider, Melissa Gilbert-Ross, Adam I. Marcus, Haian Fu, Suresh Ramalingam, Stacey A. Smith, Rabindra Tirouvanziam, Wei Zhou

Emory University

Presented by: Wei Zhou

If the cancer risk in a population follows a normal distribution pattern, current cancer studies mainly focus on individuals who are susceptible to cancer development. This assumption also suggests that there may be individuals at the other end of the spectrum who are resistant to cancer. In the formation of smoking-induced lung cancer, cancer-resistant individuals may have effective biological mechanisms to prevent cancer development, such as cytochrome P450 detoxification, DNA repair, immune system responses to eliminate tumor cells, etc. Our study specifically focuses on the immune system of these cancer-resistant individuals.

The tumor suppressor LKB1 is one of the most frequently mutated genes in lung adenocarcinoma (LUAD), and its mutations are typically found in smoking-related LUAD. Interestingly, even though women are more susceptible to developing DNA damage from smoking in their lungs, the frequency of LKB1-mutant LUAD is much lower in women than in men according to several large-scale studies. The reason for this sex-based difference is not clear, largely due to the lack of appropriate experimental models.

We previously developed a genetically engineered mouse model (GEMM) in FVB/N background, which can form $Kras^{G12D}/LKB1^{null}$ LUAD with similar tumor characteristics and microenvironments to human LUAD. A meta-analysis of our GEMM model revealed the LUAD formation at 97% in males versus 58% in females. We also established lung cancer cell lines from our GEMM, and the establishment of lung metastases through tail-vein injection in syngeneic animals was 100% in males and only ~36% in females. The adoptive transfer of pooled lymphocytes from naive female syngeneic hosts was also capable of attenuating lung metastasis in males, suggesting a potential therapeutic option.

Interestingly, the sex-based difference in lung metastasis formation was also found in nude and SCID mice, indicating that this is not a strain-specific phenomenon. No sex-based difference was observed in lung metastasis formation in NSG mice, suggesting that the innate immune cells are the key players. We are currently identifying the specific immune cells and underlying molecular mechanisms, and our long-term goal is to develop a novel therapeutic approach against LKB1-mutant LUAD based on this finding.

Spatial Mapping of the CD39/CD73 Adenosine Pathway as a Candidate Immunotherapy Target in Non-Small Cell Lung Cancer

Kerryan Ashley, Rakesh Kumar, Sarah Goldberg, Kurt Schalper

Yale University

Presented by: Kerryan Ashley

Introduction: The ectonucleotidases CD39 and CD73 act sequentially to convert ATP into highly immunosuppressive adenosine that can accumulate in the extracellular milieu and suppress NK/T-cell anti-tumor responses. Blockade of CD73 in combination with other immunostimulatory antibodies can induce anti-tumor responses in subset of patients with locally advanced non-small cell lung cancer (NSCLC). The levels, spatial distribution, clinicopathologic associations and biomarker potential of the CD39/CD73 pathway in NSCLC remains poorly understood.

Methods: Using multiplexed quantitative immunofluorescence (mQIF), we simultaneously measured the levels of CD39, CD73 protein, CD8+ T-cells and cytokeratin (CK)-expressing cancer-cells in four retrospective NSCLC cohorts represented in tissue microarrays. The mQIF analysis included compartment-based measurements based on fluorescence co-localization as well as single-cell segmentation and spatial analysis. The first three cohorts included baseline tumor samples from patients with stages I-IV NSCLC treated with non-immunotherapy regimens (Cohort #1, n=179; Cohort #2, n=172; Cohort #3, n=267). The fourth cohort (Cohort #4, n=23) included paired biopsies before and after neoadjuvant chemotherapy with or without radiotherapy. Associations between the markers with clinicopathologic variables and outcomes were studied.

Results: 90% of NSCLCs showed detectable concurrent expression of both CD39 and CD73, but the levels were highly variable across cases. CD39+ cell density was protein levels were higher in nonmalignant CK- stromal cell areas and CD73+ cell density was higher in CK+ cancer cell areas. The levels of the markers were positively associated across NSCLCs. Elevated levels of CD39 and CD73 were consistently associated with higher local CD8+ T-cell infiltration and adenocarcinoma histology across the cohorts. Levels of CD39 were associated with better 5-year overall survival. CD73+ cell density is reduced after neoadjuvant treatment. Spatially, the number of CD8+ T cells in contact with CD73 CK+ cancer-cells is reduced after neoadjuvant treatment.

Conclusion: The CD39/CD73 pathway is expressed in a subset of NSCLCs with a distinct tumor/stromal cell distribution. This pathway is associated with local adaptive anti-tumor responses and adenocarcinoma histology. Neoadjuvant therapy reduces the number of CD73+ expressing cells in the tumor microenvironment and alters their spatial relationship with effector T-cells.

Development of Immunogenic Autochthonous Mouse Lung Cancer Models

Mingrui Zhu, Jiwoong Kim, Buse Eglenen-Polat, Matthew E. Bender, John D Minna, Diego H. Castrillon, Lin Xu, and Esra Akbay

UT Southwestern

Presented by: Esra Akbay

Immune checkpoint blockade has revolutionized cancer treatment especially for non-small cell lung cancer. Biomarkers for therapeutic response to immune checkpoint blockade is unknown. Tumor intrinsic factors such as tumor mutational burden was associated with better responses to ICB in lung cancer. However not all tumors with high TMB respond well to ICB. Role of tumor mutational burden in shaping the tumor immunity and response to immune checkpoint blockade has not been mechanistically addressed in clinically relevant autochthonous lung cancer models. Because mouse models lack antigenic diversity, we induced mutations in lung cancer models by utilizing Polymerase epsilon catalytic subunit mutant mice (Pole P286R). This is an ultra-mutator variant of DNA polymerase-E (POLE) (P286R) detected in human tumors and causes elevation of TMB. We crossed this allele into the well-characterized Kras G12D;p53 L/L alleles, the two most commonly mutated genes in NSCLC. Addition of Pole significantly increased the TMB of the KP model. However, increasing TMB alone was not sufficient to induce immune responses with immune checkpoint blockade. This was in part due to mutational heterogeneity and in part due to tumor microenvironment as syngeneic models derived from these GEMMs were moderately sensitive to ICB. We also addressed the role of clonal heterogeneity in anti-tumor immunity using these models. In summary, we developed novel lung cancer GEMMs and syngeneic models with high TMB for studying immune resistance mechanisms.

Characterization of T And B Cell Antigen Specific-Engineered NSCLC Tumors to Study the Function of B Cells and TLS in Murine Models of Neoadjuvant Immunotherapy

Armando J. Ruiz-Justiz, Xin Sun, Mona Yazdani, Lili Chen, Michael Wang, John Le, Haiping Guo, Can Cui, Kelli Cannolly, Aya Tal, Wei Hu MD, Nikhil Joshi, Tina Cascone

University of Puerto Rico, UT MD Anderson Cancer Center, Yale University

Presented by: Xin Sun

Introduction: Immune checkpoint inhibitors (ICIs) have revolutionized treatment for non-small cell lung cancer (NSCLC). However, only 20-40% of patients with NSCLC benefit from these agents, highlighting the need to advance our understanding of the immune mechanisms of therapeutic resistance. Evidence suggests a correlation between tertiary lymphoid structures (TLS) in tumors and responsiveness to ICIs; however, how T and B cell function within TLS to impact ICI-based therapy in NSCLC remains unclear. To start addressing this knowledge gap, we employed the established "NINJA" and "HELLO" systems to generate neoantigen-expressing spontaneously metastatic murine models of NSCLC to study T/B cell infiltration and function, and TLS formation and maturation.

Methods: $5x10^5$ 344SQ-LentiT, -HELLO and -NINJA cells were injected subcutaneously in 129Sv mice. Tumor growth was measured until day 24 when mice were euthanized and tissues harvested for immune studies. Flow cytometry was performed on tumors and draining lymph nodes to determine T and B cell phenotypes. GraphPad Prism v.9 was used to graph the results. Statistical differences were analyzed with One-way ANOVA test.

Results: NINJA tumor growth was significantly reduced compared with that of HELLO and control tumors. NINJA tumors showed increased T cell infiltration and higher frequencies of antigen-specific T cells and CD4 TFH cells. HELLO tumors exhibited a higher number of Tregs, which may have contributed to the reduced anti-tumor activity observed in this model.

Conclusions: HELLO- and NINJA-engineered 334SQ tumors allow us to study the T/B cell function and GC formation in NSCLC for future therapeutic studies.

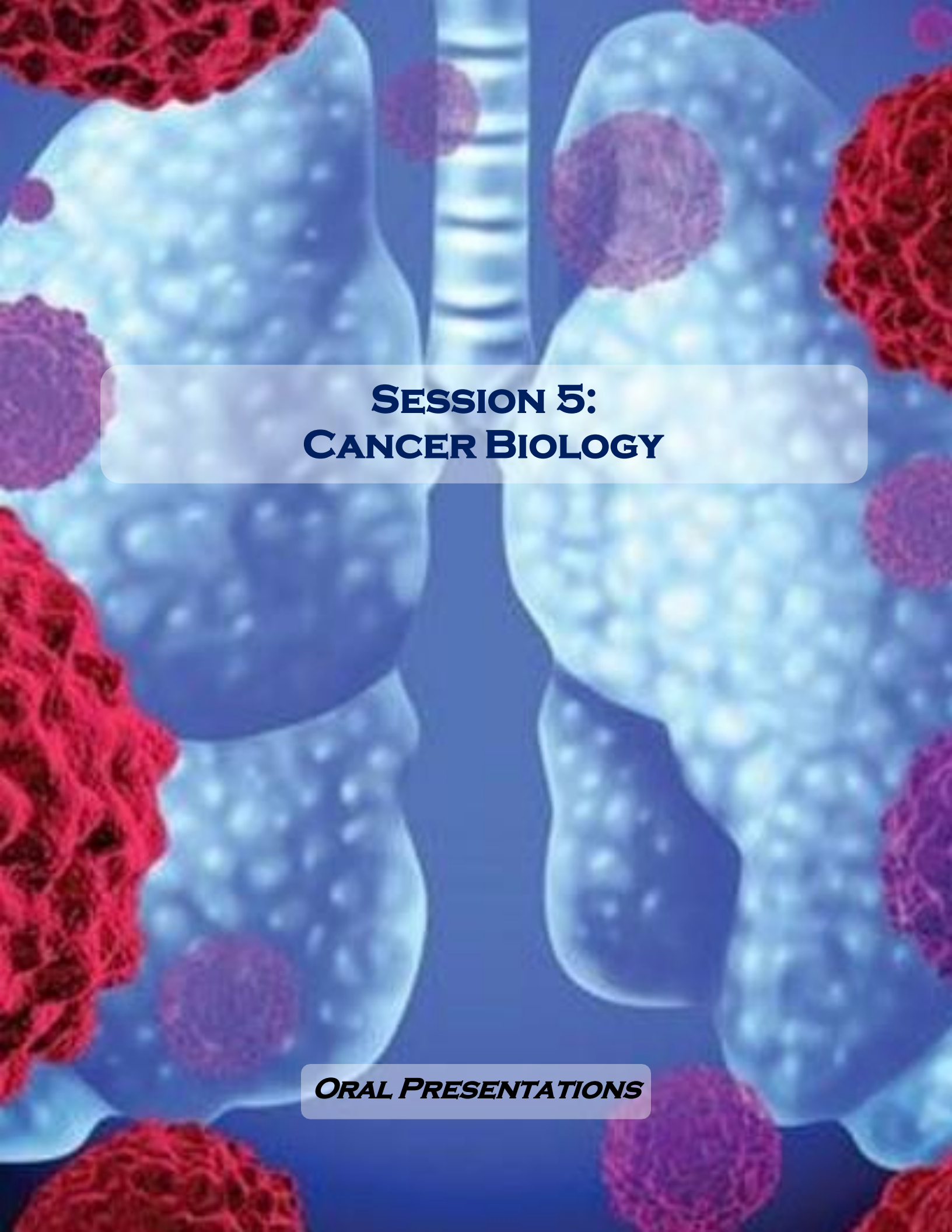
Evaluation of The Tumor Immune Microenvironment as a Contributor to Lung Cancer Disparities

Heather Gibson, Christine Lusk, Valerie Ratliff, Julie Boerner, Hirva Mamdani, Ann G. Schwartz

Karmanos Cancer Institute, Wayne State University

Presented: Heather Gibson

Racial disparities in lung cancer incidence and survival are evident, however it remains unclear whether this also extends to tumor immunotherapy response. Immune checkpoint inhibitor (ICI) clinical trials have had poor representation from African American (AA) patients (<4%), necessitating further investigation. Decades of autoimmunity and inflammation research have identified host genomic associations and racial disparities in the onset and/or severity of several immune-mediated diseases, suggesting anti-tumor immune activity may also differ by race. Biomarkers that predict response to ICI remain elusive, with PD-L1 and tumor mutational burden showing moderate, incomplete capacity to predict ICI outcomes. Our preliminary studies identified a subset of immune genes that associate with PD-L1 expression, where interferon (IFN) signaling is a common driver, in tumors from both NSCLC patients and in animal models. Interestingly, we find expression of these PD-L1-associated genes differs by race. Additionally, relative to tumors from white patients, tumors from AA patients were more likely to express components of antibody heavy and light chains, despite similar expression of general B cell markers, which may indicate a functional difference in intratumoral B cells from these populations. The presence of B cell-rich tertiary lymphoid structures (TLS) have recently been identified in lung and other cancers, and TLS formation has been linked to positive ICI response in several studies. In a cohort of 286 lung cancer patients (134 AA, 152 white), we find intratumoral TLS formation does not significantly differ by race. Bulk transcriptomic analysis identifies immune gene transcripts that associate with TLS presence, which includes several previously published TLS signature genes, as well as expression of antibody heavy and light chain genes. Interestingly, while these transcripts are expressed comparably in TLS-positive tumors from both AA and white patients, their expression is significantly higher in TLS-negative tumors from AA versus white patients. Identification of differential immune mechanisms has the potential to guide treatment decision-making and identify novel therapeutic targets for reduced disparities in lung cancer outcomes.



Session 5:
Cancer Biology

Oral Presentations

Replication Stress in BRG1 Deficient Lung Adenocarcinoma as a Vulnerability for Intervention

Maria Fernanda Trovero, Margherita Paschini, Carla Kim

Boston Children's Hospital

Presented by: Maria Fernanda Trovero

SMARCA4 encodes BRG1, a key ATPase subunit of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex. *SMARCA4* mutations are present in 9-11% of patients with non-small cell lung cancer (NSCLC), and are often found with other mutations (e.g., *KEAP1* and *STK11* mutations) that decrease sensitivity to traditional therapeutic approaches (immunotherapy, chemotherapy) and are associated with poor prognosis. Recent work from the Kim lab showed that BRG1 deficiency increased DNA replicative stress and enhanced sensitivity to ATR pathway inhibition in preclinical lung cancer models. The overall goal of our project is to identify potential therapeutic targets for *SMARCA4*-mutant NSCLC that could improve prognosis for patients with this aggressive subtype, using replication stress as a vulnerability. The aims of our proposal are: (1) characterize hallmarks of replication stress and assess immune system activation in NSCLC clinical specimens with *SMARCA4* mutations; (2) evaluate sensitivity of *SMARCA4*-mutant NSCLCs to therapies targeting replication stress defects; and (3) perform pre-clinical studies to assess the anti-tumor effects of ATR inhibition in combination with anti-PD1/PD-L1 immunotherapy. Here, we immunodetected pATR, a marker of DNA replication stress, in patient-derived xenograft (PDX) cells: MGH-1070 (BRG1 mutant, KRASG12C) and MGH-1088 (BRG1 WT, KRASG12C). We found that pATR expression is increased in the BRG1 mutant PDX. Furthermore, we detected activation of the ATR pathway in human NSCLC BRG1 KO isogenic cells by observation of phosphorylated CHK1. We also evaluated the effect of the ATR inhibitor VX970 in the same PDX cells and observed that the IC_{50} of VX970 is higher for BRG1 WT cells than for BRG1 deficient ones. We are currently optimizing multiplex immunodetection of these and other DNA replication stress markers in patient samples from DFCI and MGH. We are also performing marker analysis in genetically engineered mouse models in preparation for pre-clinical therape utic studies.

6-thio-dG Represents a New Systemic Chemotherapy Approach for Lung Cancer with Tumor Cell Selectivity Including Cancers Resistant to Current Therapies Facilitating Immune Checkpoint Blockade Anti-Tumor Immune Responses

Ilgen Mender, Silvia Siteni, John Minna, Esra Akbay, Jerry Shay

UT Southwestern

Presented by: Jerry Shay

6-thio-2′-deoxyguanosine (6-thio-dG), different from 6-thioguanine (6-TG) used to treat hematologic malignancies, enters cells and is converted to 6-thio-2'-deoxyguanosine-5'- triphosphate, which is then preferentially incorporated into the TTAGGG telomere repeats of telomerase-positive cells but not normal telomerase-silent cells. Since >95% of all lung cancers express telomerase – 6-thio-dG is incorporated into the telomeres of essentially every lung cancer cell but very few normal cells. Incorporation of 6-thio-dG results in DNA double stranded break marker □-H2AX co-localizing with a shelterin protein, TRF2 which can be visualized as telomere damage–induced foci (TIF) and rapid cell death. Since telomeres are only 1/6000th of the human genome, 6-thio-dG induced telomeric damage is significant. Even short-term 6-thio-dG treatment results in chromosome end fusions leading to an increase in micronuclei and cytosolic dsDNA, which in turn elicits immune signaling. Our studies of patient derived preclinical models showed almost all non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) lines, xenografts, as well as syngeneic mouse tumors and genetically engineered mouse models (GEMMs) are sensitive to 6-thio-dG at *in vivo* pharmacologically achievable concentrations. This 6-thio-dG sensitivity also occurs in lung cancers resistant to platin-doublet chemotherapy and various targeted therapies. Of great interest, given tumor immune signaling stimulated by 6-thio-dG induced TIFs led us to demonstrate in immune competent syngeneic mouse models, that sequential treatment of 6-thio-dG followed by immune checkpoint blockade (ICB) therapy results in dramatic innate and adaptive anti-tumor immune responses including cures. Importantly, this 6-thio-dG + ICB therapy results in long-term immunological memory since mice cured of their first tumor when later rechallenged with the same tumor do not show tumor growth. Our preclinical studies have led to a NSCLC Phase 2 clinical trial using sequential treatment with 6-thio-dG followed by ICB treatment with cemiplimab (Libtayo). We conclude that all lung cancer histologies, including those resistant to available chemotherapy and targeted therapy, remain sensitive to 6-thio-dG treatment. In addition, expression of telomerase provides a therapeutic window and that 6-thio-dG induced TIF lead to tumor and host changes facilitating responses to ICB therapy.

Brain Metastatic Outgrowth and Osimertinib Resistance Are Potentiated By RhoA in EGFR-Mutant Lung Cancer

Sally J Adua, Anna Arnal-Estapé, Minghui Zhao, Bowen Qi, Zongzhi Z Liu, Carolyn Kravitz, Heather Hulme, Nicole Strittmatter, Francesc López-Giráldez, Sampada Chande, Alexandra E Albert, Mary-Ann Melnick, Bomiao Hu, Katerina Politi, Veronica Chiang, Nicola Colclough, Richard J A Goodwin, Darren Cross, Paul Smith, Don X Nguyen

Yale University School of Medicine, AstraZeneca (UK)

Presented by: Anna Arnal-Estapé

The brain is a major sanctuary site for metastatic cancer cells that evade systemic therapies. Through pre-clinical pharmacological, biological, and molecular studies, we characterize the functional link between drug resistance and central nervous system (CNS) relapse in Epidermal Growth Factor Receptor- (EGFR-) mutant non-small cell lung cancer, which can progress in the brain when treated with the CNS-penetrant EGFR inhibitor osimertinib. Despite widespread osimertinib distribution in vivo, the brain microvascular tumor microenvironment (TME) is associated with the persistence of malignant cell sub-populations, which are poised to proliferate in the brain as osimertinib-resistant lesions over time. Cellular and molecular features of this poised state are regulated through a Ras homolog family member A (RhoA) and Serum Responsive Factor (SRF) gene expression program. RhoA potentiates the outgrowth of disseminated tumor cells on osimertinib treatment, preferentially in response to extracellular laminin and in the brain. Thus, we identify pre-existing and adaptive features of metastatic and drug-resistant cancer cells, which are enhanced by RhoA/SRF signaling and the brain TME during the evolution of osimertinib-resistant disease.

Cooperation Between PRMT1 and PRMT6 Drives Lung Cancer Health Disparities Among Black/African American Men

Pei-Ying Wu, Michelle Van Scoyk, Stephanie A. McHale, Chu-Fang Chou, Kamran Farouq, Bin Hu, Vita Kraskauskiene, Jennifer Koblinski, Vignesh Vuda- tha, Dongyu Zhang, Jose G. Trevino, Chanita Hughes-Halbert, Victoria L. Seewaldt, Ching-Yi Chen, and Robert A. Winn

Virginia Commonwealth University, University of Southern California, City of Hope Comprehensive Cancer Center

Presented by: Chang-Yi Chen

Non-Hispanic Black/African American (Black/AA) men in the United States have dis- proportionally higher incidence and mortality rates of lung cancer compared to non- Hispanic White (NHW) men. Molecular determinants including biological and genetic factors are believed to play critical roles in driving the disparities. Nevertheless, recent large-scale genomic studies fail to identify significant somatic differences in lung can- cer driver genes contributing to the observed disparities between Black/AA and NHW groups highlighting that epigenetic mechanisms may contribute to the observations. Arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) has gained considerable interest as many cancer types display elevated expression of PRMTs correlating with poorer prognosis. Here, we observed a significant difference in the expression levels of PRMT6 between Black/AA men and NHW men with lung cancer that may contribute to cancer health disparities. We demonstrated that PRMT1 and PRMT6 formed a heteromer complex and breaking this complex with a peptide inhibitor reduced cell proliferation and viability in NSCLC cell lines and lung cancer organoids. We also uncovered interleukin enhancer-binding factor 2 (ILF2) as a spe- cific substrate for the PRMT1/PRMT6 heteromer complex. Arginine methylation by the PRMT1/PRMT6 heteromer complex counteracted proteasome-mediated protein deg- radation of ILF2. Together, our results implicate that the higher PRMT6 expression which cooperates with PRMT1 to drive higher incidence and mortality rates of lung cancer in Black/AA men and targeting the PRMT1/PRMT6 heteromer complex could likely eliminate lung cancer health disparities.

Significance: Elevated expression of PRMT1/PRMT6 heteromer complex in lung ad- enocarcinoma of Black/AA men drives lung cancer development and could serve as a target to eliminate lung cancer health disparities.

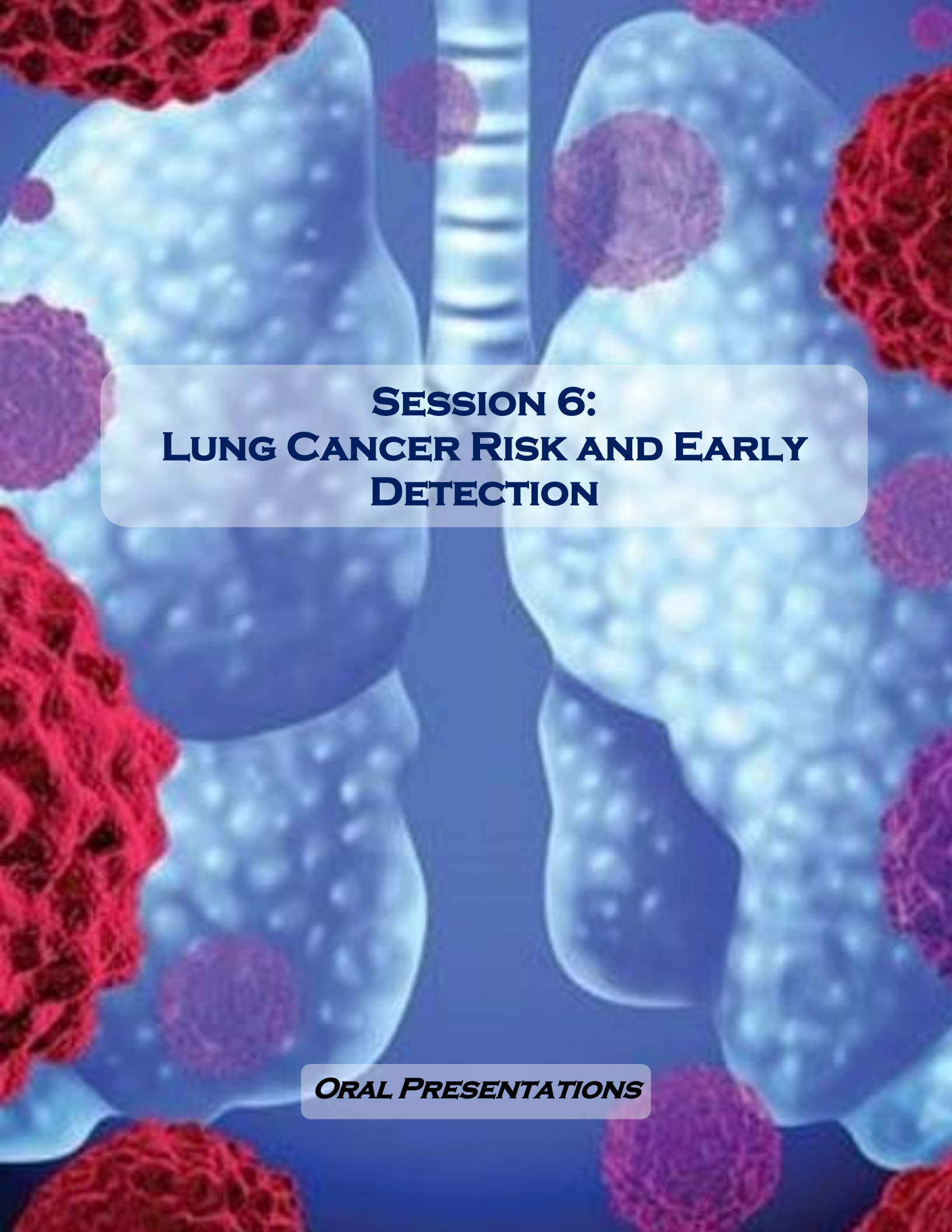
Mutation-Mediated Neo-Protein-Protein Interactions to Inform Oncogenic Rewiring and Therapeutic Vulnerability

Xiulei Mo, Qiankun Niu, Andrey A. Ivanov, Cong Tang, Changfa Shu, Qianjin Li, Kun Qian, Alafate Wahafu, Sean P. Doyle, Danielle Cicka, Xuan Yang, Dacheng Fan, Matthew A. Reyna, Lee A.D. Cooper, Carlos S. Moreno, Wei Zhou, Taofeek K. Owonikoko, Sagar Lonial, Fadlo R. Khuri, Yuhong Du, Suresh S. Ramalingam and Haian Fu

Emory University

Presented by: Haian Fu

Comprehensive sequencing of patient tumors has revealed driver genomic mutations across tumor types. Oncogenic mutations represent cancer-specific features that offer unprecedented opportunities for mutation-directed therapeutic strategies. Here we report the discovery of prevalent variant-enabled neo-protein-protein interactions (neoPPI) and validation of selected neoPPIs in the cancer cellular context. Systematic analysis of the established neoPPI network suggests unique oncogenic re-wiring mechanisms driven by distinct mutant alleles and candidate neoPPIs for therapeutic perturbation. For example, the recurrent BRAF V600E lesion led to a neoPPI with KEAP1 and VHL and created a $BRAFV^{600E}$-KEAP1 signaling axis and a $BRAF^{V600E}$-VHL signaling pathway. Further validation revealed multi-transcriptional program upregulation with a single mutational change, suggesting a novel mechanism for oncogenic mutation-driven tumors. Our work presents a new dimension of the cancer genome to inform variant neoPPI-directed therapeutic strategies for precision oncology.



Session 6: Lung Cancer Risk and Early Detection

Oral Presentations

Comparison Of Prediction Models to Diagnose Indeterminate Pulmonary Nodules Using Clinical Variables, Semantic Imaging Features And Radiomics

Wei Wu, Mladen Zecevic, Sudhakar N. J. Pipavath, Yuzheng Zhang, Kristin J Lastwika, Timothy W. Randolph, Paul D Lampe, Viswam S. Nair, A McGarry Houghton, Eric Grogan, Paul E. Kinahan

University of Washington Medical Center, Fred Hutchinson Cancer Center, Vanderbilt University

Presented by: Wei Wu

Purpose: To compare prediction models for the diagnosis of cancer in indeterminate pulmonary nodules using clinical data, radiological semantic features and radiomics.

Methods: This retrospective study included a training case-control matched cohort (based on age, gender and pack-years) (69 non-small cell lung cancers (NSCLCs) versus 66 controls with benignity) from FHCC, an unmatched refitting cohort (71 NSCLCs versus 78 controls) from FHCC and an independent validating cohort (48 NSCLCs versus 50 controls) from Vanderbilt university. The lesions on CT were contoured by a radiologist and radiomics features were calculated using the PyRadiomics package. In addition, two radiologists reviewed CT images and evaluated 54 semantic (i.e. structured reporting) features independently. Ridge models were built using LASSO selected variables on the case-control matched training cohort: "S" using semantic variables, "R" using radiomics features, and "SR" using semantic and radiomics variables. Then we refit ridge regression and generated 3 modified models "CS", "CR" and "CSR" using the selected variables with coefficients from the training model plus clinical variables (age, gender and pack-years) on the unmatched refitting cohort. We tested all 3 modified models' performance on the independent validating cohort using ROC curves. Statistical differences of model AUCs were determined by a DeLong test at a p-value < 0.05.

Results: LASSO selected 4 S features (the bounding volume maximum length (VM), smoothness, spiculation, and part-solid) 9 R features (2 shape and 7 wavelet) and 6 SR features (smoothness, spiculation, part-solid, centrilobular emphysema and 2 radiomics metrics) for each model. The AUCs on validating cohort for model (CS), model (CR) and model (CSR) were 0.78, 0.87 and 0.79 respectively. Model (CR) (AUC=0.87) was significantly more accurate at identifying cancer than models (CSR) (AUC=0.79, p=0.004) and Mayo (AUC=0.78, p=0.02).

Conclusion: Radiomics features integrated with clinical variables had the best performance for diagnosing cancer in indeterminate pulmonary nodules and performed better than existing nodule risk calculators.

Sex Differences in The Prediction Of Future Lung Cancer Risk Based on a Single Low-Dose Chest Computed Tomography Scan

Judit Simon, Peter Mikhael, Ismail Tahir, Alexander Graur, Amanda Fata, Jo-Anne Shepard, Francine Jacobson, Regina Barzilay, Lecia V. Sequist

Massachusetts General Hospital, Massachusetts Institute of Technology, Brigham and Women's Hospital

Presented by: Florian Fintelman

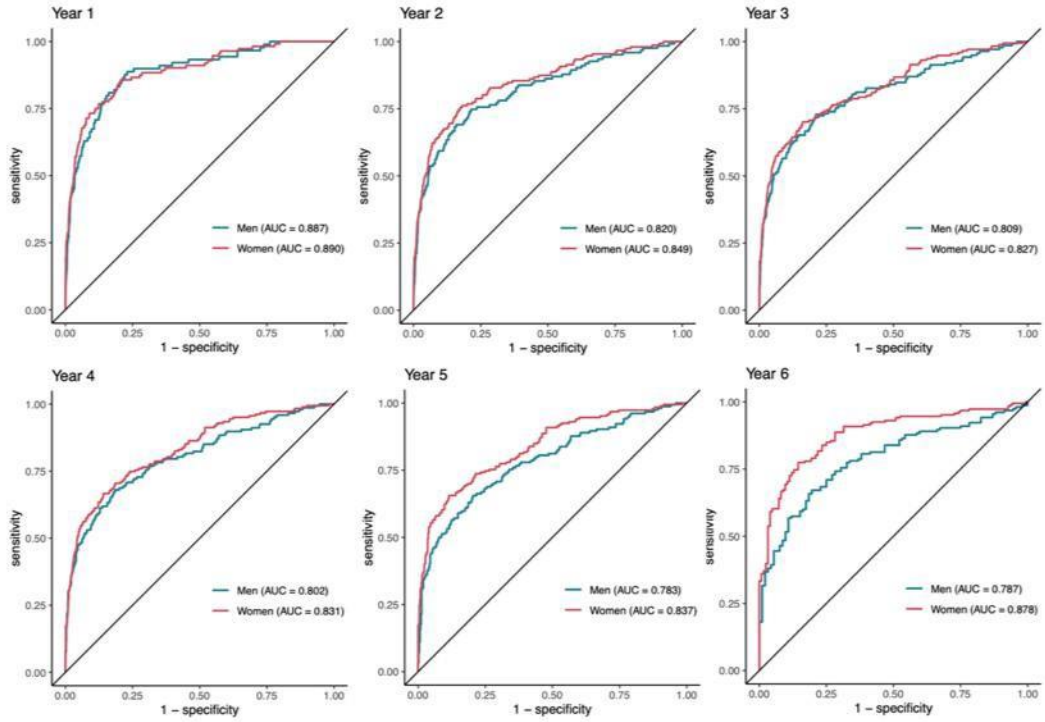
A validated open access deep learning algorithm called Sybil can accurately predict long-term lung cancer risk from a single low-dose chest computed tomography (LDCT) scan and its accuracy exceeds clinical risk assessment. Sybil was trained on predominantly (60%) men and use of artificial intelligence algorithms trained on imbalanced cohorts may lead to inequitable outcomes in real-world settings.

Aims: We aimed to study whether Sybil works equally well in both sexes.

Methods: We included participants who underwent lung cancer screening LDCT at Brigham and Women's Hospital and Massachusetts General Hospital between 2014 and 2019. Patients without follow-up were excluded. Patients diagnosed with lung cancer according to the institutional cancer registry within 6 years after the baseline LDCT were considered confirmed lung cancers. Those without a lung cancer diagnosis in the cancer registry and one or more negative follow-up LDCT were considered as negative for lung cancer. Area under the curve (AUC) values for women and men were compared with the DeLong-test.

Results: After exclusion, 10,588 LDCTs from 6,141 patients (47.1% women, mean age 64.9±6.2) were analyzed. Sybil achieved AUCs of 0.89 (95%CI: 0.85-0.93) for women and 0.89 (95%CI: 0.85-0.94) for men at 1 year, 0.85 (95%CI: 0.80-0.90) for women and 0.82 (95%CI: 0.77-0.88) for men at 2 years, 0.83 (95%CI: 0.78-0.88) for women and 0.81 (95%CI: 0.76-0.87) for men at 3 years, 0.83 (95%CI: 0.78-0.88) for women and 0.80 (95%CI: 0.75-0.86) for men at 4 years and 0.84 (95%CI: 0.79-0.89) for women and 0.78 (95%CI: 0.72-0.84) for men at 5 years; all $p>0.05$. At 6 years, AUC was 0.87 (95%CI: 0.83-0.93) for women and 0.79 (95%CI: 0.72-0.86) for men, $p=0.009$.

Conclusion: Sybil can accurately predict future lung cancer risk in women and men. For predicting long-term lung cancer risk at 6 years, Sybil performs better in women than in men.



Receiver operating characteristics curves displaying the ability of the Sybil algorithm to predict future lung cancer risk over 6 years following a single low-dose computed tomography in 2,907 women and 3,234 men who underwent lung cancer screening at Brigham and Women's Hospital and Massachusetts General Hospital between 2014 and 2019.

Lung Cancer Diagnosis Hazard in Screening and Lung Nodule Programs

Raymond U Osarogiagbon, Wei Liao, Nicholas R Faris, Carrie Fehnel, Jordan Goss, Catherine J Shepherd, Talat Qureshi, Anberitha T Matthews, Matthew P. Smeltzer, Paul F. Pinsky

Baptist Cancer Center, University of Memphis, National Cancer Institute

Presented by: Ray Osarogiagbon

Background: Guideline-concordant management of lung nodules promotes early lung cancer diagnosis, but the lung cancer risk profile of persons with incidentally detected lung nodules differs from that of screening-eligible persons. We compared lung cancer diagnosis hazard between participants receiving low-dose computed tomography lung cancer screening (LDCT cohort) and those in a lung nodule program (LNP cohort).

Methods: In a prospective community-based observational cohort study of LDCT versus LNP cohorts from 2015-2021, we compared patient, nodule and lung cancer characteristics, cumulative rates of lung cancer diagnosis, and lung cancer diagnosis hazards between the LDCT and LNP cohorts aged 50 to 80 years, with follow-up to January 1, 2022. We excluded patients with prior lung cancer, aged <50 or >80 years, LDCT cohort lacking baseline Lung CT Screening Reporting and Data System (Lung-RADS) score; stratified the LDCT cohort by Lung-RADS 1-2 versus 3-4, LNP by smoking history into screening-eligible versus ineligible sub-cohorts; updated survival at six-month intervals.

Results: There were 6,684 eligible persons in LDCT- 86% Lung-RADS 1-2, 14% Lung-RADS 3-4; 12,645 in LNP - 20% screening-eligible, 80% screening-ineligible. Black persons were 19% of LDCT and screening-eligible LNP cohorts, and 29% of ineligible LNP cohort (p-value <0.001). Median lesion size was 4mm (IQR: 2–6) for LDCT, (3mm [IQR:2–4] for Lung-RADS 1-2 and 9mm [IQR:6-15] for Lung-RADS 3-4); 9mm (IQR:6–16), and 7mm (IQR:5–11) for screening-eligible and ineligible LNP cohorts, respectively. Lung cancer was diagnosed in 1.0% of Lung-RADS 1-2, 18.6% of Lung-RADS 3-4 patients in LDCT; 21.2% in the screening-eligible, and 4.2% in the ineligible LNP cohorts. Compared to persons with Lung-RADS 1-2, the fully-adjusted hazard ratio (aHR) was 16.2(95% CI:12.67 – 20.58) for the screening-eligible, and 3.8(95%CI:2.95 – 4.97) for the ineligible cohort; compared to Lung-RADS 3-4, aHR was 1.2 (95%CI:1.01 – 1.45) and 0.3(95%CI:0.23 – 0.35), respectively; 64%, 52% and 57% of LDCT, screening-eligible and ineligible LNP cohorts, respectively, had stage I/II lung cancer.

Conclusions: The cumulative lung cancer diagnosis hazard among screening-age persons enrolled in LNP was higher than a screening cohort, irrespective of smoking history. Significantly more Black persons gained access to early detection through the LNP than LDCT.

Investigating Low Dose CT Findings in Firefighters: Results from the Fire Health Study

Erica T. Warner, PuiYee (Elaina) Chan, Isabella Galler, Audra Hite, Beza Tadess, Lian Atlas-Grayson, Jo-Anne Shepard, Amita Sharma, Lecia V. Sequist

Massachusetts General Hospital

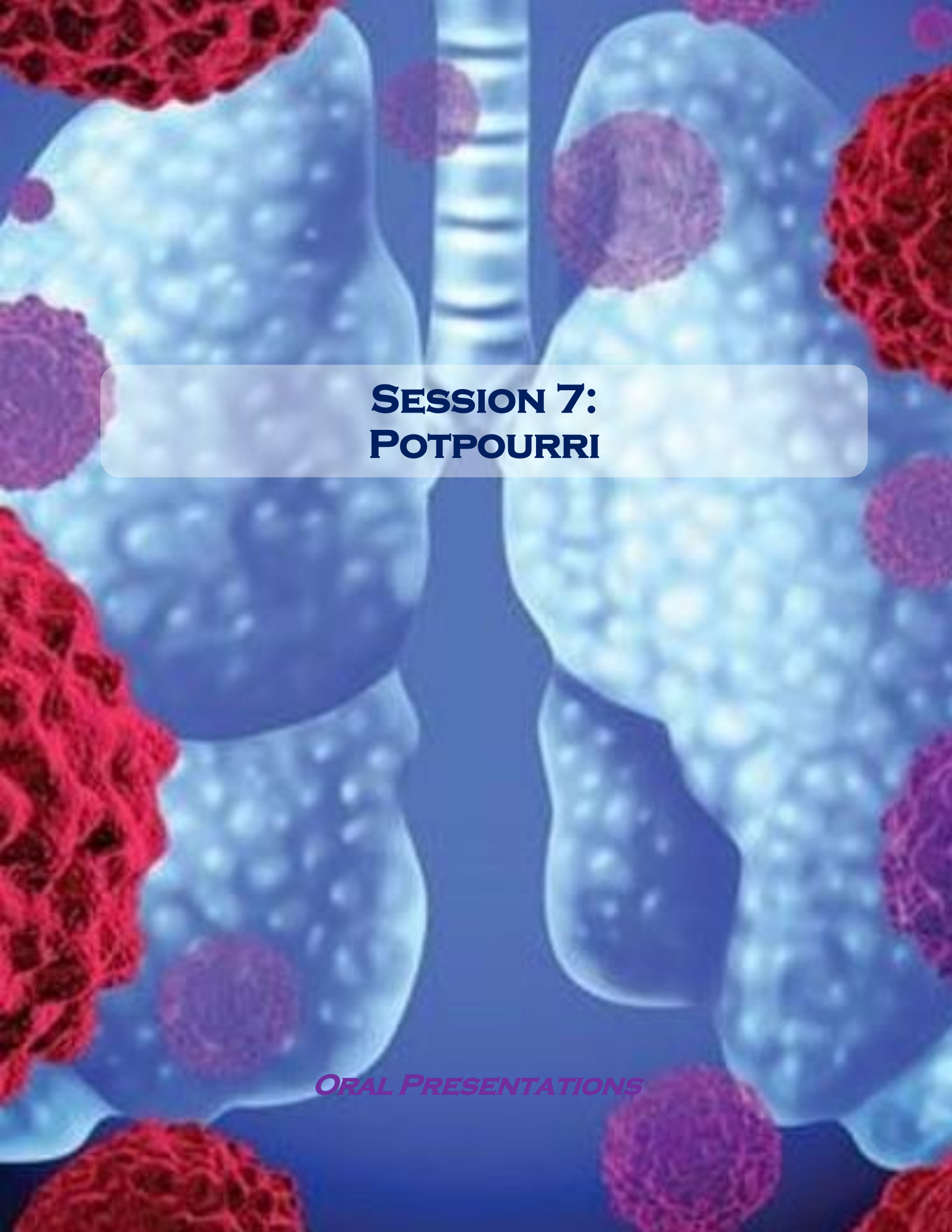
Presented by: Erica Warner

Background: While some studies suggest that firefighters are at increased risk of lung cancer compared to the general population, there is limited published medical literature about the computed tomography (CT) radiographic features of firefighters' lungs, or how variations in exposures affect CT findings. Critical data is currently unavailable to assess whether some firefighters are at sufficiently high risk for lung cancer to warrant screening due to their occupation.

Methods: In 2021 we established the Fire Health Study (FHS) (NCT04614129; https://firehealthstudy.org/) to investigate lung health and lung cancer risk in firefighters. To be eligible individuals must be an active or retired firefighter; 40 to 80 years of age (or <40 with least 10 years of firefighting experience); and have had a low-dose CT (LDCT) scan in the past year or be willing to have one during the study. Through a web-based enrollment process interested individuals complete an eligibility screener, consent form, medical release, and questionnaire. Questionnaires assess occupational and health histories and lifestyle and behavioral factors. LDCTs are evaluated by two study radiologists using the Lung Imaging Reporting and Data System (Lung-RADS).

Results: To date we have enrolled 734 firefighters. Participants mean age is 51.2 years. Among participants with available data, most are active firefighters (76.5%, n=377/493), male (96%, n=615/640), have never smoked (75.1%, n=479/638), and have 20 or more years of fire service (66.7%, n=332). Study radiologists have read and reported LDCT results for 335 FHS participants. Among this subset of the cohort, 32.2% (n=108) were Lung-RADS 1, 61.5% (n=206) were Lung-RADS 2, 5.1% (n=17) were Lung-RADS 3, and 1.2% (n=4) were Lung-RADS 4A. No lung cancers have been detected. 32.4% (n=109) of participants had clinically significant or potentially significant other findings with moderate or severe coronary calcifications most common.

Conclusion: In this population of firefighters, most of whom do not meet eligibility criteria for lung cancer screening**,** 6.3% had a positive LDCT. Other significant findings were common. Ongoing analyses will evaluate how these findings compare to matched controls and whether occupational exposures including duration of employment, cumulative fire hours, and decontamination practices are associated with LDCT results.



Session 7: Potpourri

Oral Presentations

What Next? Navigating the Dynamic, Unpredictable, and Challenging Realm of Cancer Clinical Trials During and After A Global Pandemic

UT Southwestern Medical Center

Presented by: David E. Gerber, MD

In addition to widespread disease and profound economic effects, the COVID-19 pandemic introduced several challenges to the already complex realm of cancer clinical trials. Among others, these included quarantines, site closures, travel limitations, investigational product supply chain concerns, and staff and patient illness. Recognizing these issues, the U.S. Food and Drug Administration (FDA) and the National Cancer Institute (NCI) issued interim guidance on clinical research practices during the pandemic. Recommendations included remote informed consent; telephone or video visits; local (i.e., near the patient's home) laboratory and imaging studies; delaying assessments; alternative sites for treatment administration; shipping oral study therapy directly to patients' homes; use of electronic signatures; and remote study monitoring. Taken together, these shifts arguably marked the greatest change to a longstanding status quo in recent memory. Early studies indicated that these profound changes are not only feasible, but may also be preferred.

Despite what might be considered a long-overdue flexibility in clinical trial implementation and conduct, the pandemic's early strain on healthcare systems followed by delayed staffing shortages have resulted in unprecedented operational difficulties. These challenges have disproportionately affected investigator-initiated cancer clinical trials, which offer patients access to promising Specialized Program of Research Excellence (SPORE) and other NCI-supported scientific discoveries. Compounding this worrisome trend, a growing number of trial sponsors have reverted to pre-pandemic cumbersome and costly practices, such as routine on-site trial monitoring regardless of trial design or site performance and experience. Additionally, the FDA guidance which has provided much welcome reforms to trial conduct may expire with the end of the COVID-19 Public Health Emergency on May 11, 2023.

What can we as a Lung SPORE community do to preserve these recent gains? To start, it is critical to understand that recent regulatory guidance does not represent new or temporary regulation. Indeed, practices such as remote consent and remote trial monitoring have long been permitted and in some cases encouraged by the FDA. However, awareness of these possibilities was previously quite limited. Educating our clinical trialists, challenging sponsors' inflexibility, and advocating for our patients will be critical to maintaining a productive pipeline of bench-to-bedside scientific discovery.

Sexual Dysfunction in Women with Lung Cancer: Updates from the SHAWL Study

Narjust Florez, Lauren Kiel, Kelly Meza, Zihan Wei, Emanuele Mazzola, Ana Velazquez Manana, Ivy Franco, Mary Fiddler, Ivy Elkins, Jill Feldman, Lori Seaborne, Christine Heisler, Jennifer King, Amy Moore, David Kushner

Dana-Farber Cancer Institute, UCSF, Brigham and Women's Hospital, Rush University, EGFR Resisters, University of Wisconsin Hospital, GO2 Foundation for Lung Cancer, LUNGevity Foundation

Presented by: Narjust Florez

Background: Though underdiscussed, sexual dysfunction is highly prevalent in patients with lung cancer (LC); however, most data precede the approval of targeted therapies and immune checkpoint inhibitors. We report updated data from the SHAWL study, the largest study evaluating sexual dysfunction in women with LC in our current clinical environment, focusing on sex life satisfaction.

Methods: This cross-sectional survey study was administered via the GO2 Foundation Lung Cancer Registry. We utilized the Patient-Reported Outcomes Measurement Information System (PROMIS) Sexual Function and Satisfaction Measures for data collection. Participants were recruited from June 2020- June 2021. Eligibility criteria included age > 18 years, self-identification as a woman, and a LC diagnosis within ten years. Participants were asked about sexual health pertaining to 30 days before survey completion, referred to as "recent."

Results: The survey was administered to 249 women (median age: 61 years). Most (67%) had stage IV LC and 47% were receiving targeted therapy; 66% were undergoing active treatment. Before LC diagnosis, 49% (117) reported having no sexual health issues. Most women (54%, 128) indicated having had recent sexual activity, though 77% (183) reported moderate to severe sexual dysfunction. Only 7.5% (18) reported being quite or very interested in sexual activity and the vast majority (72%, 91) reported minimal to no sex life satisfaction. Common reasons for lack of recent sexual activity were lack of interest (68%, 76) or vaginal dryness or pain (30%, 33). Most women (69%, 88) reported rarely becoming sufficiently lubricated during sexual activity, with 54% (68) indicating that it was difficult to impossible to do so. Patients with stage IV diagnoses had a lower interest in and desire for sexual activity than those with non-metastatic LC; patients not receiving active treatment reported similar rates of these measures as those undergoing active therapy. Patients on targeted therapy had similar rates of sexual dysfunction as those receiving other treatments.

Conclusions: Sexual dysfunction, dissatisfaction, and lack of interest were highly prevalent in women with LC regardless of treatment status and therapy type, suggesting that even after treatment completion, sexual issues persist. Sexual health should be integrated into thoracic oncology care.

A Radiomic Based Predictive Model of Lung Adenocarcinoma Brain Metastases

Xiancheng Wu, Maria A. Velez, Murat Ak, Nourel H. Tahon, Priyadarshini Mamindla, Vishal Peddagangireddy, Rivka R. Colen, Laura P. Stabile, Timothy F. Burns

University of California Los Angeles, University of Pittsburgh

Presented by: Xiancheng Wu

Although almost 40% of NSCLC patients will develop BM during the course of their disease, there are currently no reliable prediction tools for identifying patients at risk for BM, especially in the early-stage setting where MRI screening is not performed. Furthermore, in the later stage setting, brain MRI are only performed annually. Therefore, there is a critical need to identify high-risk patients for BM that could benefit from MRI surveillance. We identified 162 lung adenocarcinoma (LUAD) patients with (N=66) or without (N=96) BM that had treatment-naïve CT scans with a segmentable lesion. The tumor, surrounding ground glass opacity and necrosis were segmented via 3D slicer to create a volume of interest for radiomic texture analysis and 400 features were extracted. The Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression feature selection method was used to select the most relevant features and models were built using the machine learning method XGBoost classifier. Training and testing sets with random splitting was used for cross validation. We report the accuracy, sensitivity, specificity, and area under the curve (AUC) for each model. Among the extracted features that LASSO deemed as most discriminative for development of BM, we identified the most relevant features using XGBoost that predicted BM with 79% accuracy, 83% sensitivity, 72% specificity, and 79% AUC (p=0.01) in the overall population. The addition of ground glass opacity and necrosis to the model did not significantly improve performance. Furthermore, the model distinguished those with metachronous vs synchronous BM with 84% accuracy, 83% sensitivity, 86% specificity, and 83% AUC (p =0.04). Our model held up across molecular subtypes (*EGFR* and *KRAS* mutant). Importantly, the model was predictive in early-stage patients with 92% accuracy, 96% sensitivity, 83% specificity, and 95% AUC (p=0.0005). Moreover, our model predicted for high vs. low overall survival, and was BM-specific as it was not predictive of other metastatic sites. We are currently validating our model in a larger cohort and if successful, this would serve as the rationale for a future trial of MRI surveillance in early stage NSCLC patients.

Lung Cancer Information System: Enabling Precision Oncology Through Informatics

Madhusmita Behera, Jeffrey Switchenko, Suresh Ramalingam, Sorena Nadaf

Emory University, Ci4CC

Presented by: Madhusmita Behera

Digital transformation in healthcare is happening at an increasingly rapid pace, allowing the medical community to deliver high-value, high quality care and accelerate knowledge generation. While advances in biomarker development have accelerated the path towards data-driven insights for cancer care and research, most approaches do not account for integrated approaches across modalities. Multimodal integration of structured clinical data, molecular diagnostics and image derived information from radiology and histology, provide unique opportunities to advance precision oncology. Expanding upon the comprehensive thoracic oncology and data science programs at Emory, we have architected an advanced Lung Cancer focused Digital Information System (LCIS), aimed to provide an integrated precision oncology platform, further enabling collaborative research between Winship lung cancer investigators and the larger SPORE community.

In the initial phase of this work, we have developed LCIS as a cloud-based lung cancer data ecosystem, architected with a secure, scalable and compliant technology platform provided by DNAnexus Apollo (DNAnexus, CA). The LCIS aims to aggregate multiple data types in a single management and analysis environment, starting with genomic, EMR, and pathology imaging data. The ecosystem includes a large selection of existing bioinformatics tools, with the ability for users to apply their custom analysis pipelines on the data through several programmatic approaches. The aggregated lung cancer data in LCIS can be analyzed in multiple ways that suit the needs and informatics capabilities of diverse users. The platform includes graphically rich interfaces that allows for building cohorts based on clinical, genomics, and other complex data parameters, demonstrating clear, customizable, multi-dimensional views of patient-level data. The comprehensive platform also provides researchers an infrastructure suitable for building and deploying AI/ML models. We will be expanding the architecture and data model to also adhere to mCODE, as we partner with national “Minimal Oncology Data Elements” CodeX program.

With LCIS, our overarching goal is to enable lung SPORE researchers to better identify clinical and multi-omic variables that warrant further investigation, and to drive impactful research aimed at improving patient outcomes through research collaborations both internally and externally.

Artificial Intelligence-aided Clinical Annotation of a Large Multi-Cancer Genomic Dataset

Kenneth L. Kehl, Wenxin Xu, Alexander Gusev, Ziad Bakouny, Toni K. Choueiri, Irbaz Bin Riaz, Haitham Elmarakeby, Eliezer M. Van Allen, and Deborah Schrag

Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, Mayo Clinic, The Broad Institute, Memorial-Sloan Kettering Cancer Center

Presented by: Kenneth L. Kehl

To accelerate cancer research that correlates biomarkers with clinical endpoints, methods are needed to ascertain outcomes from electronic health records at scale. Here, we train deep natural language processing (NLP) models to extract outcomes for participants with any of 7 solid tumors in a precision oncology study. Outcomes are extracted from 305,151 imaging reports for 13,130 patients and 233,517 oncologist notes for 13,511 patients, including patients with 6 additional cancer types. NLP models recapitulate outcome annotation from these documents, including the presence of cancer, progression/worsening, response/improvement, and metastases, with excellent discrimination (AUROC > 0.90). Models generalize to cancers excluded from training and yield outcomes correlated with survival. Among patients receiving checkpoint inhibitors, we confirm that high tumor mutation burden is associated with superior progression-free survival ascertained using NLP. Here, we show that deep NLP can accelerate annotation of molecular cancer datasets with clinically meaningful endpoints to facilitate discovery.

Session 8: SCLC

Oral Presentations

Extrachromosomal DNAs Harboring Myc Family Proto-oncogenes Drive Acquired Cross-resistance in Small Cell Lung Cancer

Shreoshi Pal Choudhuri, Luc Girard, Stanley Lim, Jillian Wise, Braeden Freitas, Edmond Wong, Seth Hamilton, Victor Chien, Jun Zhong, Sarah Phat, David Myers, Marcello Stanzione, Nicholas Dyson, Michael Lawrence, Sihan Wu, Benjamin Drapkin

UT Southwestern Medical Center, Massachusetts General Hospital

Presented by: Benjamin Drapkin

Small cell lung cancer (SCLC) is a common malignancy with a poor prognosis that has not improved significantly over the past 40 years. Nearly all patients with SCLC are treated with chemotherapy, have dramatic tumor regression and symptomatic improvement, and relapse within months. Following relapse SCLC changes, acquiring resistance to a broad range of cytotoxic agents that renders it refractory to treatment. Despite decades of research, no recurrent drivers of cross-resistance have been identified, largely due to scarcity of relapsed samples and low fidelity of laboratory models to clinical drug responses. Here we report a panel of 51 SCLC PDX models derived from 43 patients, 19 models before treatment and 32 after at least one line of therapy. Each model was treated with three clinical regimens, cisplatin + etoposide (EP), olaparib + temozolomide (OT) and topotecan, resulting in 678 tumor-volume curves. These were compared with 1184 control xenografts from the same models to measure cross-resistance. These measurements recapitulated hallmark clinical features of cross-resistance in SCLC, including reduced sensitivity after relapse and further reduction with abbreviated chemotherapy-free interval (CTFI) or with early progression on the next therapy after model derivation. To discover candidate drivers of resistance, we compared serial models from the same patient before first-line EP and again after second-line OT. This revealed a high-level *MYC* amplification on extrachromosomal DNA (ecDNA) that was acquired after start of second-line therapy. To assess whether this alteration was recurrent in resistant SCLC, we generated comprehensive molecular profiles of each model by whole genome and transcriptome sequencing. High level amplifications of Myc family proto-oncogenes (*MYC*, *MYCN* or *MYCL*) on ecDNAs (ecMyc) were found in 9/51 models, of which 8 were derived from relapsed patients. All 9 ecMyc+ models were resistant to at least 2 regimens, and 7/9 were cross-resistant to all three. Finally, we found that within cross-resistant ecMYC+ xenografts, the cells with highest ecMYC copy number were the most resistant to DNA damage and were enriched following chemotherapy. We conclude that ecMyc amplifications may be acquired on therapy and are recurrent drivers of cross-resistance in SCLC.

Spatiotemporal Heterogeneity of Transcription Factor-Based Subtype Assignment in Small Cell Lung Carcinoma

Thomas Denize, Catherine B. Meador, Anna B. Rider, Maria L. Ganci, Mari Mino-Kenudson, Yin P. Hung

Massachusetts General Hospital and Harvard Medical School

Presented by: Catherine Meador

Background: Small cell lung carcinoma (SCLC) can be classified into transcription factor-based subtypes (ASCL1, NEUROD1, and POU2F3) with potentially specific therapeutic vulnerabilities. While *in vitro*/murine studies have suggested intratumoral heterogeneity in the expression of these markers, how SCLC subtypes vary over time and locations within a given patient remains unclear.

Methods: We searched a consecutive series of patients from our institution between 2006-2022 for those with >1 available formalin-fixed paraffin-embedded (FFPE) SCLC sample at multiple sites or time points. Immunohistochemistry for ASCL1, NEUROD1, and POU2F3 was performed and evaluated using H scores, with subtype assignment based on the highest H score.

Results: We identified 179 FFPE samples from 84 patients fitting our search criteria. Of these, there were 74 patients with 2 samples available, 9 with 3, and 1 with 4. Of the total number of samples, 75 were from lung, 51 from lymph nodes, and 53 from non-nodal metastases. Ninety-nine of 179 (55%) were ASCL1-dominant, 50/179 (28%) NeuroD1-dominant, 2/179 (1%) ASCL1-NeuroD1 co-dominant, 15/179 (8%) POU2F3-dominant, and 13/179 (7%) were triple-negative samples. While 47/75 (63%) lung tumors were ASCL1-dominant, 52/104 (50%) extrapulmonary samples including 23/51 (45%) lymph nodes, were ASCL1-dominant. Of the 74 patients with only 2 samples for pairwise comparison, 57/74 (77%) harbored concordant subtypes (37 both ASCL1-dominant, 13 both NEUROD1-dominant, 5 POU2F3-dominant, and 2 both triple-negative), while the remainder 17/74 (23%) showed discordant subtypes (10 with ASCL1- and NEUROD1- in one sample each, 2 with triple-negative and ASCL1-, 2 with triple-negative and NEUROD1-, 2 with POU2F3- and ASCL1-, 1 with POU2F3- and NEUROD1-dominant). After accounting for ASCL1/NEUROD1-double expression (H-score difference ≤50), 11/74 (14.9%) patients with two samples available harbored discordant subtypes among their samples. No significant differences in discordant rates were noted with locations (pulmonary vs extrapulmonary, thoracic vs extrathoracic) or time intervals between the samples (<3 vs ≥3 months).

Conclusion: While transcription factor-based subtype assignment was concordant among multiple SCLC samples from most patients, ~15% of patients displayed discordant subtypes. Our findings highlighted the spatiotemporal heterogeneity of SCLC in clinical samples, with implications on challenges using subtype-specific therapeutics.

Post-Translational Modifications Induce Autoantibodies with Risk-Prediction Capability In Patients With Small Cell Lung Cancer

Kristin J. Lastwika, Andrew Kunihiro, Samantha M. Sarrett, Francesca Urselli, Yuzheng Zhang, David Shelley, Timothy W. Randolph, Christopher I. Li, Eric L. Grogan, David MacPherson, Brian M. Zeglis, Justin J. Taylor, A. McGarry Houghton, Paul D. Lampe

Fred Hutchinson Cancer Center, Hunter College, City University of New York, Memorial Sloan Kettering Cancer Center, Vanderbilt University Medical Center

Presented by: Kristin J. Lastwika

A major function of our immune system is to recognize and eliminate transformed cells. Small cell lung cancer (SCLC) elicits the generation of autoantibodies that result in unique paraneoplastic neurological syndromes. The mechanistic basis for the formation of such autoantibodies is largely unknown, but is key to understanding their etiology. We developed a high-dimensional technique that enables detection of autoantibodies in complex with native antigens directly from patient plasma. Here we used our platform to screen 1,009 human plasma samples for 3,600 autoantibody-antigen complexes, finding that plasma from patients with SCLC harbors, on average, 4-fold higher disease-specific autoantibody signals compared to plasma from patients with other cancers. Across 3 independent SCLC cohorts, we identified a set of common but previously unknown autoantibodies that are produced in response to both intracellular and extracellular tumor antigens. As a proof of principle, we investigated an autoantibody-identified target, CD133, for its functionality in immuno-positron emission tomography imaging (immunoPET) and as an antibody drug conjugate (ADC) in SCLC xenografts. We found SCLC tumors can be detected by immunoPET with minimal uptake in healthy tissues and tumor growth is inhibited when treated with a CD133-ADC. We further determined several autoantibodies targeted post translational modifications (PTM) and with this knowledge created 10 PTM-peptide tetramers to enrich for PTM-specific autoantibodies from SCLC patient B cells. After 198 tetramer captured B cells were single cell sorted, 98 pairs of V_H and V_L were sequenced and 19 were cloned into human IgG expression vectors. With this approach we have successfully isolated and produced multiple human PTM-AAbs with high specificity for tumor tissue but not normal human tissue. Lastly, since most patients with SCLC have metastatic disease at diagnosis, we queried if these autoantibodies could be utilized for SCLC early detection. We created a risk-prediction model using 5 autoantibodies with an average area under the curve of 0.84 for the 3 cohorts that improved to 0.96 by incorporating cigarette smoke consumption in pack years. Taken together, our findings provide an innovative approach to identify circulating autoantibodies in SCLC with mechanistic insight into disease-specific immunogenicity and clinical utility.

Tumor and Liquid Biopsy-Based Subtyping of Small Cell Lung Cancer using Transcriptomic and DNA Methylation Classifiers

Simon Heeke, Carl M. Gay, Marcos R. Estecio, Hai Tran, Benjamin B. Morris, Bingnan Zhang, Ximing Tang[3], Maria Gabriela Raso, Pedro Rocha, Siqi Lai, Edurne Arriola, Paul Hofman, Veronique Hofman, Prasad Kopparapu, Christine Lovly, Kyle Concannon, Luana Guimaraes De Sousa, Whitney Elisabeth Lewis, Kimie Kondo, Natalie I. Vokes, Monique B. Nilsson, Allison Stewart, Yuanxin Xi, Lixia Diao, Qi Wang, Jianjun Zhang, Peter Van Loo, Jing Wang, Ignacio I. Wstuba, Lauren A. Byers, John V. Heymach

UT MD Anderson Cancer Center, Hospital del Mar, Nice Hospital, University Côte d'Azur, Vanderbilt University Medical Center, The Francis Crick Institute

Presented by: Simon Heeke

Background: Small-cell lung cancer (SCLC) is an aggressive malignancy composed of distinct transcriptional subtypes, defined by the predominant expression of one of the three transcription factors ASCL1 (SCLC-A), NEUROD1 (SCLC-N) and POU2F3 (SCLC-P) as well as an inflamed subtype (SCLC-I; see Gay et al. Cancer Cell. 2021), each with potential therapeutic vulnerabilities. Implementing subtyping in the clinic has remained challenging due to limited tissue availability, particularly for longitudinal monitoring. Given the known epigenetic regulation of critical SCLC transcriptional programs, we hypothesized that there would be subtype-specific patterns of DNA methylation that could be detected in tumor or blood from SCLC patients.

Methods: We included 179 patients with SCLC and performed RNA sequencing and genomic-wide reduced-representation bisulfite sequencing (RRBS). We further analyzed DNA methylation in 68 plasma samples including longitudinal samples to track SCLC subtype evolution over time.

Results: Using machine learning approaches, we developed a highly accurate DNA methylation-based classifier (SCLC-DMC) that could distinguish SCLC subtypes using clinical tumor samples with 95.8% accuracy in the testing set compared to mRNA-based profiling. We further adjusted the classifier for circulating-free DNA (cfDNA) to subtype SCLC from plasma. Using the cfDNA classifier (cfDMC), we could demonstrate that SCLC subtypes evolve frequently during disease progression, highlighting the need for longitudinal tracking of SCLC during clinical treatment. Furthermore, methylation-based subtyping predicted response to a wide variety of drugs in preclinical models like CDK and AURK inhibitors, and clinical outcomes were indistinguishable in cohorts of patients subtyped using mRNA or SCLC-DMC ($p = 0.95$).

Conclusions: These data establish that tumor and cfDNA methylation can be used to identify SCLC subtypes and guide precision SCLC therapy.

Molecular Predictors And Immunomodulatory Role Of Dual Checkpoint Inhibitor Blockade Using Ipilimumab/Nivolumab In Patients With Advanced Stage Small Cell Lung Cancer

Anne Chiang, Kurt Schalper, Hossein Asghari, KerryAnn Ashley, Eric Schultz, Stan Skrzypczak, Carl Kingsford, Scott Gettinger, Sarah Goldberg, Roy Herbst, Frederick Wilson

Yale University School of Medicine, Ocean Genomics

Presented by: Anne Chiang

Background: In patients with advanced small cell lung cancer (SCLC), the biological impact of immunotherapy is poorly understood with no clear predictive biomarkers to guide patient selection in this setting.

Methods: We collected paired baseline (pre-treatment), on-treatment (week 4), and progression biopsies from patients with relapsed extensive-stage SCLC treated with combination nivolumab (nivo) and ipilimumab (ipi) in a single-arm, phase 2 clinical trial (NCT03670056). Nivo 1 mg/kg and ipi 3 mg/kg were administered every 3 weeks for 4 cycles, followed by nivo maintenance until progressive disease (PD) by RECIST 1.1 or treatment-limiting toxicity. Paired pre/on-treatment samples were available from 16/22 patients, as well as 3 biopsies at progression. The tumor samples were studied using whole exome DNA sequencing (including germline DNA) and RNA-sequencing coupled to Ocean Genomics TxomeAI® data analysis pipeline.

Results: 6/11 evaluable patients had PD; 5 patients showed clinical activity of treatment (3 with stable disease, 2 with partial response). The frequency of deleterious mutations in *TP53* and *RB1* was 91% and 64%, respectively. Mutations in *HLA-A* were more common in baseline samples from patients with PD than those with clinical activity. New *TP53* and *PLEC* mutations were found 4 of 6 patients with PD in week 4 samples vs baseline. The baseline tumor mutational burden was not associated with treatment sensitivity and prominently increased in week 4 biopsies of PD patients. All four molecular SCLC transcriptomic subtypes based on the expression of *ASCL1*, *NEUROD1*, and *POU2F3* were present in the trial with SCLC-A being the most common(9/16cases). All patients with clinical activity to ipi/nivo were of SCLC-A subtype. 2/16 of cases showed a different molecular subtype after 4 weeks of treatment. Comparison of baseline and on-treatment samples showed upregulation of transcripts associated with T-cell activation and PD-1 signaling. In the 3 biopsies at progression, transcriptomic changes included reduction of neutrophil degranulation, type 1/2 interferon and interleukin-2,4,10,13 signatures, as well as down-regulation of β-2 microglobulin, while cell cycle and mitotic prophase pathways were overexpressed.

Conclusions: Dual checkpoint blockade using nivo/ipi has a prominent immunomodulatory role in extensive stage SCLC characterized by increased local adaptive immune responses, reduced HLA class-I antigen presentation and change in the molecular subtype in a subset of cases. We identified genomic features associated with treatment sensitivity/resistance.

Inference of Gene Expression and Transcription Factor Activity in Small Cell Lung Cancer Using Targeted Sequencing of Plasma cfDNA

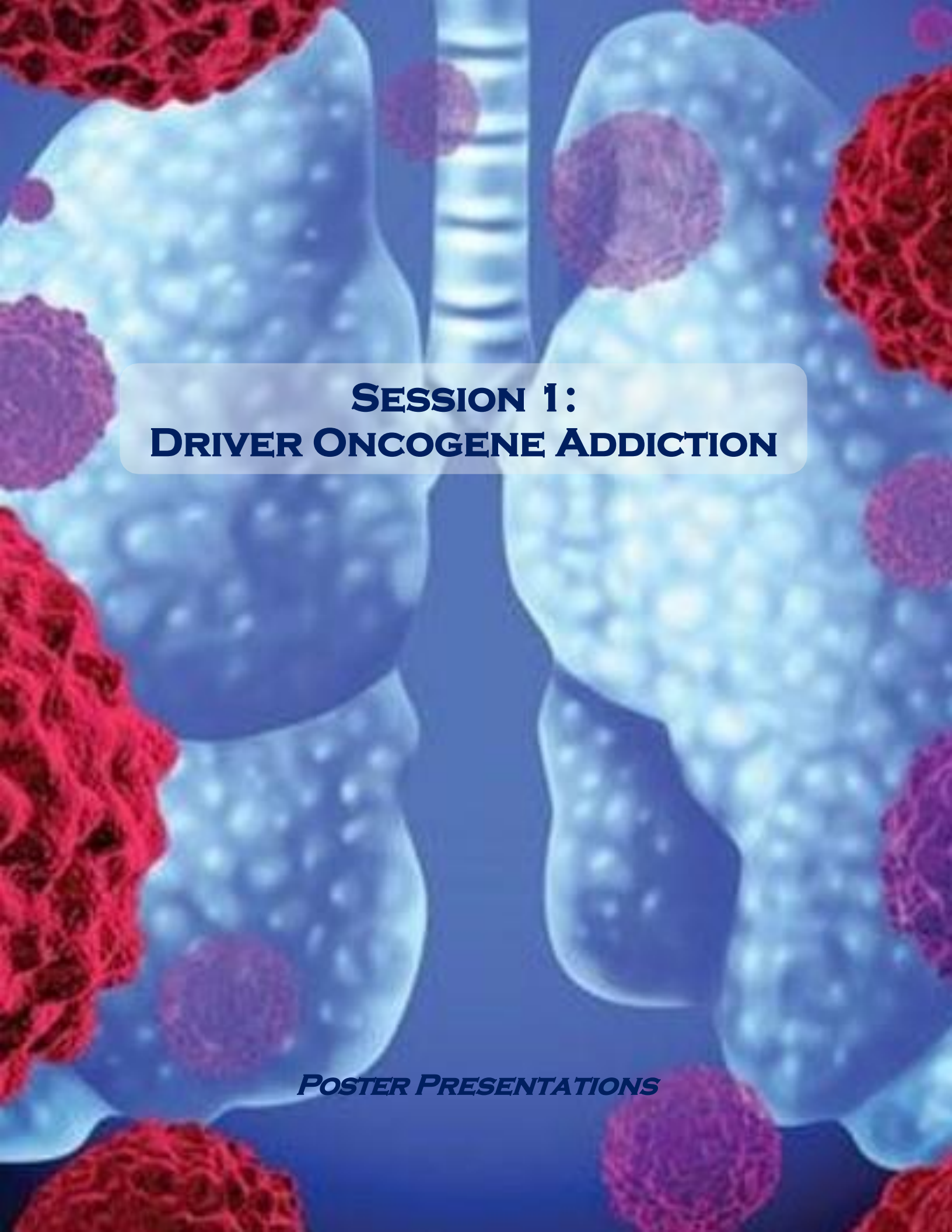
Joseph B Hiatt, Anna-Lisa Doebley, Henry U Arnold, Holly Sandborg, Minjeong Ko, Feinan Wu, Alvaro Quintanal Villalonga, Rafael Santana-Davila, Keith Eaton, Caroline Dive, Charles M Rudin, Anish Thomas, McGarry Houghton, Gavin Ha, David MacPherson

Fred Hutchinson Cancer Research Center, Memorial Sloan Kettering Cancer Center, University of Manchester, National Cancer Institute, University of Washington

Presented by: David MacPherson

Small cell lung cancer (SCLC) exhibits distinct molecular subtypes characterized by activation of transcription factors (TFs) such as ASCL1, NEUROD1, POU2F3, and REST, but clinical translation has been limited by tissue scarcity. Here, we developed a cell-free DNA (cfDNA) targeted sequencing assay that analyzes DNA fragmentation patterns to infer nucleosome profiles at 1,535 TF binding sites and 13,240 gene transcription start sites (TSSs) and also detects exonic mutations in 842 genes. Application to plasma cfDNA from SCLC patient-derived xenograft (PDX) models faithfully captured signatures of TF activity and gene expression and revealed highly informative nucleosome profiling loci including TSSs of key genes including ATOH1, POU2AF2, and targets of SCLC subtype-defining TFs. Prediction models of ASCL1, NEUROD1, and REST activity achieved AUCs (0.82-1.00) in SCLC patient samples while a predictor of SCLC vs NSCLC histology achieved an AUC of 0.99. Our approach identified previously unrecognized heterogeneity in cfDNA nucleosome profiling signals, resulting in a practical method for non-invasive SCLC genotype and phenotype assessment. In addition to utility in SCLC subtyping from liquid biopsies, our assay has immediate application towards identifying NSCLC-SCLC transdifferentiation and could be generalized to study and monitor many other cancer types.

Poster Presentations



Session 1:
Driver Oncogene Addiction

Poster Presentations

Murine EML4-ALK and TRIM24-RET Models Unveil Transcriptional Induction of the HGF-MET Pathway as a Bypass TKI Resistance Mechanism

Lynn E. Heasley, Trista K. Hinz, Sophia Jaramillo, Ana M. Selman

University of Colorado and Eastern Colorado VA Healthcare System

Presented by: Lynn Heasley

Introduction: ALK and RET gene rearrangements yield fusion oncogenes driving subsets of lung adenocarcinomas and tyrosine kinase inhibitors (TKIs) targeting ALK and RET fusions are approved as first-line therapeutics. TKI therapies extend survival in these patients, but acquired resistance drives treatment failure and tumor progression. The literature supports the importance of modeling TKI responses in immune-competent systems. Thus, we developed murine ALK and RET-driven lung cancer cell lines that can be propagated orthotopically in immune-competent hosts. Herein, mechanisms of TKI resistance in these murine cell lines that may highlight novel therapeutic strategies in human lung cancers were evaluated.

Methods: When propagated orthotopically in C57BL/6 mice, EML4-ALK (EA1, EA3) and TRIM24-RET-driven cells (TR.1) yield residual disease following TKI therapy. To identify rapidly and chronically-acquired resistance mechanisms, the cell lines were cultured in vitro with ALK inhibitor, alectinib, or RET-specific TKIs, LOXO-292 or BLU-669, until cultures resistant to ~1 mM drug were obtained. Parental cell lines treated for 1-5 days with TKIs and stable TKI-resistant cultures were submitted to RNAseq. HGF protein was measured by ELISA. Sensitivity of control and resistant cultures to ALK, RET and MET-targeting TKIs was determined in 96-well clonogenic growth assays.

Results: MET-HGF mRNA and protein were induced by alectinib within 3 days in parental EA1 cells and increased in cultures chronically resistant to 1 mM drug. MET, but not HGF was acutely and chronically induced in EA3 cells. Acute and chronic treatment of TR.1 cells with LOXO-292 or BLU-667 induced HGF and MET mRNA. Consistent with the HGF-MET pathway functioning as a bypass pathway, alectinib-resistant derivatives of EA1 cells, but not EA3 cells retained sensitivity to crizotinib. Also, TKI-resistant TR.1 cells exhibited 41-fold greater sensitivity to crizotinib relative to control TR.1 cells.

Summary: The studies support rapid transcriptional induction of the HGF-MET pathway in murine models of ALK and RET-driven lung cancer that is maintained in chronically TKI-resistant cells. These murine ALK and RET-dependent cell lines provide novel models to explore therapeutic strategies to disrupt HGF-MET pathway-mediated bypass signaling and increase the durability of responses in these subsets of lung cancers.

A Lentiviral Platform for the Identification of On-Target Resistance Mutations to Targeted Lung Cancer Therapies

Xiaofang Huo, Michael Peyton, Kimberley Avila, John Heymach, John Minna, Kenneth Westover, Ralf Kittler

UT Southwestern, UT MD Anderson

Presented by: Ralf Kittler

Discovery of mutations in a cancer drug target that confer therapeutic resistance validate the drug target, nominate clinically relevant resistance mutations, and provide chemical and structural information about the drug and target, which can be useful for developing new therapeutic strategies or drugs to circumvent clinical resistance. Thus, identification of these resistance-conferring mutations can inform both clinical and pre-clinical drug development programs. We have developed a rapid, unbiased method for facile discovery of drug resistance mutations that relies on lentiviral mutagenesis. This technique, which we termed LentiMutate utilizes error-prone lentiviral transduction to create mutations in the cDNA generated during reverse transcription of RNA packaged in lentivirus by a mutated HIV-1 reverse transcriptase (M-RT). This process results in a large population of transduced cells that harbor different single nucleotide variants and large and short indels in the integrated cDNA. If this cDNA is the open reading frame of a purported anti-cancer drug target, mutations that confer resistance can then be identified by culturing the cells in the presence of that drug, which would enrich over time cells that harbor resistance mutations that are then identified by next-generation sequencing (NGS). We have used this methodology to identify on-target resistance mutations to clinical and preclinical inhibitors and antibody-drug-conjugates (ADCs) of non-small cell lung cancer (NSCLC) driver oncogenes such as mutant EGFR, HER2, KRAS, BRAF as well as RET, ROS1 and ALK fusion genes.

Somatic Mutation But Not Aneuploidy Differentiates Lung Cancer In Never-Smokers and Smokers

Sitapriya Moorthi, Amy Paguirigan, Minjeong Ko, Mary Pettinger, Anna Hoge, Anwesha Nag, Neil Patel, Feinan Wu, Cassie Sather, Mathew Fitzgibbon, Aaron Thorner, Garnet Anderson, Gavin Ha, Alice Berger

Fred Hutchinson Cancer Center

Presented by: Sitapriya Moorthi

The genomic characterization of lung cancer has transformed its clinical management. The majority of these analyses have been done on samples from patients with a history of smoking. However a significant proportion of cases, occurs in patients who have never smoked. Lung cancer in never-smokers is distinct from that in smokers. The most frequently diagnosed histological subtype of lung cancer in never-smokers is adenocarcinoma, women are diagnosed more often than men, particularly older women and the majority of lung cancer cases in south and east Asian women occur in never-smokers. At the genetic level, lung tumors from smokers have a significantly higher overall somatic mutation rate and different somatic mutation patterns than tumors from never-smokers, suggesting alternative mechanisms of cancer development in never-smokers and smokers. Here, we sought to define the genetic landscape of lung cancer in female never-smokers along with a matched cohort of smokers. We performed a custom whole-exome genomic analysis of 73 tumor and matched normal DNA from post-menopausal women that were enrolled into the trials of the Women's Health Initiative (WHI) and developed lung cancer. We found that never-smokers displayed a unique mutational spectrum of *EGFR* and *KRAS* variants with implications for both targeted and immunotherapy. Moreover, we surprisingly did not detect chromosomal fusions in *ALK*, *RET*, and *ROS1* suggesting that lung cancers from older female never-smokers may have lower rates of these fusion oncogenes. Somatic mutation signature analysis found DNA repair defect signatures in 22% of the tumors, although we were unable to attribute this phenotype to germline cancer predisposition variants. Finally, we confirm the distinct copy number subtypes of lung adenocarcinoma, defined in the recent NCI Sherlock study, but we found that these subtypes are shared across tumors from both smokers and never-smokers. Thus, we found that aneuploidy and somatic copy number alteration are a more general features of lung cancer and are not related to a history of smoking. Overall, our study provides insights into the genetic landscape of lung cancer in female never-smokers, highlighting differences from smokers and suggesting potential avenues for diagnosis and targeted therapy.

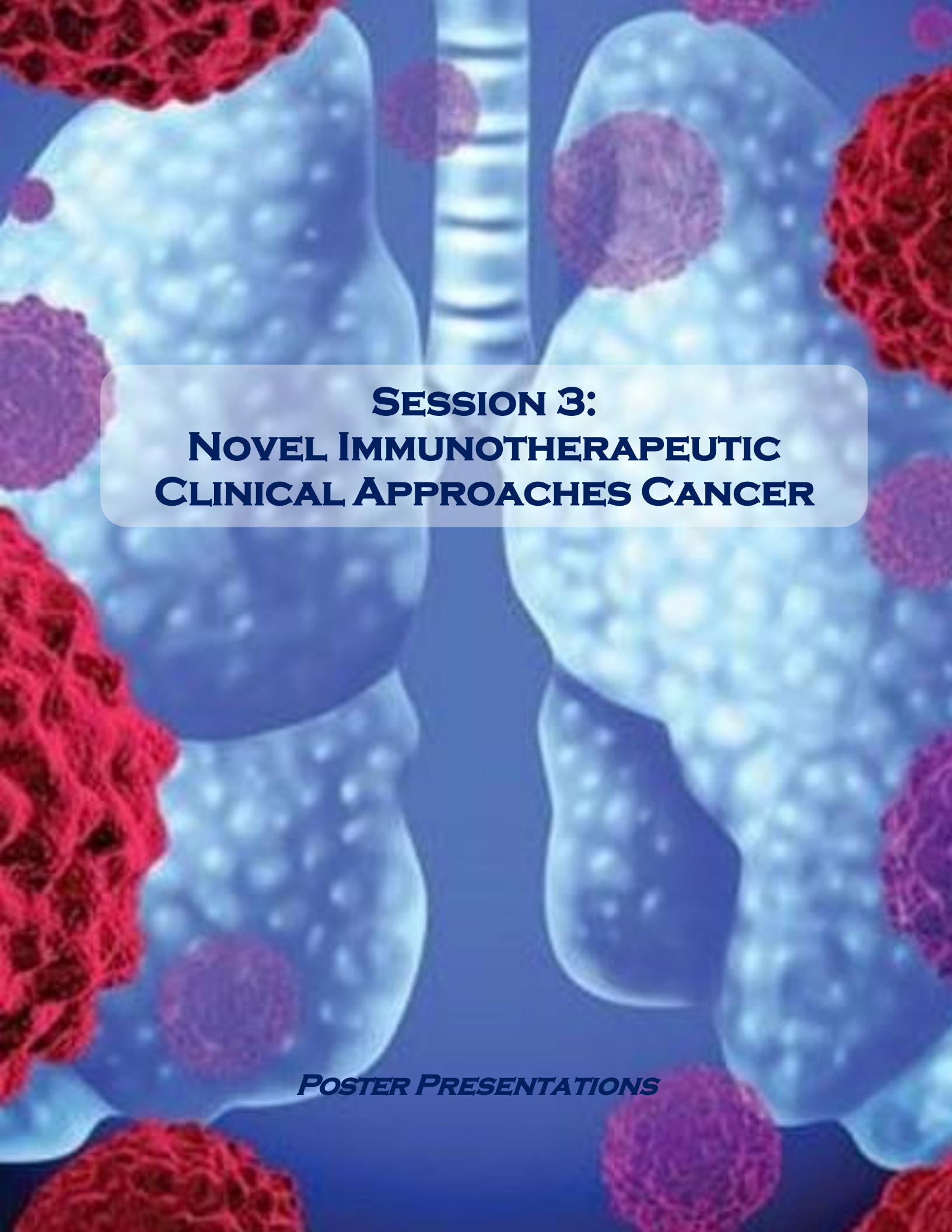
Targeting PIK3CA-Mutant Lung Squamous Cell Carcinoma

Hatim E. Sabaawy, Gina M. Castellano, Dinoop Ravindran Menon, and Sharon R. Pine

University of Colorado School of Medicine

Presented by: Sharon Pine

Despite the fact that two-thirds of lung squamous cell carcinomas (LUSC) have dysregulated PI3K/AKT/mTOR signaling, there have been very limited advancements in effective targeted therapeutics against this pathway. Over 5% of LUSC tumors harbor oncogenic mutations in PIK3CA, which encodes for the p110 alpha isoform of the catalytic subunit of PI3K. There has not been success in targeting oncogenic PIK3CA mutations in LUSC due to multifactorial reasons including toxicities, poor responses due to co-occurring oncogenic driver mutations or activation of feedback mechanisms. Our lab has been exploring combinations of drugs with PI3K inhibitors. Of note, we recently reported that CC-115, a dual mTORC1/2 and DNA-PK inhibitor, sensitizes LUSC to chemotherapy. We demonstrated that CC-115 synergizes with carboplatin in nearly half of NSCLC cell lines, primarily PIK3CA-mutant LUSC. Synergy was more potent in cell lines with decreased basal levels of activated AKT and DNA-PK, evidenced by reduced P-S473-AKT, P-Th308-AKT, and P-S2056-DNA-PKcs, independently of PIK3CA mutations. CC-115 sensitized LUSC to carboplatin by inhibiting chemotherapy-induced AKT activation and maintaining apoptosis, particularly in PIK3CA-mutant cells lacking wild-type (WT) TP53. In addition, pathway analysis revealed that enrichment in the IFNα and IFNγ pathways were significantly associated with synergy. In LUSC patient-derived xenograft and cell line tumor models, CC-115 plus platinum-based doublet chemotherapy significantly inhibited tumor growth and increased overall survival as compared with either treatment alone at clinically relevant dosing schedules. IHC and immunoblot analysis of CC-115-treated tumors demonstrated decreased P-Th308-AKT, P-S473-AKT, P-S235/236-S6, and P-S2056-DNA-PKcs, showing direct pharmacodynamic evidence of inhibited PI3K/AKT/mTOR signaling cascades. The combination treatment in immunocompromised and immunocompetent mice did not exacerbate the clinically accepted side effects of standard-of-care chemotherapy. While we found that activating mutations in PIK3CA were not a robust biomarker for effective anti-cancer activity of CC-115 plus chemotherapy, the results provide strong support for the further investigation of CC-115 plus chemotherapy in LUSC. We have also performed synthetic lethal siRNA screens against genes that are clinically targetable or have drugs in the pipeline, in PIK3CA mutant cell lines combined with Alpelisib. Thus far we have noted a wide degree of inter-tumoral heterogeneity of genes that, when targeted, significantly improve the effectiveness of Alpelisib. We are exploring novel combinations of drugs targeting these genes in our patient-derived organoid and xenograft models.



Session 3:
Novel Immunotherapeutic Clinical Approaches Cancer

Poster Presentations

Small Molecule Bax Activator Sensitizes Chemoradiotherapy and Enhances Immunotherapy In Treatment Resistant Lung Cancer

Abu Syed Md Anisuzzaman, Muhammad Waliul Talukdar, Rafi Ahmed, Suresh S. Ramalingam, and Xingming Deng

Emory University

Presented by: Xingming Deng

Induction of apoptosis is a critical mechanism underlying the durable efficacy of cancer treatment agents. Bax is a major proapoptotic protein whose activation is required for apoptotic cell death. We have recently discovered that small molecule Bax activator CYD-2-11 targets the S184 structural pocket in the c-terminal tail of Bax, directly activates its proapoptotic activity via conformational change and formation of Bax homo-oligomers in mitochondrial membranes. CYD-2-11 has potent anti-tumor activity against lung cancer in various animal models. Here we further discovered that depletion of endogenous mutant KRAS blocks Bax phosphorylation and sensitizes human lung cancer cells to radiation, cisplatin and Bcl-2 inhibitor(s). Combination of CYD-2-11 with radiation, cisplatin, BH3 mimetic Bcl2 inhibitor venetoclax (ABT-199) synergistically suppress non-small cell lung cancer (NSCLC) in vitro and in vivo. KRAS mutation(s) accompanying with LKB1- or p53-mutation(s) or deficiency are associated with therapeutic resistance to PD-1/PD-L1 checkpoint blockade immunotherapy. CYD-2-11 but not anti-PD-L1 has potent antitumor activity against immunologically “cold” mutant KRAS driven lung cancer. Combined treatment with CYD-2-11 and anti-PD-L1 synergistically produces maximum efficacy in tumor burden reduction and significantly prolongs survival of KL and KP mice. Mechanistically, combined treatment enhances intratumor T cells (T cell infiltration in tumor tissues). These findings provide preclinical evidence for pharmacologic combinations of small molecule Bax activator with chemoradiotherapy, Bcl-2 inhibitor or PD-L1 antibody as effective strategies for lung cancer therapy.

Immune Cell Profiling to Predict Future Immune-Related Adverse Events in Lung Cancer and Other Malignancies

Hong Mu-Mosley, David Farrar, Farjana Fattah, Jialiang Jia, Yaming Xue, Yang Xie, Angela Mobley, Mitchell von Itzstein, Amrit Gonugunta, Rodrigo Catalan, Jason Park, Shaheen Khan, Edward Wakeland, David Gerber

UT Southwestern Medical Center

Presented by: David Gerber

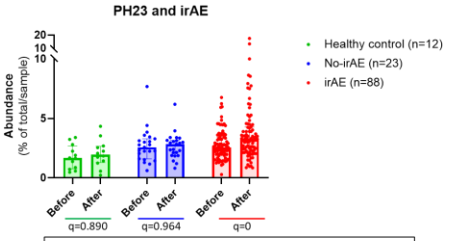
Immune checkpoint inhibitors (ICI) have revolutionized the therapeutic landscape of patients with malignancies. However, ICI are often associated with potentially severe autoimmune toxicities known as immune-related adverse events (irAE). The occurrence, type, severity, and timing of irAE remain unpredictable. Although irAE have been studied extensively in melanoma populations treated with anti-CTLA4-based therapy, far less is known about them in other malignancies, including lung cancer.

We applied mass cytometry by time of flight (CyTOF) to study peripheral blood immune cells profiles from cancer patients treated with anti-PD-1 monotherapy or combination anti-PD-1 + anti-CTLA-4 ICI. We analyzed 246 paired pre- and post-ICI initiation (≤12 weeks) blood samples from 111 cancer patients (non-small cell carcinoma 40%, melanoma 42%, other 18%) and paired peripheral samples (separated by up to 8 weeks) from healthy controls (n=12).

We found a baseline, pre-treatment abundance (% of total CD45+ cells/sample) of cluster PH.23 (CD3+CD4+CD45RO+CD45RA-CCR7-CD25+CCR4+HLA-DR+CD38+CD27+, activated effector memory CD4 T cells) in patients with eventual irAE. These patients also displayed a significant post-ICI initiation increase in cluster PH.23 compared to no-irAE cancer patients and healthy controls (**Figure1**, FDR adjusted $P<0.001$ in irAE group, but no significant change in healthy control or no irAE cases, paired t test). The increased frequency of PH.23 in the peripheral blood of patients with irAE was observed across cancer type and ICI type (**Figure 2**).

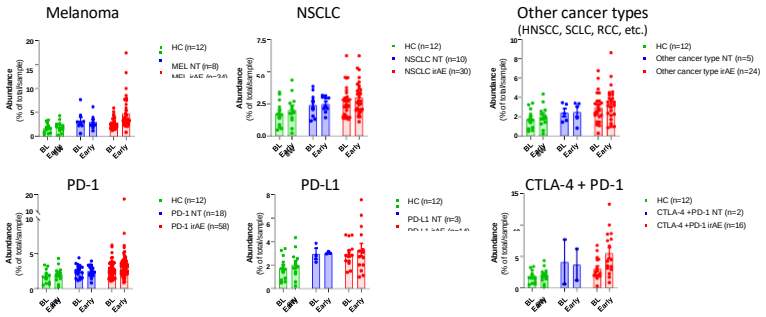
In conclusion, elevated populations of potential effector memory CD4 T cells in before and during ICI treatment may be associated with heightened irAE risk in lung cancer and other malignancies. This information could eventually inform the selection of patients, ICI regimens, and clinical toxicity monitoring.

Figure 1. **PH.23 elevated in PB of patients with irAE within 12 weeks after ICI treatment**



Adjusted p values are shown Before/After ICI treatment by Wilcoxon test
q=0 by SAM in OMIQ, FDR=0.1
Each bar represents the median with interquartile range

Figure 2. **The increased frequency of PH.23 in the PB of patients with irAE was observed across cancer type and ICI type**



Hyper-Interferon Sensitive (HIS) Influenza Virus Sensitizes Murine NSCLC To Anti-PD-1 Therapy

Ramin Salehi-Rad, Yushen Du, Tian-hao Zhang, Dongdong Chen, Yuan Shi, Jiang Hong, Tseng Yenwen, Bin Liu, Steven Dubinett, Ren Sun

University of California Los Angeles

Presented by: Ramin Salehi-Rad

Exclusion of T cells from the tumor or the presence of a dysfunctional T cell compartment within the tumor microenvironment (TME) constitute two central hallmarks of resistance to immune checkpoint inhibition (ICI) in patients with NSCLC. Seminal studies have identified that loss of LKB1 in *KRAS*-mutant NSCLC drives resistance to ICI, possibly through the suppression of STING which results in dysregulation of the interferon (IFN) signaling. Because of the critical function of host IFN signaling in activation of anti-tumor adaptive immune responses, treatment strategies that leverage the IFN pathway hold promise for combating immune resistance.

One approach to augment host IFN signaling within the TME is virotherapy with engineered viruses that retain the ability to induce well-controlled anti-tumor immune responses while harboring limited capacity for viral replication. We developed a live attenuated influenza viral vaccine, designated as 'hyper-interferon sensitive (HIS)', through systematic elimination of eight IFN evasion genes from the influenza genome. HIS virus induced robust IFN responses in human and murine NSCLCs *in vitro*, which was superior to wild-type (WT) influenza. While HIS and WT viruses had similar replication capacity in IFNAR$^{-/-}$ mice, a ~3-log reduction in viral titers was observed in the lungs of immunocompetent mice treated with HIS compared to WT, consistent with IFN-mediated abrogation of HIS replication. Intratumoral administration of HIS virus demonstrated superior efficacy compared to WT virus in multiple syngeneic murine models of NSCLC which was dependent on local induction of type 1 IFN response. HIS virotherapy induced host T cell and NK infiltration and activation in the TME and led to synergistic tumor inhibition when combined with anti-PD-1. Successful eradication of tumors following combination therapy with HIS virus and anti-PD-1 led to systematic, long-lasting anti-tumor immunity. These studies provide compelling evidence that support further clinical evaluation of HIS virotherapy for NSCLC refractory to current ICI immunotherapies.

A Therapeutic Cancer Vaccine Delivers Antigens and Adjuvants to Lymphoid Tissues Using Genetically Modified T Cells

Joshua R. Veatch, Naina Singhi, Julia Szeto, Sylvia Lee, Shivani Srivastava, and Stanley R. Riddell

Fred Hutchinson Cancer Research Center

Presented by: Joshua Veatch

Therapeutic vaccines to augment T cell responses to tumor antigens have been limited by poor potency in clinical trials. In contrast, transfer of T cells modified with foreign transgenes frequently induce potent endogenous T cell responses to epitopes in the transgene product, which are unwanted because they lead to rejection of transferred T cells. We sought to harness gene-modified T cells as a vaccine platform. We developed cancer vaccines comprised of autologous T cells modified with tumor antigens and additional adjuvant signals (T-vaccine). T cells expressing model antigens and a broad range of tumor neoantigens induced robust and durable T cell responses through cross-presentation of antigens by host dendritic cells. Providing T-vaccine with signals such as CD80, CD137L, IFN-b, IL-12, GM-CSF and FLT3L enhanced T cell priming. Co-expression of IL-12 and GM-CSF induced the strongest $CD4^+$ and $CD8^+$ T cell responses through complimentary effects on recruitment and activation of dendritic cells. We determined that autocrine IL-12 signaling in T-vaccine cells was sufficient to augment immunity, so a constitutively activated IL-12 receptor could be used to reduce potential toxicity. Therapeutic vaccination with T-vaccine and adjuvants exhibited antitumor activity in subcutaneous and metastatic preclinical models. We now describe the development of a human T-vaccine product incorporating a constitutively activated IL-12 receptor and GM-CSF targeting the cancer-testis antigen CT83, which is expressed in a significant subset of non-small cell lung cancer and known to have T cell responses in patients. We propose our plan for an upcoming first in human clinical trial in pretreated NSCLC expressing CT83.

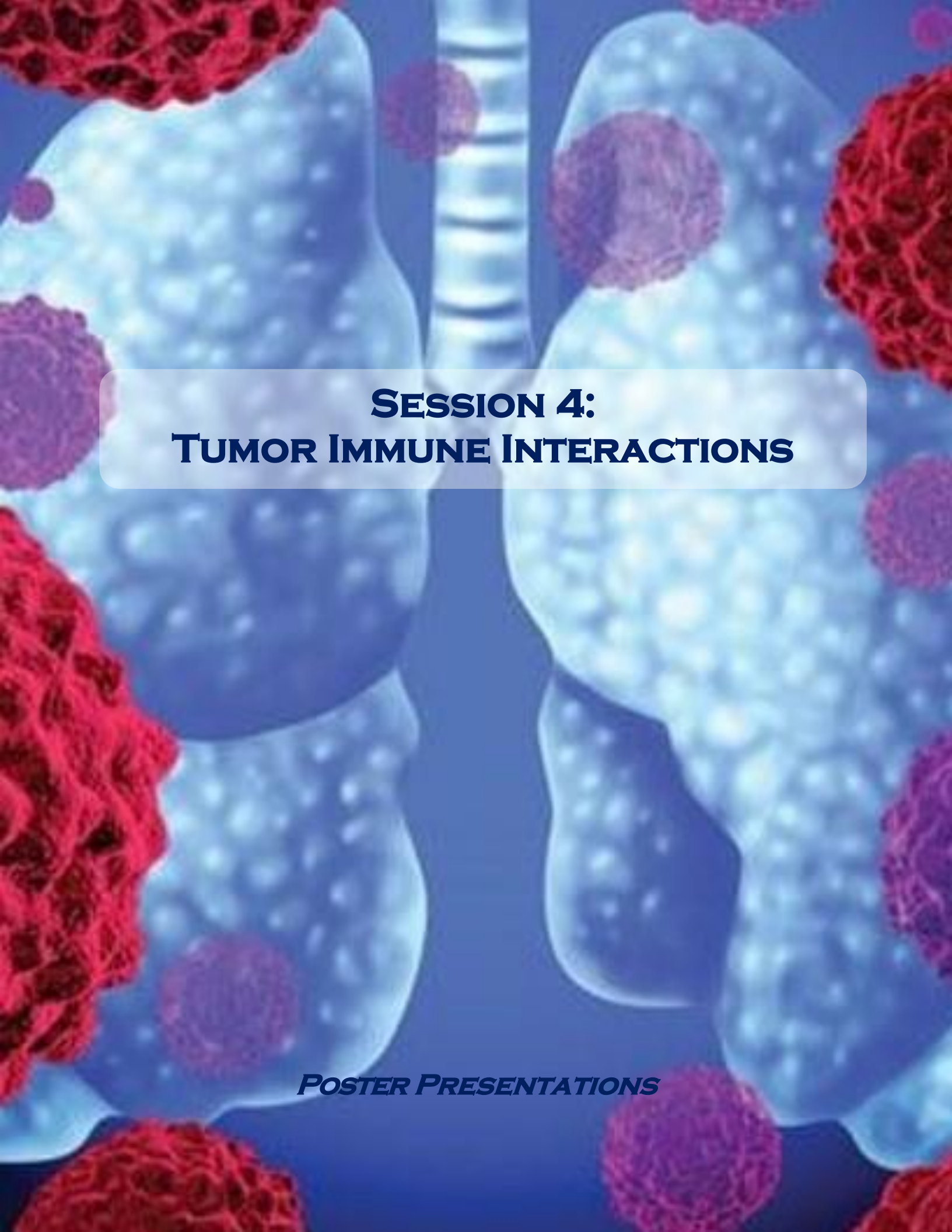
MERTK Inhibition Potentiates Host-Versus-Lung Cancer Immunity and Overcomes Resistance to Immune Checkpoint Inhibition

Dan Yan, Xiaodong Wang, Stephen V. Frye, H. Shelton Earp, Deborah DeRyckere, and Douglas K. Graham

Emory University, University of North Carolina at Chapel Hill

Presented by: Dan Yan

The TAM (TYRO3, AXL, MERTK) family receptor tyrosine kinases mediate apoptotic cell clearance to maintain tissue homeostasis and activation during efferocytosis promotes an immunosuppressive phenotype, which can be hijacked by tumor cells to promote immune evasion. MERTK inhibition on immune cells subsets in the tumor microenvironment (TME) and the role in resistance to immune checkpoint inhibitors in lung cancer has not been clearly defined. Using a subcutaneous *KRAS*-mutant lung cancer tumor model in wild-type (WT), *Axl* knockout (KO), or *Mertk* KO C57Bl/6 mice, we found that the *Mertk* KO background blocked the *KRAS*-mutant tumor growth. However, the *Axl* KO background did not alter *KRAS*-mutant tumor growth. The anti-tumor effect seen in *Mertk* KO mice was abrogated in *Mertk* KO mice which also had a background of severe combined immunodeficiency (deficient of T and B cell), suggesting MERTK as an immunotherapeutic target in this *KRAS*-mutant lung cancer. The changes in immune cell repertoire and phenotype in tumors from *Mertk* KO and WT were further assessed. Although MERTK expression was not detected on $CD11c^{+}CD11b^{-}MHCII^{+}$ tumor-associated dendritic cells (DCs), there was a significant increase in the incidence of DCs in tumors from *Mertk* KO mice compared to WT mice by day 5 after inoculation of tumor cells. Characterization of tumor-associated macrophages by flow cytometry revealed three distinct populations expressing different levels of MHCII ($CD11b^{+}MHCII^{high}$, $CD11b^{+}MHCII^{+}$ and $CD11b^{high}MHCII^{neg}$). Tumors from *Mertk* KO mice had significantly greater numbers of $MHCII^{high}$ macrophages by day 10 after tumor cell inoculation and reduced numbers of $MHCII^{neg}$ macrophages by day 19. Together, the increased incidence of dendritic cells and the shift toward a more antigen-presenting macrophage phenotype are expected to promote anti-tumor immunity in *Mertk* KO mice. MRX-2843 is a novel first-in-class MERTK-selective inhibitor and is currently being evaluated for efficacy in clinical trials. Treatment with MRX-2843 provided a dose-dependent inhibition of tumor growth. Furthermore, *Mertk* KO or treatment with MRX-2843 enhanced sensitivity to immune checkpoint inhibitors (ICIs) and overcame ICI resistance in two different murine *KRAS*-mutant lung cancer models, suggesting a role for MERTK in mediating resistance to ICIs in lung cancer models. These findings establish MERTK as a potential immunotherapeutic target in the non-small cell lung cancer (NSCLC) TME.



Session 4:
Tumor Immune Interactions

Poster Presentations

Overexpressed Malat1 Drives Metastasis Through Inflammatory Reprogramming of Lung Adenocarcinoma Microenvironment

Fernando de Miguel, Katerina Politi, Jesse Zamudio, Nadya Dimitrova

Yale University

Presented by: Nadya Dimitrova

Metastasis is the main cause of cancer deaths but the molecular events leading to metastatic dissemination remain incompletely understood. Despite reports linking aberrant expression of long noncoding RNAs (lncRNAs) with increased metastatic incidence, *in vivo* evidence establishing driver roles for lncRNAs in metastatic progression is lacking. Here, we report that overexpression of the metastasis-associated lncRNA *Malat1* (metastasis-associated lung adenocarcinoma transcript 1) in the autochthonous K-ras/p53 mouse model of lung adenocarcinoma (LUAD) is sufficient to drive cancer progression and metastatic dissemination. We show that increased expression of endogenous *Malat1* RNA cooperates with p53 loss to promote widespread LUAD progression to a poorly differentiated, invasive, and metastatic disease. Mechanistically, we observe that *Malat1* overexpression leads to the inappropriate transcription and paracrine secretion of the inflammatory cytokine, Ccl2, to augment the mobility of tumor and stromal cells *in vitro* and to trigger inflammatory responses in the tumor microenvironment *in vivo*. Notably, Ccl2 blockade fully reverses cellular and organismal phenotypes of *Malat1* overexpression. We propose that *Malat1* overexpression in advanced tumors activates Ccl2 signaling to reprogram the tumor microenvironment to an inflammatory and pro-metastatic state.

PTEN Loss Confers Resistance to Anti-PD-1 Therapy In NSCLC By Increasing Tumor Infiltration Of T Regulatory Cells

Francisco Exposito, Miriam Redrado, Maeva Houry, Katherine Hastings, Teresa Lozano, Magdalena Molero-Abraham, Ramón Amat, Jose L Solorzano, Sergio Leon, Javier Garcia, Diego Serrano, Camila Robles-Oteiza, Giorgia Foggetti, Enriqueta Felip, Juan J Lasarte, Luis Paz-Ares, Jon Zugazagoitia, Katerina Politi, Luis Montuenga, Alfonso Calvo

Yale University, University of Navarra, Hospital Universitario 12 de Octubre, Vall d'Hebron Institute of Oncology, MD Anderson Cancer Center (Spain),

Presented by: Francisco Exposito

Immune checkpoint inhibitors (ICIs) targeting PD-L1/PD-1 have dramatically improved the management of NSCLC patients. Nevertheless, more than 50% patients will present primary resistance or will develop secondary resistance within 12-25 months after treatment initiation. The mechanisms of resistance to ICIs may depend on tumor-intrinsic molecular/genetic alterations that generate an immunosuppressive tumor microenvironment (*is*TME). Using *In silico* analyses (TCGA and AACR-GENIE cohorts), and protein expression analysis in our institutional CIMA-CUN cohort, we have shown that >50% LUSC and ~28% LUAD tumors exhibited reduced *PTEN* mRNA/protein levels. Patients with PTEN-low tumors had higher levels of CXCL10, PD-L1 and PD-L2. In a cohort of stage IV NSCLC patients treated with ICIs, we found that patients with low tumor PTEN levels were significantly associated with worse OS (p=0.021). We have developed and characterized a *Pten*-null LUSC model using UN680 cells, which was also refractory to anti-PD-1 when implanted in syngeneic mice. Pten loss led to a deregulation of multiple pathways related to immunosuppression, with a positive enrichment in "IL-6/JAK/STAT3" and "TNF-α via NFkB" hallmarks, identified by GSEA. Tumors from the *Pten*-null LUSC model were highly metastatic and fibrotic, and secreted TGF-β/CXCL10, thus favoring conversion of CD4+ lymphocytes into Tregs. Human/mouse PTEN-low tumors were enriched in Tregs and immunosuppressive genes. Importantly, treatment of mice bearing *Pten*-null tumors with TLR agonists+anti-TGF-β, aimed to alter this *is*TME, led to tumor rejection and immunological memory in 100% of mice. Our results demonstrate that lack of *PTEN* causes immunotherapy resistance in LUSC by establishing an *is*TME that can be reversed therapeutically.

Induction of Tertiary Lymphoid Structures In Non-Small Cell Lung Cancer Improves B and T Cell Anti-Tumor Immunity

Hye Mi Kim, Noor Nader, Ian Macfawn, Jennifer Lue, Allison Casey, Alexandra McDonough, Sheryl Kunning, Asia Williams, Stephen Thorne, Laura Stabile, Tullia C. Bruno

University of Pittsburgh

Presented by: Hye Mi Kim

Tertiary lymphoid structures (TLS) are lymphoid aggregates that often form locally in tissues with chronic infection, autoimmune disease, and cancer. TLS correlate with favorable prognosis in patients with solid tumors, including non-small-cell lung cancer (NSCLC). Further, TLS have recently been associated with superior response to immune checkpoint blockade (ICB). B cells are predominantly located within TLS and correlate with improved survival and ICB response. Despite the therapeutic promise of B cells and TLS, they have not been investigated as immunotherapeutic targets. Moreover, a mechanistic understanding of TLS formation and function in cancer is lacking. Our studies aim to interrogate unique factors that promote or inhibit TLS formation. First, we studied TLS in human lung adenocarcinoma using multispectral imaging and spatial transcriptomics (Nanostring Digital Spatial Profiler) to uncover pathways that could improve TLS formation and subsequently B and T cell function. According to spatial transcriptomics, tumor-associated TLS have increased expression of factors such as LIGHT that initiate secondary lymphoid organs development and CXCL13, IL-21 and CD40 ligand that promote germinal center (GC) response, in comparison to TLS absent regions. We paired these studies with murine models of lung adenocarcinoma. Specifically, we utilized a physiologically relevant, carcinogen (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK) induced murine model of lung cancer that spontaneously forms TLS. A syngeneic tumor line derived from this model that can be orthotopically injected into the murine lung also forms high B cell infiltrate. We utilized these models to test if TLS formation was increased with an oncolytic virus (OV) that targets TLS initiation and GC maturation factors. As a result, we have observed increased formation and improved maintenance of TLS upon treatment with an OV that delivers LIGHT, CXCL13, IL-21 and CD40 ligand, compared to an empty vector. Tumor was reduced at varying degrees in the mice treated with different combinations of transgenes via OV. We will further identify TLS promoting factors using spatial transcriptomics and use OV to modulate TLS formation and maturation. These studies will increase our understanding of TLS formation for improved immunotherapies in NSCLC patients and will potentially provide therapeutic interventions to treat patients.

Objective Analysis and Clinical Significance Of The Spatial Tumor Infiltrating Lymphocyte Patterns In Non-Small Cell Lung Cancer

Miguel Lopez de Rodas, Yvonne Wang, Gang Peng, Jianlei Gu, Hongyu Zhao & Kurt A. Schalper

Yale University

Presented by: Miguel Lopez de Rodas

Introduction: Previous studies of spatial analysis of TILs using semi-quantitative pathology-based approaches have revealed marked differences in the spatial distribution of lymphocytes in the tumor microenvironment with expected associations with patient outcomes. Tumors are generally classified based on the relative abundance of TILs in the tumor center/core and the invasive tumor margin into three main groups: "Immune infiltrated"; "Immune excluded"; and "Immune desert". These spatial patterns are expected to reflect the underlying biology mediating tumor immune evasion and immune checkpoint inhibitor (ICI) sensitivity/resistance. However, the role of these patterns in NSCLC as well as their independence from other TIL metrics remain uncertain.

Methods: We used multiplexed quantitative immunofluorescence to study major TIL subsets (DAPI, CK, CD4, CD8 and CD20) with single-cell resolution in full-face baseline whole-tumor slides from a large multi-institutional cohort of NSCLC patients treated with ICI (n=176). The spatial TIL patterns were analyzed using a qualitative pathologist-based approach, as well as with objective analysis of TIL density ratios in tumor/stromal tissues stratified using K-means clustering. The association of the spatial patterns with ICI outcomes were studied for different TIL markers.

Results: The analysis of CD8+ TILs using qualitative assessment of pre-defined visual patterns identified prominent limitations of this approach including the presence of a broad spectrum of phenotypes within most tumors and limited association with outcomes. The utilization of an objective method to classify NSCLCs based on their predominant spatial TIL distribution showed the existence of at least three predominant subgroups with more balanced representation within the cohort and partial overlap with those defined using visual inspection patterns. Using this strategy, a subset of cases with "immune excluded-like" tumors corresponding to 22% of the cohort showed prominently worse outcomes, suggesting reduced sensitivity to ICI. The analysis of cases for other TIL subsets showed dissimilar results as compared with CD8 T-cells, underscoring the relevance of the marker selected for spatial TIL pattern evaluation and opportunities for integrating markers.

Conclusion: Our results identified major challenges associated with the qualitative spatial TIL pattern evaluation. We devised a novel objective strategy to overcome some of these limitations that has strong biomarker potential.

A Multiplexed Time-Resolved Fluorescence Resonance Energy Transfer Ultrahigh-Throughput Screening Assay for Targeting SMAD4-SMAD3-DNA Complex

Wukun (Kenny) Ouyang, Qiankun Niu, Min Qui, Haian Fu, Yuhong Du, Xiulei Mo

Emory University

Presented by: Xiulei Mo

Immune checkpoint inhibitor (ICI) therapy is a first-line treatment for advanced lung adenocarcinoma. However, most patients do not respond, and relapse after initial response represents another major challenge. Transforming growth factor-b (TGFb) signaling is an emerging and attractive cancer immunotherapy target. Multiple anti-TGFb signaling therapies have been developed to focus on inhibiting upstream TGFb and membrane receptors. However, most clinical trials failed to recapitulate the encouraging observations from preclinical *in vitro* and mouse models. These daunting challenges not only highlight the complexity of TGFb signaling, but also underscore the need for further expanding the anti-TGFb signaling therapy toolbox.

SMAD4 (Mothers against decapentaplegic homolog 4) is an essential downstream master regulator of the canonical TGFb signaling pathway. SMAD4 functions as an adaptor protein forming a protein-protein interaction (PPI) complex with the receptor regulated SMADs, such as SMAD3. Upon TGFb activation, the SMAD4-SMAD3 PPI complex translocates into the nucleus, binds to SMAD-binding elements (SBE) containing DNA sequence, and activates a spectrum of TGFb target gene expression. However, SMAD4 is still considered "undruggable" due to a lack of enzymatic activity and a large interaction interface.

Herein, to discover SMAD4 inhibitors for expanding the toolbox of anti-TGFb signaling therapy, we report the development of a multiplexed time-resolved fluorescence resonance energy transfer (TR-FRET) assay. The assay measures SMAD4-SMAD3 PPI and SMAD-SBE protein-DNA interaction (PDI) simultaneously. The assay enables us to monitor SMAD4-SMAD3-SBE PPI and PDI dynamics at a single amino acid resolution in a homogenous cell lysate-based configuration. Moreover, the assay can be miniaturized into an ultrahigh-throughput screening 1,536-well plate format for large-scale campaigns of SMAD4 inhibitors towards the future discovery of novel anti-TGFb signaling therapy drugs. Our overarching goal is to develop novel SMAD4 inhibitors that disrupt the TGFb-mediated immunosuppressive pathway, as a potential therapy for metastatic lung adenocarcinoma.

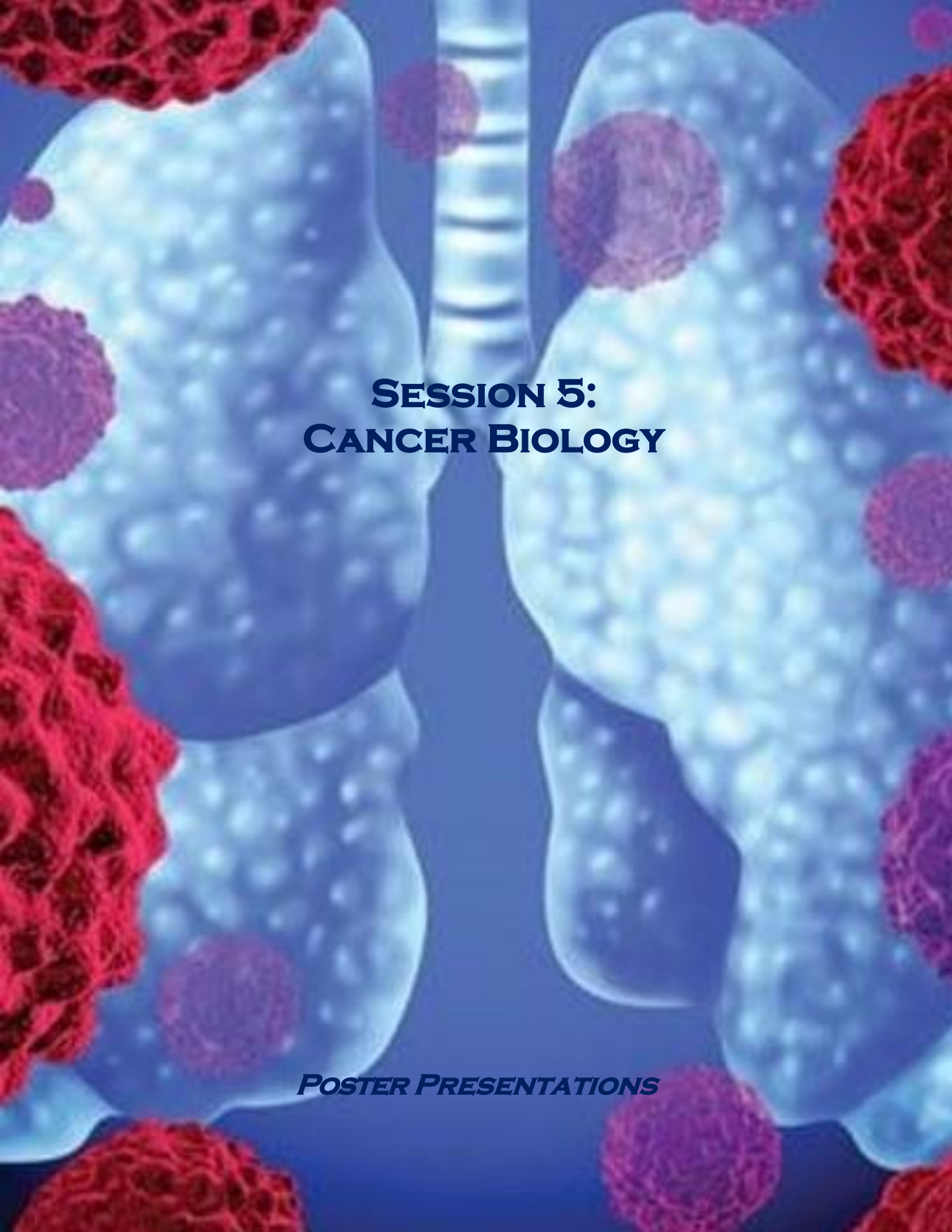
Translational Control of Immune Checkpoint Proteins By the Integrated Stress Response (ISR) Pathway In Non-Small Cell Lung Cancer

Shayna Thomas-Jardin, Shruthy Suresh, Cheryl Lewis, Chul Ahn, Bret M. Evers, John D. Minna, and Kathryn A. O'Donnell

UT Southwestern Medical Center

Presented by: Kathryn A. O'Donnell

Groundbreaking discoveries in identifying and exploiting the PD-1/PD-L1 (Programmed Death 1/ Programmed Death Ligand 1) and CTLA-4 immune checkpoints have resulted in the approval of monoclonal antibodies that disrupt these interactions as a first-line therapy for non-small cell lung cancer (NSCLC) patients. However, only ~20% of NSCLC patients derive long term benefit from immune checkpoint blockade (ICB) and there remains a disconnect between tumor expression of ICB targets and therapy response. A critical question remains as to what mechanisms facilitate resistance to PD-1/PD-L1 therapy and whether other immune checkpoints or pathways can be targeted in combination with existing therapies. The integrated stress response (ISR) pathway represents an emerging therapeutic vulnerability in lung cancer. We previously demonstrated that ISR activation potently induces PD-L1 in NSCLC (Suresh et al, *Nature Cancer*, 2020). We discovered that ISR pathway activation enhances *PD-L1* translation through the bypass of inhibitory upstream open reading frames (uORFs) in the *PD-L1* 5' UTR in a manner dependent upon the translation initiation factor eIF5B. Our latest studies demonstrate that the immune checkpoint protein, CD155 (PVR, poliovirus receptor cell adhesion molecule), is also induced by ISR activation. Both PD-L1 and CD155 are induced by multiple arms of the ISR pathway, and CD155 harbors putative inhibitory uORFs in its 5' UTR. Of clinical relevance, we observe a significant positive correlation between PD-L1 and CD155 expression in primary lung adenocarcinomas. We hypothesize that increased translation of both *PD-L1* and *CD155* due to activation of the ISR contributes to tumor cell immune escape. To address this hypothesis, we are dissecting the mechanisms of ISR mediated *PD-L1* and *CD155* translational control and examining the impact of ISR activation on immune cell responses in human cells and mouse models. We are also determining the extent to which ISR inhibition suppresses tumorigenesis by promoting anti-tumor immunity and whether this may synergize with existing immune checkpoint therapies. These studies may reveal new regulatory circuits that control immune checkpoint activity and lead to new targeted therapies for lung cancer.



Session 5:
Cancer Biology

Poster Presentations

The AVERON Platform to Discover Actionable Vulnerabilities Enabled By Rewired Oncogenic Networks

Andrey A. Ivanov and Haian Fu

Emory University

Presented by: Andrey A. Ivanov

Missense mutations lead to the rewired protein-protein interaction (PPI) networks and acquisition of cancer hallmarks. While some mutations can disrupt the PPIs, others may induce new PPIs that are not natural for the wild-type counterparts. However the translation of the landscape of oncogenic mutations into clinically actionable biological models for cancer target discovery is highly challenging. To infer clinically actionable mechanistic insights into how mutant-enabled or neomorph PPIs (neoPPIs) promote tumorigenesis, we develop a set of innovative informatics tools for discovering Actionable Vulnerabilities Enabled by Rewired Oncogenic Networks (AVERON). Implemented in a widely-used Jupyter Notebook format, the AVERON streamlines the identification of the oncogenic programs and clinically significant genes that are regulated by neoPPIs in cancer patients. The AVERON can recapitulate well-established connectivity between known mutant-dependent PPIs and specific oncogenic pathways and reveal new, previously unknown mechanisms of neoPPI-mediated oncogenic signaling. To inform new therapeutic strategies in neoPPI-dependent cancers, AVERON connects neoPPI-regulated genes with available approved drugs and clinical compounds. Together, the AVERON provides a powerful informatics environment to determine therapeutically actionable vulnerabilities created by mutant-regulated protein-protein interactions to inform new personalized therapeutic strategies in cancer.

Using Spatial Genomics to Dissect Cell: Cell Cooperation In Lung Adenocarcinoma

Raehannah Jamshidi, Adam Marcus, Frank Schneider, Vaunita Parihar, Lyra Griffiths, Rich Johnston

Emory University

Presented by: Raehannah Jamshidi

Lung cancer is the second most common cancer in the United States and kills more people in this country than any other cancer type. Unfortunately, efficacious treatments for lung cancer remain suboptimal. The diversity between and within lung cancer subtypes, as well as within a patient (i.e. metastases vs primary tumor), make treating this cancer very challenging. Lung adenocarcinoma, in particular, has collective invasion packs of cells adjacent to the primary tumor that correlate with metastatic disease in mouse models. We hypothesize that the transcriptomic profile of the collective invasion packs in lung adenocarcinoma patients varies significantly from the adjacent primary tumor and represents a targetable metastatic sub-population. This work will help to identify specific cell signaling pathways that have the translational potential to develop novel therapeutics for metastatic disease, ultimately improving patient outcomes through precision medicine. LKB1 is a serine-threonine kinase (also known as STK11) that largely functions as a tumor suppressor, and is mutated in 20-30% of non-small cell lung cancers (NSCLCs). Moreover, *LKB1* is the third highest mutated gene in lung adenocarcinoma (a type of NSCLS) after *TP53* and *RAS.* Utilizing patient lung adenocarcinoma samples with *KRAS* and *KRAS/LKB1* mutations, we identified regions of interest including bulk tumor and surrounding invasion packs. Then, using GeoMx digital spatial profiling technology (by Nanostring) and next-gen sequencing, we generated genomic profiles from bulk tumor and invasion packs. To discern the metastatic potential, genomic profiles will be compared between invasion packs, tumor bulk vs invasion packs, and finally, inter-patient differences. Successful completion of this project will characterize transcriptomes of metastatic lung cancer and has the potential to identify biomarkers of aggressive disease.

In Vivo CRISPR Screen Identifies SOAT1 as a Taxane Chemosensitizing Target For Non-Small Cell Lung Cancer (NSCLC)

Long-Shan Li, Kenneth Huffman, Huiyu Li, Michael Peyton, Hyunsil Park, Kimberly Avila, Luc Girard, Mathew Augustine, Joshua T. Mendell, John D. Minna

University of Texas Southwestern Medical Center

Presented by: Long-Shan Li

Purpose: To identify FDA druggable taxane chemosensitizing gene targets in patient derived NSCLC models.

Methods: We developed a CRISPR-Cas9 lentivirus library encompassing 12,474 sgRNAs targeting 660 FDA proved 'druggable' putative proteins (18 guides/gene) and investigated their potential to chemosensitize lung adenocarcinoma (LUAD) line NCI-H2009 (TP53 and KRAS mutant) in parallel *in vitro* & *in vivo* studies. Key elements were: the use of low doses of paclitaxel (IC10 values for *in vitro* and *in vivo* doses that barely affected tumor growth) compared to control treatment; multiple biologic replicates for each transfection and drug selection; and large representation (2,000 cells/sgRNA) for each guide. At the end time point we harvested multiple tumor replicates of the taxane and control treatments and sequenced each to identify barcodes for each guide that "remained" or "dropped out."

Results: We are looking for guides that selectively "dropped out" (taxane sensitizer) in the low dose taxane exposure comparing taxane to control treatment and compared whether they dropped out *in vitro* and/or *in vivo* (xenografts). Surprisingly, we found little overlap between guides that dropped out *in vitro* vs *in vivo*. From the top 16 genes that selectively dropped out only with taxane treatment and selectively *in vivo* compared to *in vitro*, we focused on SOAT1 (Sterol O-Acyltransferase 1). SOAT1 is a key enzyme which mediates conversion of intracellular free cholesterol to cholesteryl esters which are then stored as lipid droplets. SOAT1 is a potential cancer therapeutic target with a clinically available inhibitor, avasimibe. H2009 cells with SOAT1 hemizygously removed (CRISPR) grew well *in vitro* and *in vivo* in the absence of chemotherapy treatment, and were dramatically sensitized to taxanes compared to parental H2009 cells. While avasimibe treatment, at doses/concentrations achievable in patients, sensitized NSCLC to taxanes, this sensitization was not as dramatic as hemizygous removal by CRISPR. Immunoblotting assay revealed extended G_2M signaling served as an important factor to sensitize NSCLCs to taxanes.

Conclusions: Our results defined 'druggable' targets through CRISPR functional genomic screening with significant differences between *in vitro* and *in vivo* assays, and identified SOAT1 as a therapeutic target for sensitizing NSCLC to taxane treatment (SPORE P50CA070907).

Defining The Role of PER1 and Circadian Rhythm Dysregulation In KRAS/LKB1-Mutant Lung Adenocarcinoma

Rebecca E. Parker, Junghui Koo, Bhakti Dwivedi, Wei Zhou, Adam I. Marcus, Melissa Gilbert-Ross

Emory University

Presented by: Rebecca E. Parker

KRAS and LKB1 are frequently co-mutated in lung adenocarcinoma and characterize a particularly aggressive, metastatic, and treatment-resistant subtype of this disease. We recently published that the outputs of biological rhythm pathways downstream of the AMPK kinase are altered in KRAS/LKB1-mutant tumors. In a live-cell phenotyping screen we used RNAseq to identify the circadian clock genes *PER1* and *PER2* as uniquely upregulated in partially transformed and invasive KRAS/LKB1-mutant human bronchial epithelial cells. We have analyzed expression of individual circadian clock genes in KRAS-mutant and KRAS/LKB1-mutant patient tumors and found that expression of the PER genes, particularly *PER1*, is increased in the KRAS/LKB1 subtype of lung adenocarcinoma. At the protein level, PER1 expression is increased in KRAS/LKB1-mutant HBECs as compared to KRAS-mutant cells. Addback of wild-type LKB1 in A549 cells, which are KRAS/LKB1-mutant, results in a decrease in PER1 protein levels, confirming that LKB1 has a role in controlling PER1 levels. Multiple phosphorylation events lead to PER1 nuclear translocation to control its role as a transcriptional repressor in the core clock. In circadian-synced HBECs, PER1 enters the nucleus in KRAS-mutant cells, but remains cytosolic in KRAS/LKB1-mutant cells. In circadian-synced A549 cells, we also observed a lack of nuclear PER1 localization. Addback of wild-type LKB1 restored nuclear localization of PER1, as did expression of kinase-dead LKB1, suggesting that LKB1's regulation of PER1 localization is kinase-independent. Our current and future experiments aim to determine how clock-controlled gene expression may be altered in KRAS/LKB1-mutant cells, what the functional impact of altered PER1 expression and localization is on growth of KRAS/LKB1-mutant tumors, and whether pharmacological manipulation of LKB1-related signaling pathways can restore nuclear localization of PER1.

Glucose Deprivation Promotes Pseudo-Hypoxia And De-Differentiation In Lung Adenocarcinoma

Pasquale Saggese, Aparamita Pandey, Eileen Fung, Abbie Hall, Jane Yanagawa, Erika F. Rodriguez, Tristan R. Grogan, Giorgio Giurato, Giovanni Nassa, Annamaria Salvati, Alessandro Weisz, Steven M. Dubinett, Claudio Scafoglio

University of California Los Angeles, University of Salerno

Presented by: Claudio Scafoglio

Increased utilization of glucose is a hallmark of cancer. Several studies are investigating the efficacy of glucose restriction by glucose transporter blockade or glycolysis inhibition. However, the adaptations of cancer cells to glucose restriction are unknown. Here, we report the discovery that glucose restriction in lung adenocarcinoma (LUAD) induces cancer cell de-differentiation, leading to a more aggressive phenotype. Glucose deprivation causes a reduction in alpha-ketoglutarate (αKG), leading to attenuated activity of αKG-dependent histone demethylases and histone hypermethylation. We further show that this de-differentiated phenotype depends on unbalanced EZH2 activity, causing inhibition of prolyl-hydroxylase PHD3 and increased expression of hypoxia inducible factor 1α (HIF1α), triggering epithelial to mesenchymal transition. Finally, we identified an HIF1α-dependent transcriptional signature with prognostic significance in human LUAD. Our studies further current knowledge of the relationship between glucose metabolism and cell differentiation in cancer, characterizing the epigenetic adaptation of cancer cells to glucose deprivation and identifying novel targets to prevent the development of resistance to therapies targeting glucose metabolism.

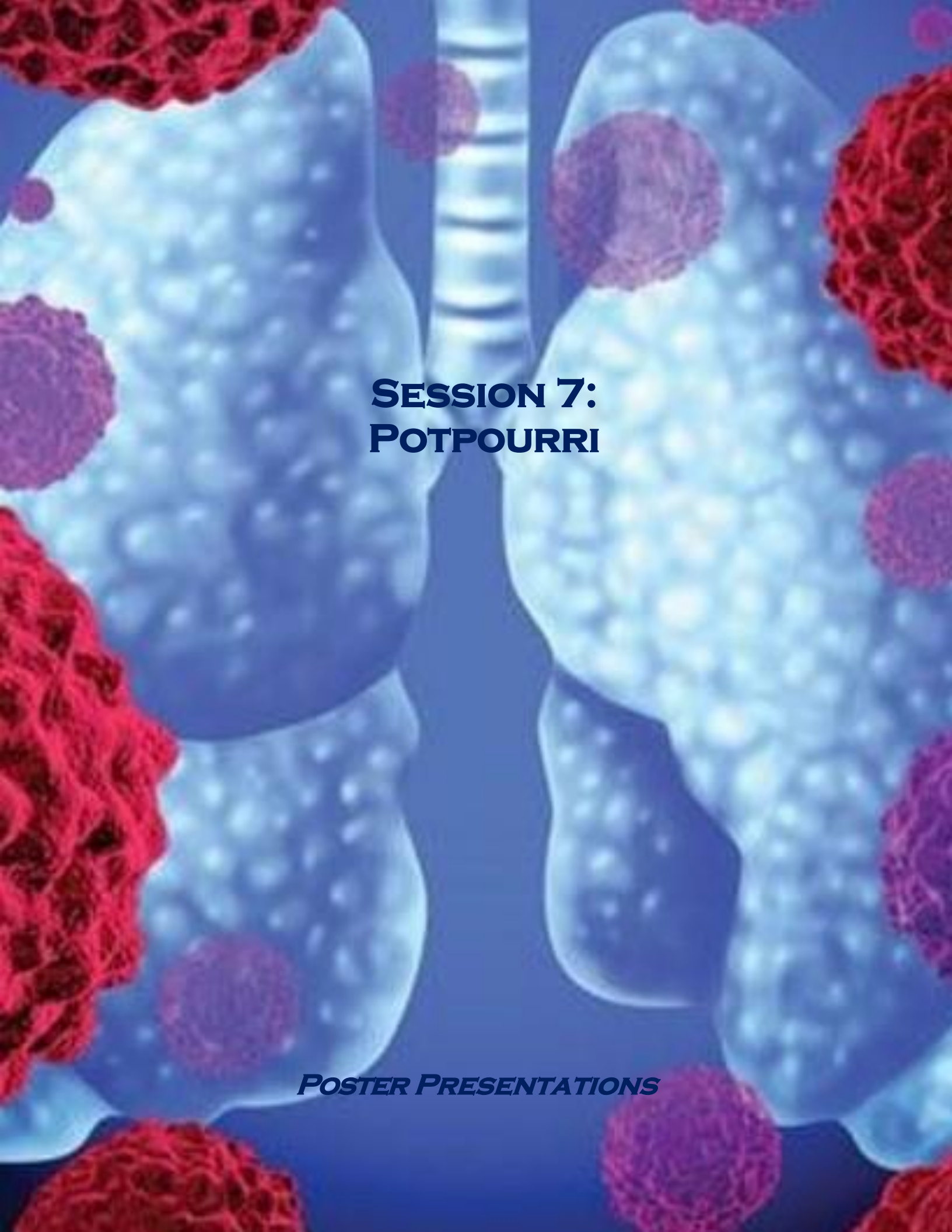
Targeting A Positive Feedback Loop Of MERTK and STAT3 During Macrophage Maturation May Activate Anti-Tumor Immunity in Lung Cancer

Dan Yan, Justus M. Huelse, Swati Bhasin, Manoj Bhasin, Deborah DeRyckere, and Douglas K. Graham

Emory University, Atlanta

Presented by: Dan Yan

MERTK tyrosine kinase is expressed on tumor-associated macrophages where it mediates clearance of apoptotic cells to maintain tissue homeostasis and promotes an immunosuppressive microenvironment. MERTK is upregulated upon maturation of monocytes to macrophages, but the mechanisms that drive MERTK upregulation during macrophage maturation are obscure. A better understanding of the mechanisms by which MERTK is upregulated during macrophage maturation may provide insight into mechanisms of disease in lung cancer and suggest novel therapeutic approaches. In our studies, MERTK was expressed at very low levels in the monocytic leukemia cell line THP1 and murine bone marrow-derived monocytes (BMDMs). Treatment of THP1 cells or murine BMDMs with PMA resulted in cell morphology changes consistent with induction of macrophage maturation. Treatment with PMA also enhanced chemokine secretion in THP1 cultures, including IL-8 and MCP-1, as determined by proteomic cytokine array analysis. Upregulation was confirmed at the gene expression level using real-time PCR. STAT3 signaling was also activated during macrophage maturation and single-cell sequencing demonstrated *Stat3* expression in murine bone marrow monocytic lineage cells. Treatment with a pan-STAT inhibitor or a STAT3-selective inhibitor abrogated MERTK expression in response to PMA treatment in THP1 cells. Conditional knockout of *Stat3* in $LysM^+$ myelomonocytic cells, including macrophages, led to decreased MERTK+ macrophages ($CD11c^+$ $Ly6c^+$ $F4/80^+$ $CD45^+$). These data suggest that STAT3 regulates expression of *Mertk* during macrophage maturation. Previously published data demonstrated regulation of STAT3 signaling downstream of MERTK. Thus, we propose that MERTK and STAT3 form a positive feedback loop during macrophage maturation. Treatment with a MERTK and/or STAT3 inhibitor may interfere with this feedback pathway, potentially reversing an immunosuppressive phenotype in the lung tumor microenvironment.



Session 7:
Potpourri

Poster Presentations

Incidence, Correlates, and Prognostic Significance of Mixed Responses to Carboplatin-Paclitaxel + Bevacizumab Induction Therapy in Advanced Non-Small Cell Lung Cancer

Sheena Bhalla, Yating Wang, Suresh Ramalingam, Zhouxin Sun, Hossein Borghaei, Julie R. Brahmer, Joan H. Schiller, David E. Gerber

UT Southwestern, Eastern Cooperative Oncology Group Statistical Center, Dana-Farber Cancer Institute, Emory University, Fox Chase Cancer Center, Johns Hopkins University, University of Virginia

Presented by: Sheena Bhalla

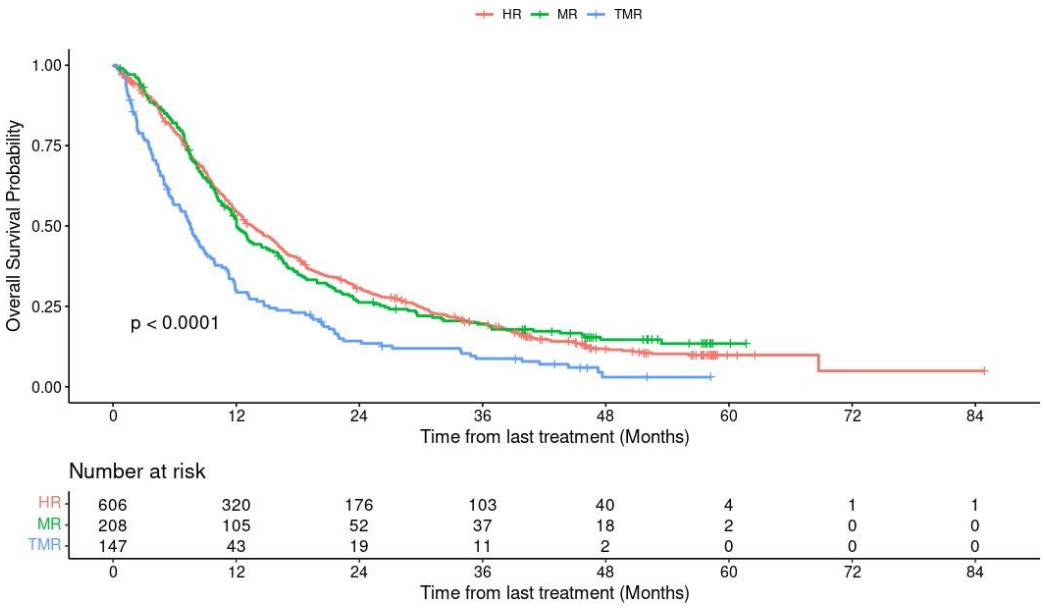
Background: Mixed response, a scenario featuring discordant tumor changes, has been reported primarily in lung cancer treated with targeted therapies or immunotherapy. We determined the incidence and prognostic significance of mixed response in advanced non-small cell lung cancer (NSCLC) treated with cytotoxic chemotherapy.

Methods: We analyzed patient-level data from ECOG-ACRIN E5508 (carboplatin-paclitaxel + bevacizumab induction followed by randomization to maintenance therapy regimens). For patients with at least two target lesions and available measurements after cycle 2, we characterized response as homogeneous response (HR, similar behavior of all lesions), mixed response (MR, similar behavior but >30% difference in magnitude of best and least responding lesions), or true mixed response (TMR, best and least responding lesions showing different behavior: ≥10% growth versus ≥10% shrinkage). We compared category characteristics using Mann Whitney U and Chi-square tests, and overall survival (OS) using log-rank test, univariate, and multivariate Cox models.

Results: Among 965 evaluable patients, HR occurred in 609 patients (63%), MR in 208 (22%), and TMR in 148 (15%). Median OS was 13.6 months for HR, 12.0 months for MR, and 7.6 months for TMR (P<0.001). Compared to HR, TMR had inferior OS among stable disease cases (HR 1.62; 95% CI, 1.23-2.12; P<0.001) and a trend toward inferior OS among progressive disease cases (HR 1.39; 95% CI, 0.83-2.33; P=0.2). In multivariate analysis, TMR was associated with worse OS (HR 1.48; 95% CI, 1.22-1.79; P<0.001).

Conclusions: “True” mixed response to induction chemotherapy + bevacizumab occurs in ~15% of NSCLC cases and independently confers poor prognosis. By contrast, mixed responses representing the same direction but quantitatively different have similar prognosis to those with homogenous responses to therapy. Further studies to understand the mechanistic underpinning to these clinical findings are warranted.

Figure 1: Overall survival according to response heterogeneity category



Loss of the Endocytic Tumor Suppressor HD-PTP Phenocopies LKB1 And Promotes RAS-Driven Oncogenesis

Chang-Soo Seong, Chunzi Huang, Austin C. Boese, Yuning Hou, Janna K. Mouw, Manali Rupji, Greg Joseph, H. Richard Johnston, Henry Claussen, Jeffrey M. Switchenko, Madhusmita Behera, Michelle Churchman, Jill M. Kolesar, Susanne M. Arnold, Katie Kerrigan, Wallace Ackerly, Howard Colman, Margaret A. Johns, Cletus Arciero, Wei Zhou, Adam I. Marcus, Suresh S. Ramalingam, Haian Fu, Melissa Gilbert-Ross

Emory University, M2Gen, University of Kentucky, University of Utah

Presented by: Melissa Gilbert-Ross

Oncogenic RAS mutations drive aggressive cancers that are difficult to treat in the clinic, and while direct inhibition of the most common KRAS variant in lung adenocarcinoma (G12C) is undergoing clinical evaluation, a wide spectrum of oncogenic RAS variants together make up a large percentage of untargetable lung and GI cancers. Here we report that loss-of-function alterations (mutations and deep deletions) in the gene that encodes HD-PTP (*PTPN23*) occur in up to 14% of lung cancers in the ORIEN Avatar lung cancer cohort, associate with adenosquamous histology, and occur alongside an altered spectrum of KRAS alleles. Furthermore, we show that in publicly available early-stage NSCLC studies loss of HD-PTP is mutually exclusive with loss of LKB1, which suggests they restrict a common oncogenic pathway in early lung tumorigenesis. In support of this, knockdown of HD-PTP in RAS-transformed lung cancer cells is sufficient to promote FAK-dependent invasion. Lastly, knockdown of the Drosophila homolog of HD-PTP (dHD-PTP/Myopic) synergizes to promote RAS-dependent neoplastic progression. Our findings highlight a novel tumor suppressor that can restrict RAS-driven lung cancer oncogenesis and identify a targetable pathway for personalized therapeutic approaches for adenosquamous lung cancer.

Effect of COVID-19 Bivalent Booster Vaccination on the Binding and Live-Virus Neutralizing Antibody Response to Currently Circulating Variants of Concern in Lung Cancer Patients

Rajesh M. Valanparambil, Margaret A. Johns, Lilin Lai, Jennifer Carlisle, Susanne L. Linderman, Akil Akthar, Estefany L Bocangel Gamarra, Mehul S. Suthar, Madhav V. Dhodapkar, Rafi Ahmed and Suresh Ramalingam

Emory University

Presented by: Rajesh M. Valanparambil

Purpose: To examine the binding and neutralizing antibody responses in NSCLC patients to currently circulating variants of concern after the monovalent and bivalent booster vaccination.

Methods: NSCLC patients who received SARS-CoV-2 monovalent (n=48) and bivalent (n=48) booster vaccination were included in the study. Blood was collected from these patients within 3 months of booster vaccination and SARS-CoV-2-specific binding and neutralizing antibody responses were evaluated by Meso Scale Discovery (MSD) assay and live virus Focus Reduction Neutralization Assay (FRNT), respectively. Patients in the cohorts infected with SARS-CoV-2 was identified by the presence of binding antibody to the nucleocapsid antigen.

Results: All NSCLC patients in the study generated detectable binding and neutralizing antibody titers to the SARS-CoV-2 WA.1 (WT) strain. Although not statistically significant, there was a slight increase (2-fold) in the neutralizing antibody titers to the WA.1 strain in patients receiving bivalent booster compared to the patients who received monovalent booster vaccination alone. The binding and neutralizing antibody response to the Omicron variants were significantly increased in patients who received the bivalent booster compared to the monovalent booster. Neutralizing antibody titers to the Omicron variants BA.5 (5-fold), and BQ1.1 (4-fold), were significantly higher (P=<0.001) in the bivalent booster recipients compared to the monovalent booster cohort. Importantly, patients who received the bivalent booster, had a significant (P=<0.0001) increase in the live-virus neutralizing antibody titers to the currently circulating XBB1.5 variant compared to their monovalent counterparts.

Conclusions: Most patients receiving the SARS-CoV-2 bivalent booster vaccination had a significant increase in the binding and live-virus neutralizing antibodies to the Omicron variants, importantly to the XBB1.5 variant which now accounts for more than 90% of COVID-19 cases in the US. These data highlight the importance of COVID-19 bivalent booster vaccination for cancer patients given the evolution of new variants of concern.

Session 8: SCLC

Poster Presentations

Molecularly Matching Patient-Derived Lung Cancer Cell Lines and Xenografts (PDX) Models with Non-small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) Tumor Specimens in Deposited Databases to Facilitate Clinical Translation

Luc Girard, Benjamin Drapkin, Kenneth Huffman, Bingliang Fang, Victor Stastny, Boning Gao, Shreoshi Pal Choudhuri, Ling Cai, Jing Wang, Lauren Byers, John Heymach, Jack Roth, John Minna

UT Southwestern, UT MD Anderson

Submitted by: Luc Girard
Presented by: Benjamin Drapkin

Background: A major issue in lung cancer translational research is to determine the relevance and molecular relationships between patient-derived lung cancer preclinical models (cell lines, PDXs) and tumor specimens such as those in the TCGA NSCLC and "George" SCLC datasets. While the preclinical models are not derived from these deposited specimens it is important to know which tumor specimen(s) each preclinical model is most closely aligned molecularly, and which tumor specimens have not yet generated models.

Methods: To address these issues, we completed whole-exome or whole-genome sequencing, together with RNAseq on 71 SCLC lines, 30 SCLC PDXs, 166 NSCLC lines, and 80 NSCLC PDX models. We used a concordance (similarity) metric to compare each preclinical model with each tumor specimen from the TCGA NSCLC (n=996) and George SCLC (n=71) datasets. This similarity metric combined RNAseq expression and somatic mutation data. The expression similarity was the Pearson correlation on ~2,000 most variably expressed genes while the mutation similarity used a concordance value on mutations in 700 "cancer genes" (COSMIC) (# genes mutated in both/ # genes mutated in either sample; with genes weighted by mutation frequency).

Results: We found: that preclinical models were most similar to tumor specimens of the same histological type; PDXs showed higher concordance values than cell lines; 69% of NSCLC and 93% of SCLC tumor specimens were matched with a preclinical model; the NSCLC (31%) and SCLC (7%) specimens that lacked a preclinical model were associated with better survival compared to those that had a model match.

Conclusions: We found: nearly all 347 patient-derived lung cancer preclinical models have a "molecular match" in deposited tumor databases; expected correlations found in tumors (histology with gene expression and mutation patterns) are maintained in preclinical models; additional models are needed to fill in "gaps" and the study of such "non-model" tumors represents an important knowledge gap; this preclinical model database will benefit from being updated with models from other centers; this information provides a new resource for guiding the use of preclinical models in translational studies such as therapy response phenotypes and rationally interrogating deposited tumor datasets.

Immune Heterogeneity in Small Cell Lung Cancer and Vulnerability to Immune Checkpoint Blockade

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Following the pivotal Phase III IMpower133 study, atezolizumab (anti–PD-L1), combined with carboplatin and etoposide (CE), was the first immune checkpoint inhibitor approved for first-line treatment of extensive-stage small cell lung cancer (ES-SCLC) and is now a standard of care. A clearer understanding of therapeutically relevant SCLC subsets is needed to improve outcomes and to identify rational combination strategies. Transcriptomic analyses and nonnegative matrix factorization were conducted on 271 pre-treatment patient tumor samples from IMpower133 and four subsets with general concordance to previously reported SCLC subtypes were identified. Deeper investigation into the immune heterogeneity relevant to clinical outcomes uncover two subsets with differing neuroendocrine (NE) versus non-neuroendocrine (non-NE) phenotypes that demonstrated hallmarks of immune cell infiltration. The balance of tumor-associated macrophage (TAM) to T-effector signals distinguished these inflamed subsets. Tumors with low TAM but high T-effector signals had a NE phenotype and demonstrated longer overall survival with PD-L1 blockade and CE versus CE alone than did tumors with high TAM and high T-effector signal, which had a non-NE phenotype. The delineation of SCLC immune heterogeneity offers a clinically relevant approach to discriminate SCLC patients likely to benefit most from immunotherapeutic approaches and highlights the complex mechanisms underlying response and resistance to immune checkpoint blockade.


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