ANNUAL LUNG CANCER SPORE WORKSHOP 2024

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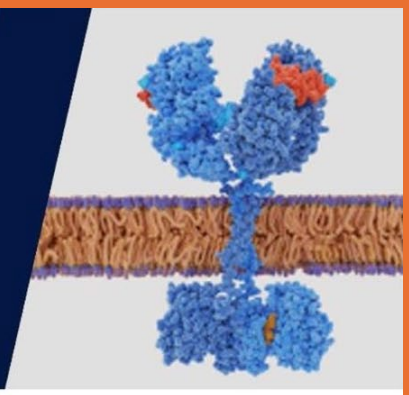
CELEBRATING THE 20TH ANNIVERSARY OF THE EGFR MUTATIONS DISCOVERY



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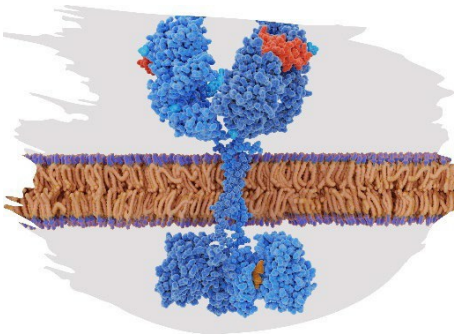


Session 1:

Oncogene-Driven Lung Cancers And Resistance To Targeted Therapies

Multi-Omic Characterization Of *KRAS/STK11/KEAP1* Co-Mutant Non-Small Cell Lung Cancer (NSCLC) Displays a Unique Metabolic Profile and Therapeutic Vulnerabilities

Triparna Sen

Icahn School of Medicine at Mount Sinai

Presented by: Triparna Sen

Background: *KRAS*-mutant NSCLC with co-occurring loss-of-function mutations in *STK11* and *KEAP1* are remarkably aggressive and unresponsive to chemo- and immunotherapy. Novel therapeutic strategies are urgently needed to improve outcomes for patients with *KRAS/STK11/KEAP1* co-mutant NSCLC (KSK). We interrogated the transcriptomic landscape using a large real-world dataset of NSCLC to identify therapeutic vulnerabilities that may help guide treatment selections in *KSK*.

Method: *KRAS* mutant NSCLC clinical samples (N=7210) were tested with NextGen Sequencing (592-gene panel or whole exome sequencing) and RNA (whole transcriptome sequencing) at Caris Life Sciences (Phoenix, AZ). Specimens were stratified into $KRAS^{MUT}/STK11^{MUT}/KEAP1^{MUT}$ (KSK; N=698), $KRAS^{MUT}/STK11^{MUT}/KEAP1^{WT}$ (KS; N=786), $KRAS^{MUT}/STK11^{WT}/KEAP1^{MUT}$ (KK; N=466), and $KRAS^{MUT}/STK11^{WT}/KEAP1^{WT}$ (K; N=4536). Additionally, an *in vitro* CRISPR screen, bulk RNA sequencing, and phospho-kinase arrays were performed in *KRAS/STK11/KEAP1* co-mutant models.

Results: *KEAP1* mutations (mOS: KK=7.83m, KSK=7.23m) were strongly associated with poor OS compared to *STK11* mutations (mOS: KS=17.6m). Pathways significantly upregulated in KSK clinical samples included fatty acid metabolism and redox pathways. KSK clinical samples had significant overexpression of genes involved in ferroptosis evasion and metabolism like *SLC7A11*(KSK/KK=1.28, KSK/KS=4.82, KSK/K=10.24; all $q<0.01$) and *SCD1* (KSK/KS=1.19, KSK/K=1.24; both $q<0.01$) compared to single mutants or wild-type groups.

CRISPR/Cas9-based genetic screening identified *SCD1* as a potential therapeutic target in the KSK cell lines. *SCD1* inhibition led to global metabolomic changes in KSK cells, including key pathways involved in lipid and glucose metabolism. Moreover, *KSK* co-mutant cells have a significantly higher expression of *SLC7A11,* an amino acid transporter that enables cystine uptake and its subsequent conversion to cysteine. Consequently, *KSK* co-mutant cells are most resistant to cysteine depletion in the media as compared to single mutants or NTC cells. SCD1 inhibition causes a decrease in SLC7A11 expression exclusively in *KSK* co-mutant cells. Finally, pharmacological inhibition of SCD1 significantly reduced the viability of KSK cells and caused significant tumor regression in KSK syngeneic mouse models.

Conclusion: We highlight the importance of the *SCD1-SLC7A11* axis in regulating unique metabolic and ferroptosis evasion pathways in *KRAS/STK11/KEAP1* co-mutant NSCLC. The study data furthers the understanding of ferroptosis in NSCLC and the potential to translate SCD1 inhibitors and ferroptosis inducers in NSCLC clinical trials.

Enhanced Sensitivity of EGFR Inhibitor Resistant NSCLC And Drug Tolerant Persister Cells to Chimeric Antigen Receptor (CAR)-NK Cellular Therapy

Yan Yang, Monique B. Nilsson, Xiaoxing Yu, Alissa Poteete, Junqin He, John V. Heymach

UT MD Anderson Cancer Center

Presented by: Yan Yang

Patients with non-small cell lung cancer (NSCLC) harboring EGFR mutations often benefit from tyrosine kinase inhibitors (TKIs) such as osimertinib. However, the emergence of drug-tolerant persister cells (DTPCs), which eventually give rise to drug-resistant cells (DRCs), remains a therapeutic challenge. Furthermore, EGFR-TKI resistant NSCLCs are refractory to immune checkpoint inhibitors. Therefore, novel treatment strategies are urgently needed. Chimeric antigen receptors (CARs) have shown promise in augmenting the anti-tumor activity of immune effector cells. In this study, we evaluated the efficacy of CAR-based therapies for parental EGFR mutant NSCLC cells, osimertinib-resistant (OR) cells, and osimertinib DTPCs. Our findings demonstrate robust cytotoxic activity of EGFR CAR-T and CAR-NK cells against parental cells, while OR cells displayed altered sensitivities: reduced response to EGFR CAR-T and enhanced susceptibility to EGFR CAR-NK treatment. Notably, DTPCs showed increased sensitivity to both EGFR CAR-T and CAR-NK cells. Mechanistically, altered EGFR levels, epithelial-mesenchymal transition (EMT) status, increased B7-H6 (*NCR3LG1*) expression, and elevated NKG2D ligands (MICA, MICB, and ULBP1) contributed to differential responses to EGFR CAR-T and CAR-NK cells. Treatment with osimertinib elevated EGFR expression on OR cells, rendering them more responsive to EGFR-CAR therapy. Additionally, elevated TGF-β levels in EGFR-TKI-resistant cells suppressed CAR-T and CAR-NK function, which was rescued by TGF-β pathway inhibition through galunisertib or dominant-negative TGF-β receptor II (DNTGFBRII) co-expression in EGFR CAR-NK cells. Furthermore, EGFR CAR-NK cells demonstrated complete responses in HCC827 xenograft models. In an osimertinib-resistant model (H1975 OR17), EGFR CAR-NK cells significantly inhibited tumor growth as compared to control NK cells ($p < 0.001$). Moreover, DNTGFBRII-expressing CAR-NK cells treatment resulted in a significantly reduced tumor volume as compared to EGFR CAR-NK cells ($p < 0.001$). Furthermore, in H1975 osimertinib DTPC *in vivo* models, EGFR CAR-NK cells demonstrated potent efficacy either in combination with osimertinib or following osimertinib treatment. In conclusion, EGFR-directed cellular therapies, particularly EGFR CAR-NK cells, are active against drug-resistant models of EGFR mutant NSCLC and DTPCs, and that this activity is enhanced by combination with TKIs or TGF-β pathway blockade.

Systematic Discovery Of Osimertinib Resistance Variants Using CRISPR Prime Editing

Alice Berger

Fred Hutchinson Cancer Center

Presented by: Alice Berger

Treatment of oncogene-driven lung cancer with small molecule tyrosine kinase inhibitors, beginning with EGFR, was a breakthrough in solid tumor precision oncology. However, resistance to targeted therapies develops rapidly through both adaptive and genetic mechanisms. Genetic mechanisms include selection for pre-existing somatic variants that enable continued cell proliferation in the presence of the therapeutic agent, eventually leading to disease progression. Resistance variants can include second-site variants in the targeted oncogene but may also include selection for genetic bypass variants that reconstitute oncogenic programs. Due to tremendous intra-tumor genetic heterogeneity and cancer evolution, the possible number of independent resistance mechanisms is very large and methods for prediction of resistance variants at scale are needed. Moreover, the ability to profile the resistance landscape of cancer therapies still in pharmaceutical and clinical development would enable prioritization of therapies with non-overlapping patterns of resistance, which would open a path to effective combination therapy development.

Here we develop a method, prime-SGE ("prime saturation genome editing"), for CRISPR prime-editing based discovery of drug resistance mutations in cancer. Unlike CRISPR knockout screening, prime-SGE can model specific single-nucleotide variants which may include both inactivating or activating variants. Moreover, unlike traditional CRISPR saturation genome editing which can study only one genomic loci in each experiment, prime-SGE can install libraries of specific mutations throughout the genome in a single, multiplexed experiment. We applied prime-SGE to assay thousands of single nucleotide variants in eight genes to determine their ability to confer drug resistance to osimertinib, the current standard-of-care EGFR tyrosine kinase inhibitor, as well as two EGFR inhibitors in clinical development. We identify known and novel resistance variants and establish prime-SGE as a framework for multiplex identification of cancer resistance variants at scale.

Systematic Targeting of Protein Complexes with Staples

Stefan Harry, Diane Yang, Liron Bar-Peled, John Iafrate, Brian Liau

Harvard University and MGH

Presented by: Stefan Harry

Small molecule glues that stabilize protein complexes have transformed the study of signal transduction, having a direct impact on clinical oncology. However, the systematic identification of new classes glues and their corresponding protein targets has remained largely serendipitous. Herein, we report the development of staples–small molecule probes endowed with two cysteine-reactive warheads and STAPLR–a complementary chemical proteomic platform for target deconvolution. By profiling the targets of staples across 11 cell cancer cell lines, we uncovered hundreds of stapled targets, including mutant selective stapling. We focused on stapling of EML4-ALK, developing an advanced staple, which engages the onco-fusion outside of its kinase domain disrupting the signaling of EML4-ALK resistant mutants. Stapling of EML4-ALK restricts protein dynamics and leads to its proteasome-mediated degradation suggesting, a mechanism to tune signaling independent of kinase-directed inhibition. Collectively, by intersecting covalent staples with chemical proteomics, this modality substantially expands the scope of small molecule glues providing an unbiased opportunity to target protein dynamics.

Identifying Resistant Mechanisms To Direct KRAS-Inhibitors

Samrat Kundu

UT MD Anderson Cancer Center

Presented by: Samrat Kundu

Mutations in the KRAS oncogenic driver gene are frequently found in malignancies such as pancreatic, colorectal, and lung cancers. The replacement of the amino acid glycine at position 12 (e.g., G12C, G12D, G12V) is a frequent mutation that traps the protein in an active state and promotes uncontrolled cell proliferation. KRAS was regarded as "undruggable" for many years due to insufficient drug-binding pockets on the protein's surface. Recent breakthroughs, however, have resulted in the development of covalent inhibitors capable of selectively targeting the KRAS G12C mutation, like Sotorasib (AMG510) and Adagrasib (MRTX849) which are approved by the FDA due to their encouraging effects in clinical trials. The identification of selective inhibitors of other oncogenic KRAS alleles, such as the noncovalent KRAS-G12D inhibitor, MRTX1133, and a pan-KRAS-inhibitor drug, BI3706674 is also a promising next step in the treatment of KRAS-dependent malignancies. While these inhibitors have shown initial success, with some receiving FDA approval, their use frequently leads to resistance, the mechanisms of which largely remain unknown. To fill this essential research gap, we are employing our pre-clinical syngeneic mouse models and KRAS mutant allele-specific cell lines to investigate the underlying molecular processes of acquired resistance to direct KRAS allele-specific inhibitors. Using these murine syngeneic cell lines and human NSCLC cell lines, we have generated a panel of cell lines with acquired resistance to these direct KRAS inhibitors. To elucidate the molecular underpinnings of acquired resistance to direct KRAS inhibitors, we performed proteomic profiling of the sensitive and resistant cells by RPPA analysis. Among the several proteins whose expressions were altered in the KRAS-inhibitor-resistant cells, we identified the YAP/TEAD1 pathway that was commonly upregulated in the cells resistant to MRTX849 (G12Ci) or MRTX1133 (G12Di). We also observed significant re-sensitization of the resistant cells to the specific KRAS inhibitors upon co-treatment with a TEAD inhibitor, *in vitro.* Tumors from syngeneic mice that were implanted with KRAS inhibitor-resistant cells or their sensitive versions and treated with the specific KRAS inhibitors for 3-4 weeks, also exhibited increased nuclear YAP1 localization in the resistant tumors. We are currently performing in vitro and In vivo studies to understand the therapeutic efficacy of a TEAD inhibitor (VT107) in combination with KRASi (MRTX849 or MRTX1133) to either reverse or prevent resistance to the direct KRAS inhibitors. Successful completion of this research will help address the urgent need to understand ways to overcome resistance to KRAS inhibitors and increase their clinical efficacy.

The ATM Kinase as a Therapeutic Target in Drug Resistant NSCLC with Brain Metastasis

Don X. Nguyen
Yale University

Presented by: Don X. Nguyen

NSCLCs with activating mutations in the epidermal growth factor receptor (*EGFRmut*) represent a significant subset of lung cancers that develop brain metastases and are treated with the blood brain barrier (BBB)-penetrant tyrosine kinase inhibitor (TKI) osimertinib. A proportion of these tumors become resistant without secondary *EGFRmut* and seem to have worse outcome when compared to TKI-resistant tumors that are driven by secondary mutations. To improve the clinical outcomes of NSCLC patients, new combination therapies are needed that effectively treat extra-cranial tumors as well as metastases in the central nervous system (CNS).

The **ataxia-telangiectasia mutated serine/threonine kinase (ATM)** is a canonical regulator of DNA damage response (DDR). It has been proposed that ATM inhibition sensitizes treatment-naïve tumors to radiation and targeted therapies, via double strand break mediated cell death. However, ATM also controls redox homeostasis and inflammation. It is currently unclear which, if any, effector functions of ATM are necessary for drug resistance and brain metastasis in NSCLC.

Using novel xenograft models, we found that, despite widespread penetration of drug in the brain, *EGFRmut* brain metastases become resistant to osimertinib in a manner that is dependent on the tumor microenvironment (TME) but does not require secondary gene mutations. Drug-resistant micrometastases can be preferentially located within perivascular regions of the CNS that are associated with neuro-inflammation. ATM activation is increased in drug-resistant brain metastatic cells relative to treatment-naive cells**,** especially under conditions which detects ATM multimers that are formed by disulfide bonds under oxidative stress and osimertinib treatment. Thus, *EGFRmut* metastatic cells may conditionally activate ATM for drug resistance when under selective pressure from the TME and osimertinib. With the ongoing development of novel ATM inhibitors in the clinic, these findings provide new avenues for combinatorial therapy in patients with TKI resistant *EGFRmut* NSCLC including, but not limited to, cases with progressing brain metastases.

The On-Target MRTX1133 Resistance Mutation KRAS(R68S) Acts by Enhancing Lung Adenocarcinoma Cell Fitness

Ken Westover, Dhiraj Sinha, Deekshi Angira, Xiaofang Huo, Michael Peyton, Kimberley Avila, John Minna, Ralf Kittler, Ken Westover
UT Southwestern

Presented by: Ken Westover

MRTX1133 represents a breakthrough as the first clinical-stage drug targeting KRAS G12D, a mutation driving a significant portion of lung adenocarcinomas (LUADs). However, as with most targeted therapies, on-target resistance mutations (as well as activation of potentially other MRTX1133 resistance pathways) are an anticipated hurdle. This study utilizes the LentiMutate system to discover and characterize on-target resistance mechanisms against MRTX1133. KRAS plays a critical role in cancers, with mutations found in roughly 20-30% of all cases, particularly enriched in aggressive cancers like pancreatic, colorectal, and lung cancers. Functioning as a molecular switch, KRAS cycles between inactive (GDP-bound) and active (GTP-bound) states. Oncogenic mutations, like G12D, lock KRAS in a constitutively active GTP form, promoting uncontrolled cell proliferation. Initially deemed "undruggable" due to a lack of clear binding pockets, RAS has been successfully targeted through various approaches, including the inducible switch 2 pocket (SW2P) near the nucleotide binding site. We employed LentiMutate, a rapid and unbiased method for discovering on-target drug resistance mutations, and after discovery, validated each putative resistance mutation for their ability to confer MRTX1133 resistance. We identified and validated KRAS R73P and R68S as the most prevalent mutations conferring primary resistance against MRTX1133. Notably, R73P mutations haven't been observed with other SW2P binders, while R68S has, indicating potential MRTX1133 specificity for this resistance mutation. Interestingly, molecular dynamics simulations did not predict a significant decrease in inhibitor binding for these mutations. To elucidate this finding, we performed biochemical and functional characterization. These mutations were observed to decrease both intrinsic and GAP-mediated GTP hydrolysis, mimicking the behavior of RAS oncogenes. Additionally, R68S evaluation in cellular models revealed activation of MAPK signaling. These findings suggest that R68S contributes to drug resistance, at least partially, by enhancing cell fitness. This biologic activity of a drug resistance mutation thus has major potential implications for the development of future strategies to combat drug resistance mechanisms in KRAS G12D-driven cancers, including lung cancers. (SPORE P50 CA070907).

Epigenetic Priming Promotes Acquisition Of Tyrosine Kinase Inhibitor Resistance And Oncogene Amplification In Human Lung Cancer

Rebecca Starble, Eric Sun, Rana Gbyli, Jonathan Radda, Jiuwei Lu, Tyler Jensen, Ning Sun, Nelli Khudaverdyan, Bomiao Hu, Mary Ann Melnick, Shuai Zhao, Nitin Roper, Greg Wang, Jikui Song, Katerina Politi, Siyuan Wang, Andrew Xiao

Yale University, University of California, Riverside, Duke University School of Medicine, National Cancer Institute

Presented by: Rebecca Starble

In mammalian cells, gene copy number is tightly controlled to maintain gene expression and genome stability. However, a near-universal molecular feature across cancer types is oncogene amplification, which promotes cancer progression by dramatically increasing the copy number and expression of tumor-promoting genes. For example, in tyrosine kinase inhibitor (TKI)-resistant lung adenocarcinoma (LUAD), oncogene amplification occurs in over 40% of patients' tumors. Despite the prevalence of oncogene amplification in TKI-resistant tumors, the mechanisms facilitating oncogene amplification are not fully understood. Here, we find that LUADs exhibit a unique chromatin signature demarcated by strong CTCF and cohesin deposition in drug-naïve tumors, which correlates with the boundaries of oncogene amplicons in TKI-resistant LUAD cells. We identified a global chromatin priming effect during the acquisition of TKI resistance, marked by a dynamic increase of H3K27Ac, cohesin loading, and inter-TAD interactions, which occurs before the onset of oncogene amplification. Furthermore, we have found that the METTL7A protein, which was previously reported to localize to the endoplasmic reticulum and inner nuclear membrane, has a novel chromatin regulatory function in binding to amplified oncogenes and regulating cohesin recruitment and inter-TAD interactions. Surprisingly, we discovered that METTL7A remodels the chromatin landscape prior to any noticeable oncogene copy number gains. Furthermore, while METTL7A depletion has little effect on the chromatin structure and proliferation of drug-naïve cells, METTL7A depletion prevents the formation of TKI resistant-clones, highlighting the specific role of METTL7A as cells are becoming resistant. In summary, we discovered an unexpected mechanism required for the acquisition of TKI resistance regulated by a largely uncharacterized factor, METTL7A. This discovery sheds light into the maintenance of oncogene copy number and paves the way to the development of new therapeutics for preventing TKI resistance in LUAD.

Partnering with Patient Advocates to Enhance Research

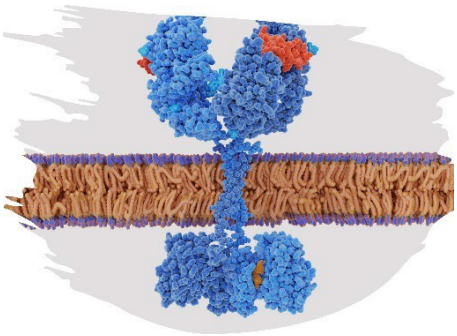
Janet Freeman-Daily and Elda Railey
Fred Hutchinson (Advocate), Research Advocacy Network

Presented by: Janet Freeman-Daily

Increasingly, funders of cancer grants now require the research team to include a patient/survivor or patient advocate on the research team. The intent is for this person to be involved in all aspects of the research project, from concept development through post-study communications. Incorporating the patient voice in cancer research has the potential to help research progress faster and further. After a project is concluded, patients and advocates can also help smooth the transition of basic scientific discoveries into clinical practice.

Thanks to newer lung cancer treatments such as targeted therapies, an increasing number of people who have or had lung cancer are living long enough to become active advocates and take an interest in the research process. Some of them have become patient research advocates (PRAs) who partner with investigators to help define research questions, provide perspective on the impact of the study topic on patients, and offer suggestions for refining trial design and protocols to facilitate enrollment. These patients, some of whom are experts in their own disease, offer valuable insights into the patient experience.

This presentation will focus on ways to encourage patients to learn about the research process, incorporate patient research advocates into research teams, and gather patient perspectives as part of the research process.



Session 2:

Health Disparities in Lung Cancer

Reducing Racial Residential Segregation Lowers the Risk of Lung Cancer in African Americans

Yi Xiao, Xiaoke Zou, Calvin Tribby, Peter, Christina Chang, Richard Curley, Olutola Akande, Melinda Aldrich, Sophia Wang, Rick Kittles, Kimlin Ashing, Lennie Wong, Marta Jankowska, Tarik Benmarhnia, Loretta Erhunmwunsee

City of Hope, VUMC, Morehouse School of Medicine, Scripps Institution of Oceanography

Presented by: Loretta Erhunmwunsee

Background: Residential segregation is a known driver of lung cancer mortality in African Americans (AAs), but its role in lung cancer etiology remains unclear. Our objective was to examine the relationship between residential segregation and lung cancer incidence in AAs and to identify modifiable factors mediating this relationship.

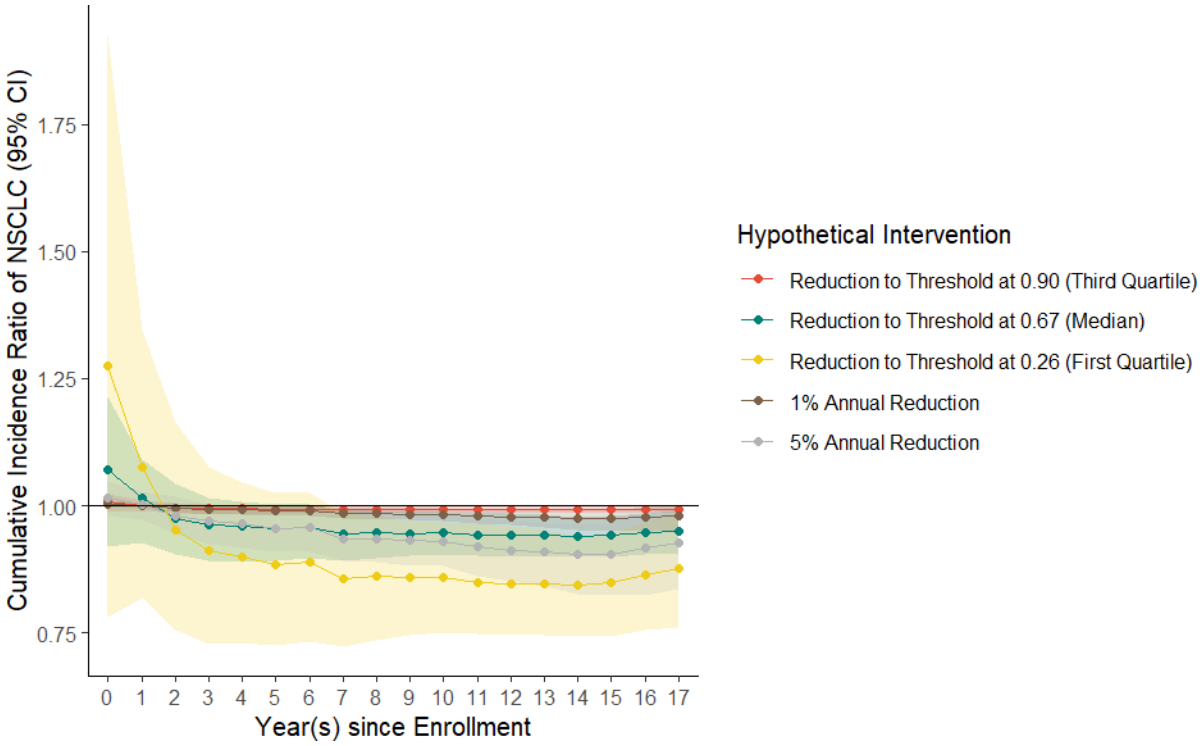
Methods: Data from the Southern Community Cohort Study (SCCS) were analyzed, encompassing AA and non-Hispanic white (NHW) participants without prior cancer diagnoses. Analysis was performed from April 2022 to January 2024. The SCCS, spanning 12 southeastern states, enrolled over 85,000 participants from community health centers or random sampling. The cohort comprises 71,634 participants (50,898 AA, 20,736 NHW) enrolled between 2002-2009. Residential segregation, measured by the isolation index using 2010 census block group data, was linked to baseline census tracts within the SCCS.

Main Outcome(s)/ Measure(s): Incident lung cancer cases were identified through linkages with state cancer registries and the National Death Index as of Dec 31, 2016, to Dec 31, 2019, depending on the state. Parametric g-computation estimated cumulative lung cancer risk under various hypothetical interventions reducing residential segregation. Mediation analyses were performed using inverse propensity weighting and marginal structural models to estimate the direct and indirect effects of mediators.

Results: AAs resided in more segregated areas (median isolation index: 0.81) than NHWs (median: 0.15), p-value (<0.001). Among AAs, all hypothetical scenarios of lowering the isolation index led to lower 17-year culminative incidence of lung cancer. For example, decrease in isolation index from above 0.26 (first quartile) to exactly 0.26 led to a 12.35% (95% CI: 1.18%, 23.83%) reduction in lung cancer incidence in AAs (Figure). No such reduction occurred among NHWs. Approximately 27.69% of the isolation index-lung cancer incidence effect in AAs was mediated by personal smoking, 12.39% by $PM_{2.5}$, 4.85% by second-hand smoke, and 4.41% by education.

Conclusion: Lower residential segregation significantly decreased lung cancer risk in AAs but not NHWs. Structural racism, driving segregation, likely impacts lung cancer risk through smoking and air pollution exposure. These findings suggest the need for policy and research interventions addressing structural racism to reduce lung cancer risk and promote equity in population health.

Figure 1: Cumulative Incidence Ratio of Lung Cancer and 95% CI Comparing Different Strategies to Lower Isolation Index to Natural Course among AAs



Racial And Ethnic Differences In The Tumor Immune Microenvironment Of Lung Adenocarcinomas

Lenora Loo, S. Lani Park, Alexandra Binder, Brenda Hernandez, Owen Chan, Lynne Wilkens, Kim Siegmund, David Conti, Loic Le Marchand, S. Lani Park

University of Hawaii Cancer Center, University of Southern California

Presented by: S. Lani Park

Lung cancer is the second most common cancer and the leading cause of cancer-related death for both men and women in the United States. There are race and ethnic differences in lung cancer risk and survival, independent of smoking history. Specifically, African Americans and Native Hawaiians are at high risk for all major histologic lung cancer cell-types, even after controlling for known risk factors. A prior study identified differences in gene expression profiles in stage and histologic subtype-matched lung tumors from African American and White patients, suggesting that underlying differences in lung tumor biology contribute to the observed race and ethnic disparities in risk and survival (PMID: 29196495). To date, there are no studies that have examined race and ethnic differences in tumor biology across a large multiethnic population. To build on these findings, we conducted genome-wide methylation profiling (Infinium MethylationEPICv1, Illumina) for 279 lung adenocarcinoma FFPE tumor tissue from diverse race and ethnic backgrounds collected by the University of Hawaii Cancer Center and University of Southern California SEER tumor repositories (37 African Americans, 55 Japanese Americans, 23 Latino American, 38 Native Hawaiians/Pacific Islanders, 75 Non-Hispanic Whites, 21 Filipino Americans, and 30 other Asian Americans (primarily Chinese and Koreans). These samples were examined for differences in the tumor immune microenvironment using the *in silico* tumor cell fraction deconvolution approach (MethylCIBERSORT; PMID: 30389940). Our preliminary results indicated that when compared to Non-Hispanic Whites (referent group), we observed differences in the DNA methylation-based estimates of immune cell composition of the non-White groups, adjusting for sex and age (**Figure 1**). We found that Filipino Americans, Japanese Americans, Latinos and the Other Asian American group, but not Native Hawaiians and African Americans, had significantly lower levels of Treg cells (p=0.01). We also found that Filipino Americans and the Other Asian American group had higher levels of endothelial cells (p=0.04). In conclusion, our study found differences in the tumor immune microenvironment for lung adenocarcinomas across diverse race and ethnic populations. Future directions include evaluating these profiles in relation to lung cancer-related survival.

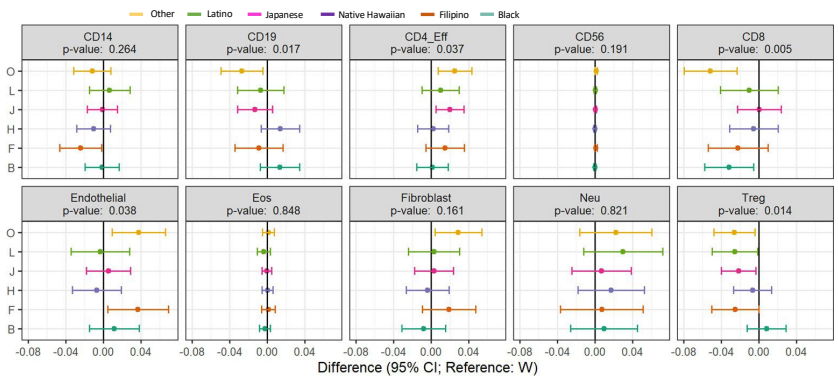


Figure 1. Differences in the extent of immune cell infiltration by race and ethnicity. Plotting the difference (95% confidence interval (CI)) from Non-Hispanic White (W) referent category based on model adjusting for age and sex; p-value is for the overall likelihood ratio test for race/ethnicity.

Real-World Molecular Testing Disparities at a Large Academic Institution in Los Angeles

Robert Hsu, Darin Poei, Jacob Thomas, Jorge Nieva

University of Southern California

Presented by: Robert Hsu

Background: With the use of targeted therapies, next generation sequencing has become a part of the recommended workup for the treatment of non-small cell lung cancer (NSCLC). However, the capabilities of molecular testing vary from single mutation testing of tissue using PCR-DNA to comprehensive genomic profiling (CGP) involving whole transcriptome sequencing testing for hundreds of mutations. We sought to distinguish molecular testing patterns and evaluate for frequency of mutations detected across two major sites at a large academic center in Los Angeles.

Methods: We evaluated 606 NSCLC patients treated at Los Angeles General Medical Center (LAGMC) (n=171) and University of Southern California Norris Comprehensive Cancer Center (Norris) (n=435) between July 2017- June 2023 who received molecular testing. Fisher's exact test was performed to compared between the incidence of tissue testing and liquid biopsy testing, if patients received a la carte/limited panel testing versus CGP, and the incidence of *EGFR*, *ALK, BRAF, ROS1, KRAS,* and *MET* Exon 14 skip mutations between patients at LAGMC and Norris. A p-value <0.05 was significant.

Results: Norris patients were more likely to receive tissue testing along with CGP tissue-based testing. LAGMC patients were more likely to receive a la carte tissue testing and liquid biopsy and receive both tissue and liquid biopsy. LAGMC patients had a significantly greater prevalence of *ALK* alterations and *BRAF* V600E mutations while Norris patients had a significantly greater prevalence of *KRAS* mutations and a trend towards greater prevalence of *ROS1*, *KRAS* G12C, and *MET* Exon 14 skip mutations. When evaluating race/ethnicity, Non-Hispanic Whites had much higher rates of tissue testing (92.3%) compared to Hispanic (79.6%) and an even greater difference in comprehensive NGS tissue testing (90.5%) compared to other race/ethnicities (Hispanic: 44.6%, African American 67.7%, and Asian 70.8%). (p<0.0001). Meanwhile, Hispanics had a greater rate of liquid biopsy (58.2%) compared to other race/ethnicities.

	LAGMC (n=171)	Norris (n=435)	p-value
Tissue testing	**122 (71.3%)**	**387 (88.9%)**	<0.0001
A la carte/limited panel testing	120 (70.2%)	24 (5.5%)	<0.0001
CGP	2 (1.2%)	363 (83.4%)	<0.0001
Liquid Biopsy	130 (76.0%)	157 (36.1%)	<0.0001
Received both tissue and liquid biopsy	88 (51.5%)	128 (29.4%)	<0.0001
Mutation Prevalence			
EGFR	56 (32.7%)	134 (30.8%)	0.6973
ALK	21 (12.3%)	21 (4.8%)	0.0021
ROS1	1 (0.6%)	10 (2.3%)	0.1945
BRAF	10 (5.8%)	13 (3.0%)	0.1031
BRAF V600E	7 (4.1%)	5 (1.1%)	0.0445
KRAS	15 (8.8%)	71 (16.3%)	0.0194
KRAS G12C	5 (2.9%)	23 (5.3%)	0.2832
MET Exon 14 skip	0 (0.0%)	10 (2.3%)	0.0695

		Total (n=606)	Hispanic (n=141)	Asian (n=204)	African American (n=35)	Non-Hispanic White (n=182)	Other (n=39)	Unknown (n=6)
Hospital								
LAGMC		171 (28.2%)	69 (48.9%)	61 (29.9%)	13 (37.1%)	20 (11.0%)	7 (17.9%)	1 (16.7%)
Norris		435 (71.8%)	72 (51.1%)	143 (70.1%)	22 (62.9%)	162 (89.0%)	32 (82.1%)	5 (83.3%)
Tissue Testing		528 (87.1%)	112 (79.4%)	178 (87.3%)	31 (88.6%)	168 (92.3%)	32 (82.1%)	6 (100.0%)
Liquid Testing		287 (47.4%)	82 (58.2%)	103 (50.5%)	17 (48.6%)	64 (35.2%)	18 (56.3%)	3 (50.0%)
Tissue testing		**Total (n=528)**	**Hispanic (n=112)**	**Asian (n=178)**	**African American (n=31)**	**Non-Hispanic White (n=168)**	**Other (n=32)**	**Unknown (n=6)**
NGS		381 (72.2%)	50 (44.6%)	126 (70.8%)	21 (67.7%)	152 (90.5%)	27 (84.3%)	5 (83.3%)
A la carte/limited testing		147 (27.8%)	62 (55.4%)	53 (29.8%)	10 (32.3%)	16 (9.5%)	5 (15.6%)	1 (16.7%)

Conclusion: The disparity molecular testing patterns and subsequent differences in mutation prevalence between patients at LAGMC and Norris and also reflected among race/ethnicity highlights the need for equality in molecular profiling testing, as this could have therapeutic implications on NSCLC patients.

The Impact of Removing the 15-year Since Quitting Smoking Criterion on Lung Cancer Screening Eligibility

Alexander Potter, Priyanka Senthil, Quiana Guo, Arian Mansur, Uma Sachdeva, Hugh Auchincloss, Chi-Fu Jeffrey Yang
Massachusetts General Hospital

Presented by: Alexandra Potter

Objective

Current lung cancer screening eligibility criteria exclude individuals with heavy smoking histories who quit smoking more than 15 years ago ("15-year since quitting requirement"). We evaluated the impact of removing the 15-year since quitting requirement on lung cancer screening eligibility using data from the Southern Community Cohort Study.

Methods

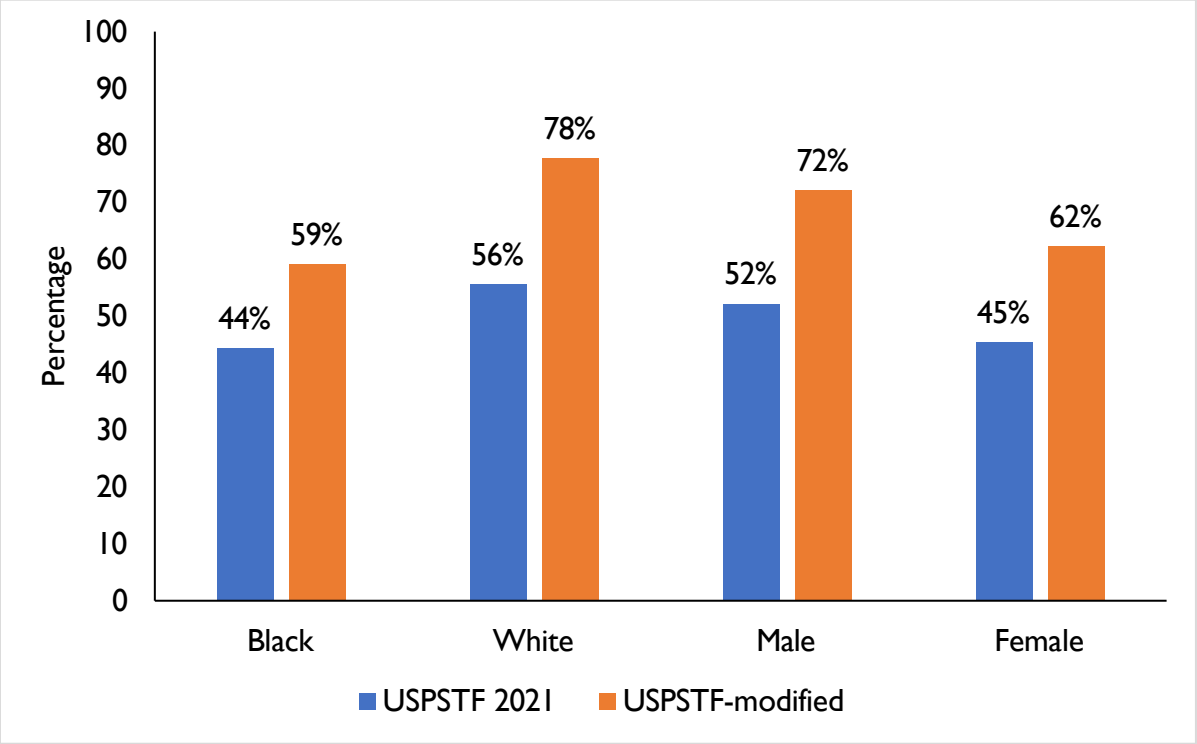
Individuals who formerly smoked in the Southern Community Cohort Study (SCCS)—a large prospective cohort study comprising 85,000 predominately low-income Black and white individuals from 12 southeastern U.S. states—were identified for analysis. The proportions of individuals diagnosed with incident lung cancer who would have qualified for screening under the 2021 United States Preventative Services Task Force (USPSTF) guideline vs. that of a modified guideline that removes the 15-year since quitting requirement were compared using McNemar's test. Subgroup analyses were conducted by race and by sex.

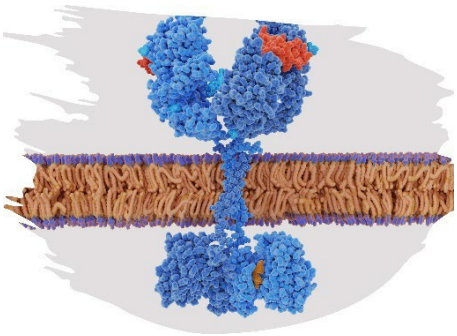
Results

A total of 22,151 individuals formerly smoked in the SCCS, of which 594 were diagnosed with lung cancer during follow-up. Among individuals with lung cancer, only 48.8% (n=290) would have qualified for lung cancer screening under the 2021 USPSTF guideline. Quitting smoking more than 15 years ago was the predominant reason for ineligibility, with 65.5% (n=199) of individuals ineligible for screening having quit smoking more than 15 years ago. Under the modified guideline—which removes the 15-year since quitting smoking requirement—67.2% (n=399) of individuals with lung cancer would have qualified for lung cancer screening, representing a significant increase in eligibility compared to the 2021 USPSTF guideline ($P<0.001$). Increases in screening eligibility under the modified guideline vs. 2021 USPSTF guideline occurred in all patient subgroups evaluated; however, the largest increases in eligibility occurred among males and White patients (**Figure**). Many lung cancer patients, especially those who were Black and female, remained ineligible due to having smoked fewer than 20 pack-years.

Conclusion The 15-year since quitting criterion excluded many individuals who formerly smoked and were diagnosed with lung cancer. Removing the 15-year since quitting criterion increased the proportion of lung cancer patients who formerly smoked that would have qualified for screening but did not reduce race- or sex-based disparities in screening eligibility.

Figure1.: Eligibility Under the 2021 USPSTF Guideline vs. Modified Guideline ("USPSTF-modified")—which Removes the 15-year Since Quitting Smoking Criterion—Among Individuals Who Formerly Smoked and Were Diagnosed with Lung Cancer in the Southern Community Cohort Study





Session 3:

Immuno-oncology

Real-World Practice Patterns of Immunotherapy, Chemotherapy, and Targeted Therapy with Radiation Therapy in Early-Stage Node-Negative Non-Small Cell Lung Cancer

Patrick Oh, Alexander Sasse, Nicholas Wells, So Yeon Kim, Sarah Henry Park

Yale School of Medicine

Presented by: Patrick Oh

Background
Large prospective studies have demonstrated the clinical benefit of chemotherapy and immunotherapy with radiotherapy (RT) in unresectable locally advanced non-small cell lung cancer (NSCLC) to improve disease-related outcomes. The use of systemic therapy, including targeted therapy, has also been shown to improve outcomes for resectable disease. However, the value of combining systemic therapy with RT for early-stage node-negative disease is less well-defined. We evaluated real-world trends in systemic therapy use with RT in this context.

Methods
The Flatiron Health de-identified electronic health record-derived database was utilized to conduct a real-world retrospective study. Inclusion criteria were diagnosis of NSCLC (cT1-3N0M0) between 2019-2023 and treatment with definitive RT with or without first-line systemic therapy. Chi-square, Wilcoxon rank-sum, and multivariable regression analyses were used to identify variables associated with receipt of systemic therapy with RT. The Cochran-Armitage test was used to assess for trends in systemic therapy use over time.

Results
Among 1,699 patients treated with RT, systemic therapy was administered to 260 patients (15%), including chemotherapy to 207 (10% neoadjuvant, 77% concurrent, 33% adjuvant) and immunotherapy to 105 (6% neoadjuvant, 9% concurrent, 95% adjuvant). Targeted therapy was used in 33% of patients with an EGFR mutation. There were no significant differences in age, sex, race, smoking status, insurance, or socioeconomic status associated with systemic therapy use. Higher T stage was significantly associated with systemic therapy on multivariable analysis ($p<0.001$). Among patients with T3N0 disease (n=198), 24% received immunotherapy compared to 4% in those with T1-2N0 disease ($p<0.001$), while 51% received chemotherapy for T3N0 disease compared to 7% in those with T1-T2N0 disease ($p<0.001$). The use of immunotherapy was not significantly associated with PD-L1 status ($p=0.3$). From 2019 to 2023, there was a trend towards increased use of any systemic therapy for T1-T2N0 ($p=0.022$).

Conclusion
Despite the lack of high-level evidence supporting the use of systemic therapy in patients with early-stage node-negative NSCLC treated with RT, modern real-world data suggests frequent off-label use of systemic therapy especially in T3N0 tumors, but increasingly even for T1-T2N0 disease. Therefore, additional prospective data on the benefit of systemic therapy in early-stage node-negative NSCLC is warranted.

Targeting The CXCR1/2 Axis Inhibits Neutrophil Function, And Not Recruitment, in NSCLC

Jeff Kwak, Xiaodong Zhu, Helena Nguyen, Naia Kenney, Christina Baik, McGarry Houghton

Fred Hutchinson Cancer Center, University of Washington

Presented by: Jeff Kwak

The level of tumor and circulating CXCR1/2-expressing neutrophils and CXCR1/2 ligands correlate with poor patient outcomes, inversely correlate with tumoral lymphocyte content, and predict immune checkpoint inhibitor (ICI) treatment failure. Accordingly, CXCR2-selective and CXCR1/2 dual inhibitors exhibit activity both as single agents and in combination with ICI treatment in mouse tumor models. Based on such reports, we have launched an investigator-initiated Phase II clinical trial combining the dual CXCR1/2 antagonist, SX-682, with pembrolizumab for non-small cell lung cancer (NSCLC) patients in the first line. It has been assumed that CXCR1/2 blockade impacts tumors by blocking neutrophil chemotaxis and reducing neutrophil content in tumors. Here, we show that while CXCR2 antagonism does slow tumor growth, it does not preclude neutrophil recruitment into tumor. Instead, CXCR1/2 inhibition alters neutrophil function by blocking the polarization of transcriptional programs towards immune suppressive phenotypes and rendering neutrophils incapable of suppressing lymphocyte proliferation. This is associated with decreased release of reactive oxygen species and Arginase-1 into the extracellular milieu. These findings will critically inform correlative studies performed on pre-treatment and on-treatment tumor biopsies as simply assessing for a decrease in tumoral neutrophil content will not be achievable and would not be an appropriate measure of on-target activity. Remarkably, these therapeutics do not impact the ability of neutrophils to phagocytose and kill ingested bacteria. Taken together, these results mechanistically explain why CXCR1/2 inhibition has been active in cancer but without infectious complications.

Development and Induction of Tertiary Lymphoid Structures in Lung Cancer for Improved Immunotherapeutics

Hye Mi Kim, Medard Kaiza, Ian MacFawn, Noor Nader, Elaine Byrnes, Alexandra McDonough, Caleb Lampenfeld, Sheryl Kunning, Asia Williams, Ashwin Somasundaram,
Kelsey Ertwine, Stephen Thorne, Laura Stabile, Tullia Bruno

University of Pittsburgh, KaliVir Immunotherapeutics

Presented by: Hye Mi Kim

Tertiary lymphoid structures (TLS) are ectopic immune structures that often form locally in cancer. TLS correlate with favorable prognosis in patients with solid tumors, including lung cancer. Further, TLS are 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, the mechanisms of TLS development remain poorly understood due to a paucity of murine models with spontaneous TLS formation. Thus, our project utilizes a carcinogen (NNK) induced murine model of lung adenocarcinoma (LUAD) that recapitulates human LUAD and spontaneously develops TLS with approximately 20-30% of TLS containing germinal centers (GCs). In this model, TLS maturity i.e. GC formation is associated with an increase in tumor-infiltrating immune cells while the size of tumor correlates with the number of anti-tumor immune cells within TLS. According to our temporal assessment of TLS formation, we learned that B cells are the first to arrive for initial TLS formation, subsequently followed by T cells. Furthermore, inhibition of TLS formation via B cell depletion demonstrated a loss of effective antitumor immunity and increased tumor size, indicating the critical role of B cells in TLS formation and activity. In parallel with our mouse studies, our lab evaluates the complexity of TLS within human LUAD using multispectral imaging and spatial transcriptomics to uncover pathways that could improve TLS formation and subsequently immune cell function. According to our spatial transcriptomic data, TLS in patients can have incomplete expression of inducing factors such as LIGHT/LTβ, CXCL13, CD40 ligand, and IL-21. Thus, we have generated an oncolytic virus (OV) which can deliver these factors while also generating immunogenic antigens and stromal space for TLS to thrive. We are utilizing two syngeneic lung cancer murine models to test our OV; (1) FVBW-17, derived from NNK induced LUAD model, and (2) Lewis lung carcinoma (LLC). These studies will increase our mechanistic understanding of TLS development for improved immunotherapies and will potentially provide new therapeutic interventions to treat lung cancer patients.

Uncovering the Rewired IAP-JAK Regulatory Axis as an Immune-Dependent Vulnerability of LKB1-Mutant Lung Cancer

Changfa Shu, Jianfeng Li, Rui Jin, Dacheng Fan, Qiankun Niu, Danielle Cicka, Sean Doyle, Alafate Wahafu, Xi Zheng, Yuhong Du, Andrey A. Ivanov, Deon B. Doxie, Kavita M. Dhodapkar, Jennifer Carlisle, Taofeek Owonikoko, Gabriel Sica, Yuan Liu, Suresh Ramalingam, Madhav V. Dhodapkar, Wei Zhou, Xiulei Mo, Haian Fu

Emory University

Presented by: Jianfeng Li

Harnessing the power of immune system to treat cancer has become a core clinical approach. However, rewiring of intrinsic circuitry by genomic alterations enables tumor cells to escape immune surveillance, leading to therapeutic failure. Uncovering the molecular basis of how tumor mutations induce therapeutic resistance may guide the development of intervention approaches to advance precision immunotherapy. Here we report the identification of the LKB1-IAP-JAK dynamic complex as a molecular determinant for immune response of LKB1-mut lung cancer cells. LKB1 alteration exposes a critical dependency of lung cancer cells on IAP for their immune resistance. Indeed, pharmacological inhibition of IAP re-established JAK-regulated STING expression and DNA sensing signaling, enhanced cytotoxic immune cell infiltration, and augmented immune-dependent anti-tumor activity in an LKB1-mutant immune-competent mouse model. Thus, IAP-JAK-targeted strategies, like IAP inhibitors, may offer a promising therapeutic approach to restore the responsiveness of “immunologically-cold” LKB1-mutant tumors to immune checkpoint inhibitors or STING-directed therapies.

Upregulated PLA2G10 in Cancer Impairs T-Cell Infiltration to Dampen Immunity

Tianxiang Zhang, Weiwei Yu, Xiaoxiao Cheng, Jacky Yeung, Viviana Ahumada, Paul Norris, Mackenzie Pearson, Xuan Yang, Ala Nassar, Mathew Vesely, Yu Zhang, Jianping Zhang, Lan Ji, Dallas Flies, Linda Liu, Solomon Langermann, William LaRochelle, Rachel Humphrey, Dejian Zhao, Qiuyu Zhang, Jindong Zhang, Runxia Gu, Kurt Schalper, Miguel Sanmamed, Lieping Chen

Yale University, Sciex Demo Lab, NextCure Inc., Normunity Inc., University of Navarra

Presented by: Tianxiang Zhang

T cells are often absent from human cancer tissues during both spontaneously-raised immunity and therapeutic immunotherapy, even in the presence of a functional T cell-recruiting chemokine system, suggesting the existence T cell exclusion mechanisms that impair infiltration. Using a genome-wide *in vitro* screening platform, we identified a role for phospholipase A2 group 10 (PLA2G10) protein in T cell exclusion. PLA2G10 upregulation is widespread in human cancers and is associated with poor T cell infiltration in tumor tissues. PLA2G10 overexpression in immunogenic mouse tumors excluded T cells from infiltration, resulting in resistance to anti-PD-1 immunotherapy. PLA2G10 can hydrolyze phospholipids into small lipid metabolites thus inhibiting chemokine-mediated T cell mobility. Ablation of PLA2G10's enzymatic activity enhanced T cell infiltration and sensitized PLA2G10-overexpressing tumors to immunotherapies. Our study implicates a role for PLA2G10 in T cell exclusion from tumors and suggests a potential target for cancer immunotherapy.

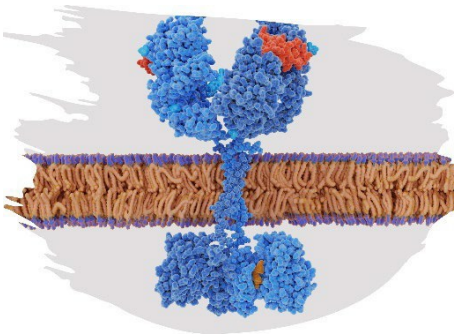
Loss Of Mir-29 as a Mechanism of Diminished Anti-PD-1 Response in Lung Cancer

Jessica Konen, Lixia Diao, Jing Wang, Don Gibbons

Emory University, UT MD Anderson Cancer Center

Presented by: Jessica Konen

Immunosuppressive checkpoint inhibitors (ICI), like those that block the PD-1/PD-L1 axis, have revolutionized oncological therapy, including for patients with non-small cell lung cancer (NSCLC). Clinical studies demonstrated objective response rates in 28-40% of advanced stage NSCLC patients depending on PD-L1 status. While encouraging, these data indicate that most patients demonstrate no clinical benefit or rapidly acquire resistance even in PD-L1-high populations. Therefore, there remains a critical need to dissect key tumor cell survival dependencies to overcome treatment resistance. To discover tumor-driven mechanisms of anti-PD-1 resistance, we performed single cell RNA-sequencing on KRAS/p53 mutant murine lung tumors with sensitivity or resistance to anti-PD-1 (PD1S or PD1R, respectively) and focused on predicted upstream regulators of differentially expressed genes (DEGs) in PD1R tumors, specifically within the malignant cell cluster. We found that the micro-RNA miR-29 was strongly predicted to be a post-transcriptional suppressive molecule of numerous DEGs in the dataset that were upregulated with resistance. Thus, we hypothesized that miR-29 downregulation associated with anti-PD-1 resistance causes vast tumor cell intrinsic and extrinsic effects on the microenvironment that diminish anti-tumor immune responses. Analysis of genes that correlate with miR-29 expression in TCGA lung adenocarcinoma datasets revealed positive enrichment in immune-related pathways, including adaptive immune response and T cell activation, by gene set enrichment analysis. Irrespective of treatment, patients with low miR-29 expression have significantly shortened overall survival compared to miR-29-high patients. In the PD1R murine models, we confirmed miR-29 downregulation compared to PD1S models and probed for several predicted/known miR-29 target genes. Specifically, the PD1R models had significantly increased expression of several immunosuppressive molecules including *Enpp2*/ATX (as published recently by our group) and *Cd276*/B7-H3, and genes involved in tumor: extracellular matrix (ECM) interactions and metastasis like *Lamc1*, *Itgb1,* and *Snail1*. Re-expression of miR-29 in PD1R cells was sufficient to downregulate expression of these genes and importantly, reinvigorate CD8+ T cell proliferation while decreasing expression of exhaustion markers PD-1 and LAG-3. Together, our data provide evidence that the miR-29 axis serves as a regulator of a vast transcriptional network that functions to promote anti-tumor immunity and ICI response in lung cancer.



Session 4:

Small Cell Lung Cancer

Plasma Autoantibodies Identify Citrullinated Transferrin Receptor Neoepitopes in Small Cell Lung Cancer

Kristin Lastwika, Sophia Lauer, Francesca Urselli, Justin Taylor, Zachary Samuels, Brian Zeglis, McGarry Houghton, Paul Lampe

Fred Hutchinson Cancer Center, Hunter College

Presented by: Kristin Lastwika

Small cell lung cancer (SCLC) is the 6th leading cause of cancer-related deaths with fewer than 6% of patients surviving 5 years post diagnosis. We have identified 22 autoantibody-antigen complexes upregulated in 3 independent cohorts of SCLC and hypothesized these autoantibodies could elucidate tumor-specific neoepitopes. One of the validated autoantibody-antigen complexes target transferrin receptor (TFRC, CD71), a highly expressed, cell surface protein present in many types of cancer. We found TFRC widely expressed in SCLC tissue microarrays and TFRC transcript was present across SCLC subtypes in 53% of $ASCL1^+$, 27% of $NEUROD1^+$, 71% of $POU2F3^+$, and 33% of $YAP1^+$ cell lines. While TFRC is expressed in rapidly proliferating normal tissues, we have found that TFRC present in SCLC tumors contain post-translationally modified citrulline residues. We also found peptidyl arginine deiminases, the enzymes responsible for converting citrulline from arginine, were highly expressed in SCLC. We identified 5 citrullinated residues in the extracellular domain of TFRC (cit-TFRC) that act as neoantigens targeted by autoantibodies in SCLC patient plasma. Using this information, we isolated cit-TFRC-specific B cells directly from SCLC peripheral blood mononuclear cells via cit-TFRC-peptide tetramers. After single cell sorting and targeted sequencing of the B cells' antibody variable binding domains, expression in a human IgG backbone allowed us to make antibodies that are specific for cit-TFRC. These human cit-TFRC antibodies recognize cit-TFRC in SCLC cell lines and tumors, but not TFRC expressed in normal human tissues. Using click chemistry the antimitotic drug monomethyl auristatin E (MMAE) was attached to a cit-TFRC antibody to create an antibody drug conjugate with the capability of killing SCLC cell lines *in vitro*. Finally, a far-red fluorescent-labeled cit-TFRC antibody injected by tain vein honed to SCLC sub-cutaneous tumors suggesting the potential for *in vivo* efficacy. Using our unique approach to identify and isolate autoantibodies directly from SCLC patients, we provide early preclinical evidence for the potential to specifically target TFRC in cancer cells- a protein previously thought to be undruggable.

Jumonji Histone Demethylases are Therapeutic Targets in Small Cell Lung Cancer

Aiden Nguyen, Clarissa Nunez, Tram Anh Tran, John Minna, Elisabeth Martinez

UT Southwestern

Presented by: Elisabeth Martinez

Small cell lung cancer (SCLC) is a recalcitrant cancer of neuroendocrine (NE) origin with little change in therapeutic approaches over decades. Here, we use preclinical models to identify a new therapeutic vulnerability in SCLC consisting of select members of the targetable Jumonji lysine demethylase (KDM) family. We show that Jumonji demethylase inhibitors block malignant growth and that etoposide-resistant SCLC cell lines are particularly sensitive to Jumonji inhibition *in vitro* and *in* xenografts *in vivo*. Mechanistically, small molecule-mediated inhibition of Jumonji histone demethylases activates endoplasmic reticulum (ER) stress response genes, upregulates ER stress pathway signaling, and triggers PARP cleavage. Furthermore, Jumonji inhibitors downregulate expression of oncogenic proteins, and decrease SCLC NE markers including INSM1 and Secretogranin-3. Genetic knockdown of KDM4A, a Jumonji demethylase highly expressed in SCLC and a known regulator of ER stress genes, induces ER stress response genes and inhibits proliferation of SCLC *in vitro* and *in vivo*. Lastly, we demonstrate that two different small molecule Jumonji inhibitors (JIB-04 and SD-70) block the growth of SCLC xenograft tumors. Our study highlights the translational potential of Jumonji KDM inhibitors, a clinically feasible approach in light of recently opened clinical trials evaluating the anticancer properties of this drug class.

Cyclin A/B RxL Macrocyclic Peptide Inhibitors as a Therapeutic Strategy for Small Cell Lung Cancers with High E2F Activity

Shilpa Singh, Catherine Gleason, Min Fang, Vishal Khivansara, Shanhai Xie, Yavuz Durmaz, Aniruddha Sarkar, Yasmin Laimon, Varunika Savla, James Aggen, Li-Fen Liu, Bernard Levin, Evelyn Wang, Sabina Signoretti, Alexander Spektor, Constantine Kreatsoulas, Rajinder Singh, David Earp, Pablo Garcia, Deepak Nijhawan

Dana-Farber Cancer Institute, Circle Pharma, Brigham and Women's Hospital, UT Southwestern

Presented by: Matthew Oser

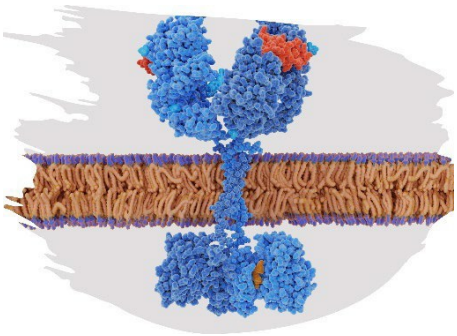
Macrocyclic peptides, or macrocycles, block protein-protein interactions (PPIs) and can be used as a strategy to target undruggable proteins in cancer. While E2F1 activity drives cancer cells to proliferate, E2F1 overactivity promotes apoptosis. Blocking a cyclin A/E2F1 RxL-mediated PPI causes E2F1 hyperactivation leading to apoptosis in transformed cancer cell lines. Here we developed cell-permeable macrocycles that block RxL-mediated binding of cyclin A and/or cyclin B to their substrates thereby inhibiting cyclin-substrate interactions. We found that small cell lung cancers (SCLCs) were hypersensitive to macrocycles that inhibit both cyclin A and cyclin B RxL interactions due to their inherent high E2F activity where dual cyclin A/B RxL macrocycles induced mitotic arrest and apoptosis in SCLC cell lines. Genome-wide CRISPR/Cas9 knockout screens and genome-wide random mutagenesis screens found that cyclin A/B RxL macrocycles induced apoptosis by hyperactivating, rather than inhibiting, cyclin B, which activated the spindle assembly checkpoint (SAC) leading to mitotic arrest. Mechanistically, cyclin B RxL macrocycles blocked a cyclin B:Myt1 inhibitory interaction in turn hyperactivating cyclin B; while cyclin A RxL macrocycles blocked a cyclin A:E2F1 RxL inhibitory interaction hyperactivating E2F1 further sensitizing cells to cyclin A/B RxL macrocycles. Cyclin A/B RxL macrocycles induced SAC activation, apoptosis, and tumor regressions in SCLC xenograft models. This work suggests that cyclin A/B RxL macrocycles should be tried as a new therapeutic strategy for SCLCs with high E2F activity.

The Chromatin Remodeling Complex PBAF Functions as a Bona Fide Tumor Suppressor Complex In SCLC

Arnaud Augert
Yale University

Presented by: Arnaud Augert

Small cell lung cancer (SCLC) is a recalcitrant, metastatic neuroendocrine carcinoma that represents the most aggressive lung cancer histology, with a five-year survival rate of less than 7%. Despite recent progress, the molecular mechanisms that promote the development of SCLC remain incompletely delineated and there is an urgent need for refined, more effective therapies. We analyzed publicly available genomic databases of SCLC and found recurrent inactivating mutations in genes that code for subunits of the polybromo-associated BAF (PBAF), a member of the SWI/SNF chromatin remodeling complexes. To build upon this initial discovery and dissect the functions of PBAF during SCLC development, we have generated a series of unique and complementary models including a novel PBAF-deficient genetically engineered mouse model (GEMM). Interestingly, we found that PBAF deficiency in a novel autochthonous mouse model of SCLC leads to a marked acceleration of SCLC development and a stark reduction in overall survival. Transcriptional and epigenomic analyses of human and mouse PBAF-deficient SCLC models pinpoint to an upregulation of gene expression programs associated with stemness, growth and metastasis. Altogether, our study identifies PBAF as a major tumor suppressive complex in SCLC.



Session 5:

Early Detection and Screening

Elaine Shum, MD
New York University

Significance of Image Acquisition Parameters for Sybil's Ability to Predict Future Lung Cancer Risk on Low-Dose Chest Computed Tomography

Judit Simon, Peter Mikhael, Alexander Graur, Regina Barzilay, Lecia Sequist, Florian Fintelmann

Mass General Hospital, Massachusetts Institute of Technology, Harvard

Presented by: Florian Fintelmann

Purpose

Sybil is a validated open access deep learning-based algorithm that can accurately predict long-term lung cancer risk from a single low-dose chest computed tomography (LDCT). We aimed to study the effect of reconstruction filter and reconstruction thickness on Sybil's performance.

Methods and Materials

We used LDCTs of the National Lung Screening Trial participants who were included in the test set for the development of Sybil (Figure 1). Series from the same LDCT examination were paired by matching kilovoltage peak, milliampere-seconds, and either reconstruction filter or reconstruction thickness, interval, and diameter. We considered any LDCT positive for future lung cancer if cancer was subsequently confirmed by needle biopsy or surgical resection. We compared the area under the curve (AUC) for each series pair using DeLong's test.

Results

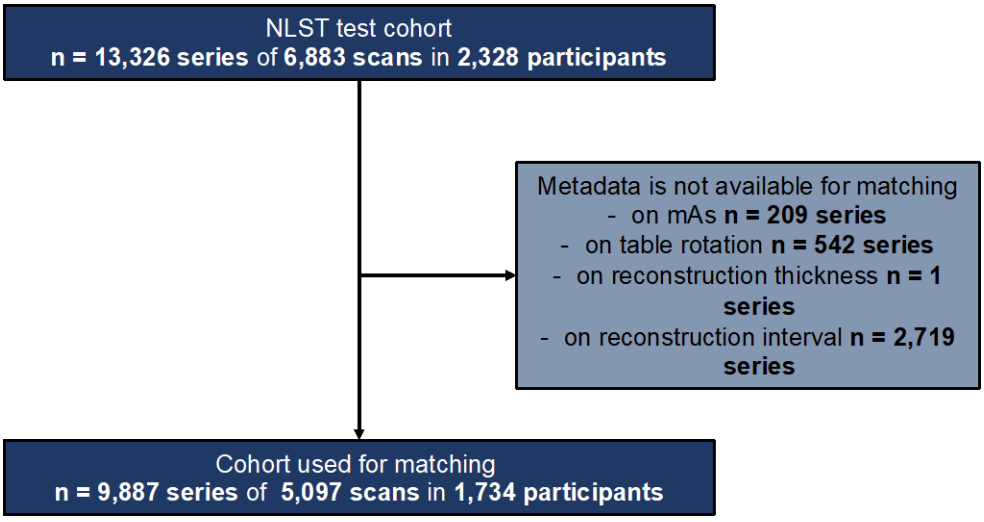
We were unable to detect a significant difference in Sybil's performance between the 1,049 pairs of bone vs standard reconstruction filter (AUC at 1 year 0.73 [95%CI: 0.66-0.80] vs 0.72 [95%CI: 0.65-0.79]; p=0.87) and the 1,961 pairs of lung vs standard reconstruction filter (AUC at 1 year 0.80 [95%CI: 0.75-0.85] vs 0.81 [95%CI: 0.76-0.85]; p=0.77). Similarly, we were unable to detect a significant difference between the 1,288 pairs of 2 mm vs 5 mm reconstruction thickness (AUC at 1 year 0.72 [95%CI: 0.65-0.79] vs 0.70 [95%CI: 0.62-0.78]; p=0.75) and the 158 pairs of 1.25 mm vs 2.5 mm reconstruction thickness (AUC at 1 year 0.75 [95%CI: 0.58-0.93] vs 0.73 [95%CI: 0.52-0.94]; p=0.86).

Conclusion

We did not detect a difference in Sybil's performance across different reconstruction filters and thicknesses, emphasizing the robustness of this tool for early lung cancer prediction across diverse clinical scenarios.

Figure 1.

Study flowchart. NLST = National Lung Screening Trial.



Rationale and Design of the Assessment of a Radiomics-based Computer-Aided Diagnosis Tool for Pulmonary NodulES (ARCADES) Pragmatic Randomized Controlled Trial

Roger Kim, John Salsini-Tobias, Katharine Rendle, Nandita Mitra, Anil Vachani

University of Pennsylvania

Presented by: Roger Kim

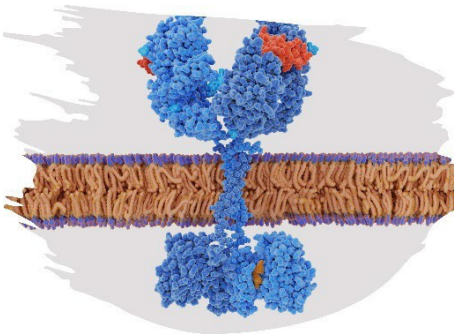
Introduction: Pulmonary nodules (PNs) pose a diagnostic challenge for clinicians, who must perform a complex risk assessment for malignancy to determine whether to pursue a lung biopsy. Advancements in PN risk stratification have not been realized despite the development of validated novel biomarkers due to a lack of clinical utility and effectiveness data. Preliminary data from our retrospective clinical utility study suggest increased diagnostic performance and theoretical net benefit when clinical PN risk assessment is supplemented by a commercially available artificial intelligence radiomics-based computer-aided diagnosis (CAD) tool (Optellum Virtual Nodule Clinic). Here we describe the protocol for ARCADES (NCT05968898), the first NCI-funded, investigator-initiated pragmatic randomized controlled trial (RCT) assessing the clinical effectiveness of this CAD tool.

Objectives: ARCADES aims to study the effect of the CAD tool on clinicians' PN management.

Study Design: Adults with a newly discovered 8-30mm PN evaluated at Penn Medicine are eligible. Key exclusion criteria include imaging data incompatible with the CAD tool software and a history of lung cancer. Subjects undergo 1:1 randomization stratified by participating clinician to 1) usual care or 2) usual care + use of the CAD tool. In the intervention arm, clinicians receive a report with the Lung Cancer Prediction score computed by the CAD tool, representing estimated PN malignancy risk. The primary outcome is appropriate PN management, defined as the composite proportion of benign PNs managed with imaging surveillance and malignant PNs managed with lung biopsy or empiric treatment. Final PN diagnosis will be determined based on pathologic evaluation. If pathology is unavailable or inconclusive, PN resolution, shrinkage, or diameter stability at 12 months will be defined as a benign diagnosis.

Study Analysis: The target sample size of 300 subjects powers the study to detect a 15% percentage-point difference in the primary outcome between arms. We will use a group sequential design to accommodate an interim analysis for efficacy at 25% enrollment. The primary intention-to-treat analysis will include an independent proportions Z-test and a multivariable logistic regression analysis adjusting for relevant patient and PN clinical characteristics.

Conclusions: The ARCADES pragmatic RCT is currently actively enrolling at Penn Medicine.



Session 6:

Cancer Biology

Monoclonal antibodies targeting PCDH7 inhibit tumor growth and enhance response to MAPK pathway-targeted therapies in non-small cell lung cancer

Nicole Novaresi, Poorva Ghosh, Hui Deng, Xuejun Fan, Zhiqiang Ku, Wei Xiong, Xiaorong Zhou, Jingfei Zhu, Huiyu Li, Mahesh S. Padanad, Bethany Smith, Chul Ahn, John D. Minna, Zhiqiang An, Ningyan Zhang, Kathryn A. O'Donnell

UT Southwestern, UT Health Science Center at Houston

Presented by: Nicole Novaresi

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-associated deaths worldwide. Given the efficacy of cell surface proteins as therapeutic targets in human malignancies, we examined transmembrane proteins that may act as drivers of lung tumorigenesis. We previously identified a critical oncogenic role for Protocadherin 7 (PCDH7), a cell surface protein and member of the Cadherin superfamily in NSCLC. PCDH7 is frequently overexpressed in lung adenocarcinoma (LUAD) and associates with poor clinical outcome. Using both *in vitro* and *in vivo* models, we demonstrated that PCDH7 modulates the MAPK signaling pathway and promotes lung tumorigenesis (PMC5410365). Using a Cre-inducible *PCDH7* transgenic mouse model, we demonstrated that PCDH7 accelerates mutant *Kras*-mediated tumorigenesis. CRISPR/Cas9 depletion of *Pcdh7* reduces lung tumor development, prolongs survival, and diminishes phospho-activation of ERK1/2 in $Kras^{LSL\text{-}G12D}$; $Tp53^{fl/fl}$ (KP) mice (PMC6359939). These results, together with our finding that *Pcdh7* knockout mice exhibit no overt developmental defects, establish a critical oncogenic function for PCDH7 *in vivo* and suggest that PCDH7 is an actionable therapeutic target. Here we report the development and characterization of high affinity anti-PCDH7 monoclonal antibodies (mAbs) that inhibit downstream MAPK pathway activation and suppress tumor growth in multiple mouse models, including *KRAS*- and *EGFR*-mutant models. A lead mAb (mAb7) sensitized tumors to the FDA-approved MEK inhibitor trametinib and the $KRAS^{G12C}$ inhibitor adagrasib. Moreover, a humanized mAb7-IgG1 exhibited antibody dependent cellular cytotoxicity (ADCC) and Fc-mediated immune effector killing of tumor cells *in vivo*. These findings provide an important step towards the clinical development of PCDH7-targeting antibodies for the treatment of NSCLC and other tumor types with high PCDH7 expression. Funding: NCI (R01CA207763), CPRIT (RP190610), and University of Texas SPORE in Lung Cancer (P50CA070907).

Characterization Of Tumor-Neuron Communication In Lung Adenocarcinoma Brain Metastasis

Sampada Chande, Atsushi Yamasaki, Don Nguyen

Yale University, Daiichi Sankyo Co., Ltd

Presented by: Sampada Chande

30-40% of cancer patients with lung cancer develop brain metastasis with poor prognosis. The incidence of brain metastasis has increased in patients that have received standard of care treatment, including targeted therapies. The mechanisms which promote lung cancer cell dissemination and colonization of the central nervous system (CNS) remain understudied and identifying novel drug targets to treat brain metastasis is an unmet clinical need.

By using a variety of animal models of lung cancer metastasis, we previously identified shifts in epithelial and neuronal like lineage gene expression programs in malignant cells as they adapt to the brain tumor microenvironment (TME) *in vivo*. Gene set enrichment analysis (GSEA) revealed that most of genes are involved in neuronal guidance and cell adhesion pathways (L2FC > 0.5, padj < 0.05, Log2RPKM > 1 compared to 2D culture). We also examined tumor-neuron interactions in patient derived surgical biopsies and found active areas of interaction between tumors and neurons in human lung adenocarcinoma (LUAD) brain metastasis. Spatial transcriptomics of human brain metastasis also revealed differential profiles for areas of active interaction. *In vitro*, we established co-culture conditions which demonstrate that primary neurons can directly interact with brain metastatic LUAD cells and improve their fitness.

Based on these observations, we performed a custom Clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 loss of function screen targeting 470 neuronal and cell adhesion genes that are up-regulated in LUAD cells that seed the brain *in vivo*. Moreover, we performed our screens under different conditions which recapitulate tumor cell-tumor cell clustering in 3-dimensional culture conditions (3D) or tumor-neuron co-clustering in 3D. Results from this differential screening approach suggests that LUAD cells are increasingly, but not exclusively, dependent on several neuronal genes as they are co-cultured with neurons.

In conclusion, we have modelled direct tumor-neuronal cell interaction *in vitro*, shown that it is an important component of brain metastasis, and identified putative targets that can be inhibited to disrupt tumor-neuron interactions and potentially limit brain metastatic progression.

The AVERON Notebook: A Computational Approach to Druggable Cancer Dependencies Enabled by Mutant-Directed Protein-Protein Interactions

Hongyue (Nicole) Chen, Brian Revennaugh, Haian Fu, Andrey Ivanov

Emory University

Presented by: Andrey Ivanov

Cancer is the second leading cause of death that takes more than 10 million lives every year. The genomic alterations, such as mutations in cancer driver genes, can dysregulate the essential networks of protein-protein interactions (PPIs), re-wiring major oncogenic pathways and inducing the transformation. While some mutations can disrupt existing PPIs, others can create PPIs, which are unnatural for wild-type counterparts. Discovery of both lost hypomorph (PPIs) and mutant-created neomorph PPIs (neoPPIs) is vital for understanding the molecular mechanisms of mutant-driven tumorigenesis and developing new personalized clinical strategies in cancer. However, systematic experimental interrogation of the clinical significance and biological functions of neo- and hypoPPIs is highly challenging. To address this critical challenge, we develop a novel computational platform called AVERON Notebook for identifying new Actionable Vulnerabilities Enabled by Rewired Oncogenic Networks. The AVERON employs specially designed algorithms and statistical techniques to

i) Assess and compare the levels of neoPPIs in cancer patients

ii) Examine their impact on clinical outcomes

iii) Identify distinctive sets of signature genes and oncogenic pathways regulated by individual neomorph PPIs

iv) Discover clinically significant neoPPI-regulated genes with available clinical compounds and approved drugs.

Together, the AVERON Notebook provides a powerful tool for elucidating the functional consequences of tumor-driving mutations mediated through the mutant PPI networks and sheds new light on previously unexplored clinically actionable dependencies enabled by neomorph PPIs in cancer patients.

The AVERON Notebook is freely available on GitHub: https://github.com/aivanovlab/averon_notebook and Ocean Code: https://doi.org/10.24433/CO.7986101.v1

Identifying Unique Challenges and Opportunities in Incorporating Patient Advocates into the Lung Cancer SPORE Program

Elda Railey, John Minna

Research Advocacy Network, UT Southwestern

Presented by: Elda Railey, Marcia Horn, Merel Mountain-Grey, Lisa Spain

Abstract:
Incorporating advocates into the Lung Cancer SPORE program has been a top priority since its inception. While there has been progress in achieving meaningful involvement, qualitative research conducted by the Research Advocacy Network Advocate Institute has identified some unique challenges for both the patient advocates and the SPORE scientific research team members.

Patient Advocate Challenges:
- Understanding the science, methodology and statistical approaches used in translational studies involving preclinical models, patient biospecimens, and early/late phase clinical trials.
- Learning how to apply critical thinking to analyze the presented science to understand and evaluate the validity, significance, quantitative impact of the findings and likelihood of eventual translation into patients
- Learning how to provide and communicate a patient's perspective that applies to translational science settings, including team members of variable degrees of experience and expertise, and that actually can help scientific team members improve and extend their findings.

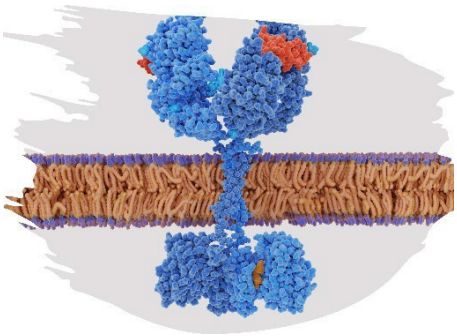
Scientific Team Challenges:
- Understanding how to collaborate with patient advocates – learning what constitutes different types of interaction (learning from patients, teaching advocates, information sharing, joint decision-making)
- Addressing communication and terminology challenges to improve communication between advocates and scientists
- Teaching young, clinical translational research inexperienced scientific team members how to interact beneficially with patient advocates (Career Enhancement, Developmental Projects)
- For non-clinical scientific team members how to appreciate dealing with lung cancer from a patient and family's perspective

Resources Developed: To address these challenges, the Research Advocacy Network has developed several resources (https://researchadvocacy.org/pdf-resources):
- Piloted a training with virtual Learning Labs with topic expert and online curriculum covering translational science-specific topics, such as an overview of translational science, biomarkers, cell biology, correlative research, as well as a skill-building session on critical thinking.
- A video for researchers to explain how to collaborate with advocates
https://vimeo.com/790243746?share=copy

The UT Southwestern/MD Anderson Lung SPORE (P50 CA070907) members played a significant role in contributing to the qualitative research, curriculum development, and delivery. Plans to offer the Translational Science curriculum for Advocates to all SPORES and expand the distribution of the video are underway. Evaluating the program's impact will be essential to its success.

Poster Presentations

Contents



Session 1:

Oncogene-Driven Lung Cancers And Resistance To Targeted Therapies

Kras G12D Inhibition Causes Tumor Regression And Synergizes with Immune Checkpoint Blockade in Immunogenic Lung Cancer Models

Esra Akbay
UT Southwestern
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Presented by: Esra Akbay

The discovery of Kras inhibitors represents a significant step forward in the treatment of Kras mutant tumors. The efficacy of the Kras G12D inhibitor, particularly demonstrated in autochthonous GEMM models and syngeneic models driven by G12D, highlights its therapeutic potential. Notably, the response to MRTX1133 was durable in autochthonous models. Further investigation into the efficacy of MRTX1133 with syngeneic models revealed a therapeutic response in both immunocompetent wildtype mice and immunocompromised NSG mice. However, syngeneic tumors responded significantly better to MRTX1133 in wildtype mice compared to NSG mice. This led us to explore of potential immune mediators contributing to this durable therapeutic response. In our analysis, MRTX1133-treated tumors exhibited a substantial increase in the infiltration of immune cells, T cells, cytotoxic T cells, and NK cells compared to the vehicle-treated controls. Within the CD8+ T cells, an increase in activation markers was observed after MRTX1133 treatment, suggesting that inhibiting KrasG12D in lung tumors has the potential to enhance effector T cell activity. G12D inhibitor had single agent activity in all Kras G12D models tested. We also evaluated the efficacy of G12D inhibitor in the newly developed Genetically engineered mouse lung model derived syngeneic lung cancer models with high tumor mutational burden (TMB).

In summary, our research sheds light on the promise of the G12D inhibitor in treating established GEMM tumors and sheds light into novel immune-mediated biology associated with Kras G12D inhibition.

Development and Effectiveness of TROP2 CAR T In Egfrm NSCLC Including in TROP2 ADC Refractory Settings

Elliott Brea, Eric Smith, David Barbie, Sachiv Chakravarti, Alexis Mottram, Prafulla Gokhale, Ken Ng, Cloud Paweletz, Pasi JÃnne, Simon Baldacci, Elena Ivanova, Ha Vo, Tran Thai, Timothy Haggerty

Dana-Farber Cancer Institute

Presented by: Elliott Brea

The majority of patients with stage IV or metastatic lung *EGFR* mutant (*EGFRm*) NSCLC are unable to achieve durable responses to EGFR inhibitors and do not respond to checkpoint blockade. Development is needed for immunotherapy-based strategies for *EGFRm* NSCLC. TROP2, a cell surface protein normally expressed during fetal development and at low levels in some normal adult tissue, and over expressed on a variety of solid tumors including NSCLC, has emerged as an ideal target. TROP2 is the target of the recently FDA approved antibody-drug conjugate (ADC) sacitizumab-govitecan for TNBC as well as for patients with locally advanced or metastatic urothelial cancer and has been evaluated in phase II/III trials in NSCLC. While high response rates have been seen with TROP2 ADC, durable responses are rare. Acquired resistance to the TROP2 ADC has been described with on target mutations in TROP2 as well as off target mutations in Topoisomerase pathway genes, which confer resistance to the chemotherapy drug conjugate. One approach is utilizing T-cells as an effector to target TROP2, which may offer more durable responses when compared to ADC based approaches. We designed a second generation CAR vector utilizing TROP2 scFv derived from the ADC sacitizumab-govitecan as well as from datopotumab. Our data demonstrate that TROP2 CAR T demonstrate high cytolytic activity in vitro on cell line (cytotoxicity of 90% at E:T 1:1 after 24h $p<0.0001$) and patient derived EGFR mutant samples including in the setting of acquired resistance to the third generation EGFR inhibitor osimertinib. TROP2 CAR T also demonstrate high activity against cell line (eradicating 5/5 PC9 and HCC827GR6 tumors at day 100 compared to 0/5 in control) and patient derived xenograft models. We also demonstrated that TROP2 CAR T are effective in models of acquired resistance to TROP2 ADC. We subsequently developed novel TROP2 VH based CAR T constructs including biparatopic CAR T which are effective in models of emerging resistance to TROP2 based therapies by targeting multiple domains. TROP2 CAR T may serve as an effective therapeutic strategy to pursue in *EGFRm* NSCLC.

Dihydrouridine Synthase 2 Sustains Levels Of Trnacys to Inhibit Ferroptosis in Lung Cancer

Austin Draycott, Matthew Wang, Diana Martínez Saucedo, Luisa Escobar-Hoyos, Wendy Gilbert

Yale University

Presented by: Austin Draycott

Dihydrouridine is a universal tRNA modification installed by conserved enzymes that are dysregulated in cancer. High dihydrouridine synthase 2 (DUS2) expression predicts poor patient outcomes in lung adenocarcinoma for reasons as yet unclear. Here, we investigated the mechanisms using human and mouse models and found that DUS2 suppresses ferroptosis, a metal-dependent non-apoptotic form of cell death that is emerging as a therapeutic target. DUS2 loss caused increased sensitivity to ferroptosis inducers with concomitant accumulation of toxic lipid peroxides, a hallmark of ferroptotic cell death. Mechanistically, DUS2 was specifically required to maintain tRNACysGCA levels to support translation of cysteine-rich proteins. We identified metallothioneins, which are 35% cysteine, as key regulators of ferroptosis downstream of DUS2 via their effects on metal and redox homeostasis. Combining DUS2 depletion with ferroptosis induction prolonged survival in a mouse model highlighting the therapeutic potential of targeting DUS2 in lung cancer.

Investigating *Lkb1* Loss as Novel Synthetic Vulnerability In *EGFR*-Driven Lung Cancer

Francisco Exposito, Emily Shuldiner, Steven Lopez, Minwei Wang, Mariana Do Carmo, Laura Andrejka, Giorgia Foggetti, Christopher W. Murray, Dimitri Petrov, Monte Winslow, Katerina Politi

Yale University, Stanford University, IRCCS Ospedale San Raffaele,

Presented by: Francisco Exposito

The discovery of mutations in *EGFR* that drive lung adenocarcinoma growth and confer sensitivity to tyrosine kinase inhibitors (TKIs) has transformed the treatment of lung cancer. Despite these encouraging clinical results, targeted therapies are not curative and the depth and duration of responses to these agents are variable. We know that multiple factors can cause these heterogeneous responses. The genomic landscape of *EGFR*-driven lung adenocarcinomas is complex, and responses to therapy can be influenced by different combinations of genomic alterations that impact tumor fitness and therapeutic sensitivity. We have shown that *Lkb1* inactivation in mouse models of KRAS-driven and EGFR-driven *Trp53*-deficient lung adenocarcinomas had opposite effects. While the loss of *Lkb1* strongly promoted growth of oncogenic KRAS-driven lung tumors, its inactivation reduced EGFR-driven tumor growth. Remarkedly, these effects align with the mutational patterns of these in human lung cancer, where *LKB1* mutations rarely co-occur with *EGFR* mutations. To gain mechanistic insights into this synthetic lethal interaction, we have generated a new conditional lung cancer model based on the inducible expression of $EGFR^{L858R}$ and deletion of *Lkb1* upon activation of a tamoxifen-inducible CreER allele. While *Lkb1* inactivation in established *EGFR*-driven tumors, did not lead to overt tumor regression (assessed by magnetic resonance imaging), we observed increased overall survival in comparison to mice with *Lkb1* wild-type tumors. *Lkb1* wild-type tumors also had increased tumor growth rates. Following *Lkb1* inactivation, morphological changes in some tumors were observed at the histological level, indicative of compromised cell viability. Further histological characterization of these lesions will provide information on the underlying mechanisms driving these morphological changes. To mechanistically dissect why LKB1 is deleterious in EGFR-driven lung adenocarcinomas, we are using multiplexed CRISPR-Cas9-mediated genome editing in our $EGFR^{L858R}$;$p53^{flox/flox}$;*Cas9* mouse model to establish which LKB1 effectors (AMPK, BRSK, MARK, NUAK, and SIK) are required for the survival EGFR-driven lung cancers. This work will uncover the therapeutic potential of LKB1 pathway inhibition and dissect the downstream effect of the synthetic lethal relationship of LKB1 and oncogenic EGFR.

Efficacy of Afatinib in Patients with Tumors Harboring the Uncommon EGFR Exon 19 Deletion, L747_A750delinsp: A Pooled Analysis

Michael Grant, Fernando de Miguel, Michael Kane, Yong Kong, Jieling Miao, Mary Redman, Zenta Walther, Katerina Politi, Sarah Goldberg
Yale University, Fred Hutchinson Cancer Research Center

Presented by: Michael Grant

Background: The uncommon *EGFR* exon 19 deletion (del19) L747_A750delinsP is less sensitive to inhibition by 1st and 3rd generation EGFR tyrosine kinase inhibitors (TKI) than the common del19 E746_A750del owing to a higher ATP binding affinity associated with L747_A750delinsP. Accordingly, EGFR L747_A750delinsP is associated with worse outcomes with erlotinib and osimertinib treatment compared to E746_A750del. In preclinical studies, L747_A750delinsP and the common del19 E746_A750del do not differ in sensitivity to afatinib, a 2nd generation TKI, which may be more effective for targeting L747_A750delinsP.

Methods: To evaluate outcomes in patients with advanced NSCLC harboring a L747_A750delinsP mutation treated with afatinib, we analyzed data pooled from 4 clinical trials (LUX-Lung 2, LUX-Lung 3, LUX-LUNG 7, SWOG S1403) of afatinib for patients with *EGFR* mutant (*EGFR*+) non-small cell lung cancer (NSCLC). We investigated objective response rate (ORR), progression free survival (PFS), and overall survival (OS) for patients with tumors with E746_A750del vs. L747_A750delinsP. Median PFS and OS were estimated using Kaplan-Meier method.

Results: Eighty-seven patients with advanced *EGFR*+ NSCLC treated with afatinib monotherapy were evaluated, 78 harboring E746_A750del and 9 with L747_A750delinsP. Sex, race, and ECOG performance status were similar, but L747_A750delinsP was associated with older age (median 60 vs. 69 years). ORR to afatinib was 76% for E746_A750del vs. 87.5% for L747_A750delinsP (ORR Odds Ratio 0.85 [80% CI, 0.18-2.97]). Median PFS was 10.1 months for E746_A750del vs. 10.1 months for L747_A750delinsP (Figure 1) with PFS Hazard Ratio (HR) 1.68 [80% CI, 0.90-3.11], and median OS was 25.3 months for E746_A750del vs. 29.0 months for L747_A750delinsP (Figure 2) with OS HR 1.79 [0.97-3.28].

Conclusion: These data support a potential role for afatinib as the preferred TKI for patients with EGFR+ NSCLC harboring L747_A750delinsP, corroborating prior preclinical and clinical findings. Prospective data comparing afatinib to osimertinib for patients with tumors harboring EGFR L747_A750delinsP is needed.

Figure 1.

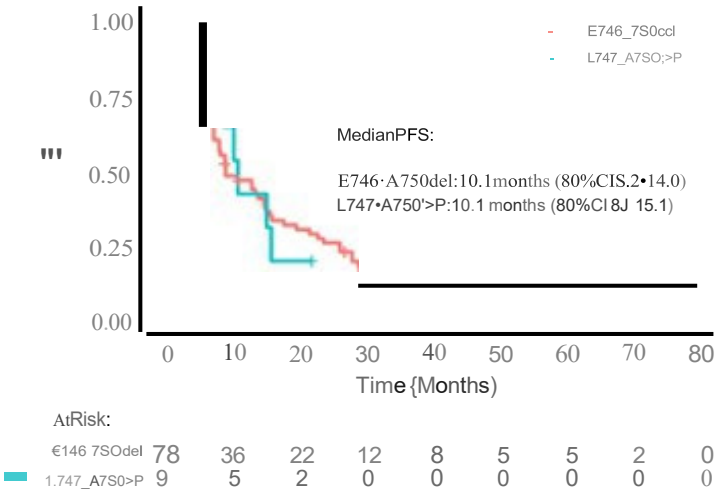


Figure 1. Progression Free Survival for patients withNSCLC harboring *EGFR* E746_A750del vs.L747_A750>P mutations treated with afatinib, Abbreviations: PFS= progress.ion free survival; CI= confidence interval

Figure 2.

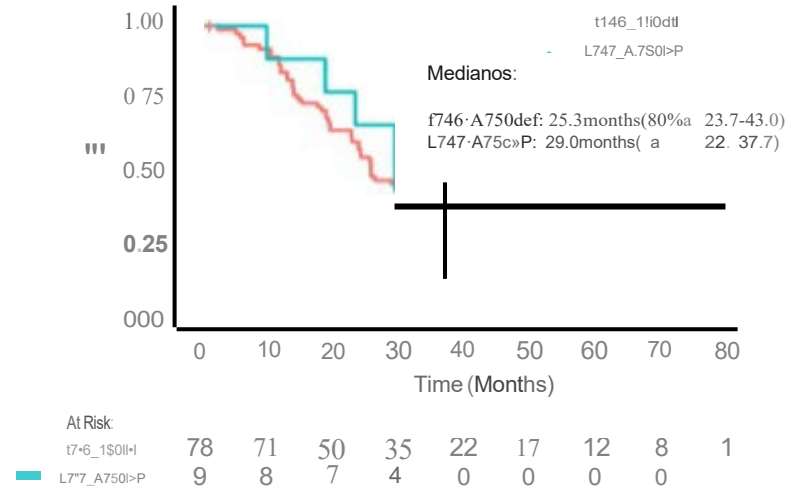


Figure 2.OverallSurvival for patients with NSCLCharboring *EGFR* E746_A750del vs. l747_A750>Pmutations treated with afatinib. Abbreviations: OS= overall survival; CI=confidence interval

Deep Mutational Scanning of EGFR Reveals Potential Domain-Specific TKI Sensitivities in Lung Cancer

Tikvah K. Hayes

University of California, Los Angeles

Presented by: Tikvah K. Hayes

The epidermal growth factor receptor, EGFR, is frequently activated in lung cancer by genomic alterations including missense mutations. The different mutation spectra in these diseases are reflected in divergent responses to EGFR inhibition: significant patient benefit in lung cancer, but limited in glioblastoma. Here, we report a comprehensive mutational analysis of EGFR function. We performed saturation mutagenesis of EGFR and assessed function of ~22,500 variants in a human EGFR-dependent lung cancer cell line. This approach revealed enrichment of erlotinib-insensitive variants of known and unknown significance in the dimerization, transmembrane, and kinase domains. Multiple EGFR extracellular domain variants, lacking approved targeted therapies, were sensitive to dacomitinib. In summary, this comprehensive screen reveals novel functional EGFR variants and suggests broader clinical investigation of EGFR inhibition for cancers harboring extracellular domain mutations.

Unlocking Immunochemotherapy Potentials for KRAS and LKB1 Mutant Lung Adenocarcinoma through FAK Inhibition and Immunotherapy with Anti-PD1 Antibody

Junghui Koo, Carol Tucker-Burden, Melissa Roy, Chunzi Huang, Wei Zhou,
Melissa Gilbert-Ross, Haian Fu, Suresh Ramalingam, Adam Marcus

Emory University

Presented by: Junghui Koo

Lung cancer presents a global health challenge demanding innovative preclinical models for research and therapeutic advancement. Genetically engineered mouse models (GEMMs) have seen remarkable growth in complexity and application over the past two decades. Our work leverages these models and is specifically designed to address the pressing need for effective immunochemotherapies in the context of KRAS and LKB1 (KL) mutant lung adenocarcinoma, which shows resistance to most immunotherapies. Our study centers around a preclinical orthotopic mouse model we developed using mouse lung and metastatic tumor cell lines derived from KL-GEMM. Furthermore, we have shown the LKB1 mutant cell lines and tumor models are uniquely sensitive to FAK inhibitors, and therefore we wanted to test whether the combination of a FAK inhibitor with anti-PD1 therapy can improve sensitivity to immunotherapy. The combinatory administration of FAK inhibitor (VS-6063) with anti-PD1 mAb increased anti-tumor response and survival outcome compared with single-agent therapy in the KL-orthotopic lung cancer model. By using GEMM-derived cell lines, we have evaluated the potential for creating innovative immunochemotherapies, customized for KL co-mutant lung adenocarcinomas with promising clinical potential. Furthermore, utilizing this model allows for a deeper understanding of the pathogenesis of KL mutant lung adenocarcinoma and the specific tumor microenvironment associated with KL mutation. It also facilitates the assessment of novel immunochemotherapy treatment strategies tailored to the KL genetic subtype of lung adenocarcinoma.

LKB1-SIK Axis Controls SHMT-Mediated Antioxidant Defense In KRAS-Mutant Lung Cancer

Hyunmin Lee, Constantinos Chronis, Kailong Li, Brandon Faubert, James Kim, Jiyeon Kim

Yale University, University of Chicago, Peking University, UT Southwestern

Presented by: Hyunmin Lee

Non-small cell lung cancer (NSCLC) with concurrent mutations in KRAS and the tumor suppressor LKB1 (KL NSCLC) is refractory to most therapies including immune checkpoint inhibitors and thus, has one of the worst predicted outcomes. Using human NSCLC metabolomics data, we uncovered upregulation of serine-glycine one carbon (SGOC) metabolism via serine hydroxymethyltransferase (SHMT) in KL NSCLC. A prior study in murine pancreatic tumor[1] showed that the LKB1-AMPK axis inhibits SGOC metabolism which is necessary for DNA methylation. Here we report that in NSCLC, LKB1, by collaboration with KEAP1, impedes SGOC metabolism through salt-induced kinase (SIK)-NRF2 axis, and one carbon units are required for antioxidant defense. SHMT suppression increased cellular sensitivity to oxidative stress and cell death. Further, the SHMT inhibitor enhanced the therapeutic efficacy of paclitaxel (first-line NSCLC therapy inducing ROS) in KL tumor growth in vivo. Collectively, the data reveal how KL NSCLC cells fulfill their metabolic requirements and provide insight into therapeutic strategies.

Continued EGFR Dependency in a Patient with Lung Cancer Harboring an EGFR Kinase Domain Duplication

Kevin Levine, Alice Berger, Colin Pritchard, Christina Baik

Fred Hutchinson Cancer Center, University of Washington

Presented by: Kevin Levine

.

Here we report an update to a previously published case of a patient with non-small cell lung cancer harboring an *EGFR* kinase domain duplication (EGFR-KDD)[1]. This case highlights a decades-long dependency on EGFR signaling in the setting of a rare *EGFR* activating alteration. Briefly, this is a 70-year-old woman without significant smoking history who presented in 1995 with a persistent cough, found to have local disease. After left lower lobectomy, she subsequently developed bilateral pulmonary metastases and began treatment with her first EGFR tyrosine kinase inhibitor in 2003 with gefitinib. At the time of the previous case report in 2015, her original tumor was found to have an EGFR-KDD involving exons 18-25 and a progressive lesion was found to have an *EGFR* T790M mutation, as determined by the UW-OncoPlex sequencing platform (sequencing results summarized in Table 1). In the interim, she was treated with osimertinib from 2016 to 2021 until she developed progression in bilateral lung nodules. Guardant ctDNA testing was negative, but UW-OncoPlex sequencing of a RLL biopsy sample was positive for the EGFR-KDD and an *EGFR* L792R mutation, which is known to contribute to osimertinib resistance. Following a short course of chemotherapy, she was started on treatment with amivantamab plus osimertinib in 1/2022 until progressive disease in early 2023. She had both radiographic and symptomatic benefit to the addition of amivantamab. Biopsy of a growing RLL lesion was positive for the EGFR-KDD and two additional *EGFR* mutations: L718R (6% VAF) and L718Q (1% VAF). Of note, the previously identified L792R mutation was not seen in this biopsy sample. Recently her treatment was switched to afatinib, and she quickly obtained clinical improvement. Overall, this n-of-1 case demonstrates several important points about the current treatment paradigm of EGFR-mutant lung cancer. First, the *EGFR* kinase domain duplication can lead to pronounced EGFR dependency and should be screened for in routine practice. Second, amivantamab is a reasonable treatment option for patients with an EGFR-KDD. Lastly, EGFR-targeted resistance mechanisms can evolve over time and space, and biopsies at the time of resistance can help inform treatment recommendations.

Date Sample Collected	Sample Type	Type of Sequencing Performed	*EGFR* Mutations Identified
1999	LLL lobectomy	UW-OncoPlex	Kinase Domain Duplication (KDD) involving exons 18-25
8/2014	RLL Biopsy	UW-OncoPlex	KDD, T790M
6/2021	Peripheral Blood	Guardant 360 ctDNA	None
6/2021	RLL Biopsy	UW-OncoPlex	KDD, L792R (VAF 12%)
4/2023	RLL Biopsy	UW-OncoPlex	KDD, L718R (VAF 6%), L718Q (VAF 1%)

Table 1. Summary of sequencing results using UW-OncoPlex (next-generation sequencing platform) and Guardant 360 ctDNA testing. VAF: Variant Allele Frequency.

References:

1. Baik CS, Wu D, Smith C, Martins RG, Pritchard CC. Durable response to tyrosine kinase inhibitor therapy in a lung cancer patient harboring epidermal growth factor receptor tandem kinase domain duplication. J Thorac Oncol. Elsevier; 2015;10(10):e97–e99.

Elucidate Epigenetic Mechanism Underlying Tyrosine Kinase Inhibitors (TKIs) Resistance in Lung Adenocarcinoma (LUAD)

Yichen Li, Andrew Xiao
Yale University

Presented by: Yichen Li

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer (NSCLC), which is observed with activating mutations in epidermal growth factor receptor (EGFR) occur in approximately 27% of patients. As such, multiple tyrosine kinase inhibitors (TKIs) have been developed to target these EGFR mutations. However, despite the initial efficacy of TKIs, acquired resistance to these drugs is a major limitation that hinders treatment. The oncogene amplification, especially in form of extrachromosomal DNA (ecDNA), has been reported to be a common mechanism for acquired therapy-resistance in cancer. My studies have demonstrated that TKI-resistant LUAD cells exhibit oncogene amplification and their hyperactive transcription.

It is well-known that a small population of cells, termed drug-tolerant persisters (DTPs), survives initial TKI treatment. A preliminary screening of ~100 nuclear factors identify an epigenetic regulator, METTL7A, that is critical for the adaption and clonal selection from DTP to acquired resistance, as well as the long-term maintenance of the resistance cells. My studies suggest METTL7A is essential for the acquisition of TKI-resistance by promoting hyperactive transcription and chromatin remodeling of oncogene amplicons. My studies indicate that the nuclear hubs of oncogene ecDNA play critical roles in supporting hyperactive transcription of the oncogenes. And I hypothesize that METTL7A plays critical role in chromatin remodeling, which facilitate the transcription of ecDNA.

To investigate roles of oncogene amplification and ecDNA in acquired TKIs in LUAD and screen the roles of the epigenetic factors in clonal selection from DTP to acquired TKIs resistance, I developed live-cell DNA imaging systems to characterize the dynamics of oncogene ecDNA and their transcription. Further, I investigate the potential factors that regulate ecDNA organization and its hyperactive transcription via using chemical or genetic inhibition approaches. Additionally, I detect the chromatin deposition of METTL7A and chromatin looping via genetics and genomic approaches to fully characterize the functions of METTL7A in chromatin remodeling and transcription. Further, I detect METTL7A interactome via biochemistry approaches to fully characterize the mechanism of METTL7A by investigating the function of the interacting proteins. This study contributes to our understanding of the mechanism for resistance in anti-EGFR therapy.

Clinicopathologic and Genomic Features of Patients with Mucinous Lung Adenocarcinoma and Response to Systemic Therapies

Alessandro Di Federico, Lingzhi Hong, Arielle Elkrief, Rohit Thummalapalli, Biagio Ricciuti, Joao Alessi, Pooja Gogia, Federica Pecci, Malini Gandhi, Marc Ladanyi, Jianjun Zhang, Don Gibbons, John Heymach, Mizuki Nishino, Lynette Sholl, Mark Awad, Kathryn Arbour, Adam Schoenfeld, Natalie Vokes, Jia Luo

Dana-Farber Cancer Institute, UT MD Anderson Cancer Center, Memorial Sloan Kettering Cancer Center, Brigham and Women's Hospital

Presented by: Jia Luo

Background

10% of lung adenocarcinomas (LUAD) have mucinous features ($LUAD^{Muc}$). The efficacy of immune checkpoint inhibitor (ICI)-based therapies and KRAS G12C inhibitors for patients with $LUAD^{Muc}$ is undefined.

Methods

Clinicopathologic, genomic, and outcomes data were abstracted from patients with LUAD at three academic centers: DFCI, MSKCC, and MDACC. LUAD with any mucinous component as assessed by a thoracic pathologist at each institution was classified as $LUAD^{Muc}$ and compared to LUAD without mucinous components ($LUAD^{Non\text{-}muc}$).

Results

$LUAD^{Muc}$ represented 9.9% of LUAD cases (N=406/4,106). Compared to $LUAD^{Non\text{-}muc}$, patients with $LUAD^{Muc}$ had lower cigarette smoking history (median pack-years: 15 vs 20, P=0.002) and PD-L1 tumor proportion score (median: 0% vs 5%, P<0.0001). Compared to $LUAD^{Non\text{-}muc}$, $LUAD^{Muc}$ had higher contralateral lung metastasis (50% vs 39%, P=0.02) and lower brain metastasis (30% vs 43%, P=0.02) in stage IV disease.

Among 3,577 patients with available genomic profiling, compared to $LUAD^{Non\text{-}muc}$ (N=3,206), $LUAD^{Muc}$ (N=371) had a lower tumor mutational burden (TMB) (median: 6.8 vs 8.5 mut/Mb, P<0.001), a higher prevalence of *KRAS*, *STK11*, *SMARCA4*, *NKX2-1*, and *GNAS* mutations, and a lower prevalence of *TP53*, *EGFR*, and *BRAF* mutations (q<0.05).

Among patients who received ICIs for advanced disease, compared with $LUAD^{Non\text{-}muc}$ (N=1,359), $LUAD^{Muc}$ (N=96) cases had a lower objective response rate (ORR 10% vs 25%, P<0.001), shorter median progression-free survival (mPFS 2.6 vs 4.0 months, P<0.001) and median overall survival (mOS 10.9 vs 18.1 months, P<0.001). Among patients who received chemo-immmunotherapy, compared with $LUAD^{Non\text{-}muc}$ (N=1,102), $LUAD^{Muc}$ (N=127) had lower ORR (23% vs 38%, P<0.001), shorter mPFS (5.0 vs 7.0 months, P<0.001) and mOS (11.9 vs 20.0 months, P<0.001). Among KRAS G12C LUAD who received KRAS inhibitors, compared to $LUAD^{Non\text{-}muc}$ (N=126), there were no significant differences in ORR (17% vs 36%, P=0.17) or mPFS (4.6 vs 5.7 months, P=0.31) among $LUAD^{Muc}$ (N=12), while mOS was significantly shorter (7.3 vs 13 months, P=0.04).

Conclusions

Compared to $LUAD^{Non\text{-}muc}$, $LUAD^{Muc}$ is enriched for *KRAS*-driven disease, a lighter smoking history, lower PD-L1 TPS and TMB. In advanced NSCLC, $LUAD^{Muc}$ has more contralateral lung metastasis and worse outcomes to standard treatments compared to $LUAD^{Non\text{-}muc}$.

Trastuzumab Deruxtecan Resistance can be Mediated by Payload Resistance or Secondary Extracellular ERBB2 Mutations but Sensitivity to HER2 Tyrosine Kinase Inhibitors is Maintained

Monique Nilsson, Xiuning Le, Junqin He, Xiaoxing Yu, Xiaofang Huo, Ashwani Kumar, Alissa Poteete, Qian Huang, Ralf Kittler, John Heymach

UT MD Anderson Cancer Center, UT Southwestern

Presented by: John Heymach (for Monique Nilsson)

Among non-small cell lung cancer (NSCLC) patients, the most common HER2 mutation is the exon 20 insertion mutation, Y772dupYVMA, which accounts for over 40% of all HER2 mutations in lung cancer. Trastuzumab deruxtecan (T-DXd), a HER2 antibody-drug conjugate (ADC), received FDA approval for the treatment of HER2 mutant NSCLC yielding a confirmed objective response of 52% and a median duration of response of greater than 9 months. Unfortunately, patients that initially respond to T-DXd will eventually acquire resistance, and the mechanisms of resistance as well as effective targeting strategies to overcome resistance are not yet elucidated. We generated Ba/F3 cells expressing the HER2 YVMA insertion mutation with acquired resistance to T-DXd by culturing cells in T-DXd until resistance occurred. T-DXd resistant cells retained expression of HER2, but were resistant to the payload, deruxtecan, as well as other topoisomerase inhibitors. T-DXd resistant cells exhibited loss of topoisomerase I, a previously reported mechanism of topoisomerase inhibitor resistance. T-DXd resistant cell lines were sensitive to payloads with alternate mechanisms of action including maytansine and likewise retained sensitivity to the HER2 ADC trastuzumab emtansine (T-DM1) which utilizes DM1 as a payload. Given that T-DXd resistant cells retained HER2 expression, we assessed whether they were sensitive to HER2 tyrosine kinase inhibitors (TKI). T-DXd resistant cells remained highly sensitive to HER2 TKIs including poziotinib, afatinib, and zongertinib. To investigate whether genomic alterations of HER2 could facilitate HER2 ADC resistance, we utilized the LentiMutate approach which employs an error-prone HIV-1 reverse transcriptase to produce a high frequency of mutations to identify resistance-associated mutations in cells treated with a HER2 ADC. Sequencing analysis revealed that resistant cells had an enrichment in point mutations within HER2 domain IV (D582N, F595C/S, E580K, C623Y), which includes the binding site of trastuzumab. To validate the impact of these mutations on HER2 ADC resistance, we generated cells expressing a HER2 exon 20 insertion in combination with the observed domain IV mutations. D582N and F695C/S co-mutations conferred resistance to T-DXd and T-DM1 but not to HER2 TKIs. Next, we generated a PDX model of acquired T-DXd resistance and observed that resistant tumors expressed a truncated form of HER2 which lacked the extracellular portion. These data demonstrate that resistance to T-DXd can be mediated by multiple mechanisms including loss of sensitivity to the ADC payload in which case ADCs bearing payloads with alternate mechanisms of action as well as HER2 TKIs retain anti-tumor cell activity. Moreover, resistance to trastuzumab-based ADCs can also be mediated by secondary mutations within domain IV of HER2 or loss of the extracellular terminal of HER2. However, these alterations do not diminish HER2 TKI activity.

Pan-HER Inhibition Overcomes Feedback Adaptation Resistance to KRAS G12C Inhibition in KRAS G12C Mutant Non-Small Cell Lung Cancer

Yuji Shibata, Hibiki Udagawa, Monique Nilsson, Jacqulyne Robichaux, Ana Galan-Cobo, David Molkentine, Junqin He, Qian Huang, Marcelo Negrao, Xiaoxing Yu, Alissa Poteete, Qian Yu, Ferdinandos Skoulidis, John Heymach

UT MD Anderson Cancer Center

Presented by: Yuji Shibata

Activating mutations in KRAS are found in approximately 30% of non-small cell lung cancer (NSCLC), and among all KRAS mutations, KRAS G12C is the most frequent variant, with a prevalence of approximately 14%. KRAS G12C inhibitors sotorasib and adagrasib are FDA approved for advanced/metastatic KRAS G12C-positive NSCLC, although the duration of benefit from these drugs is relatively modest and therapeutic resistance typically emerges relatively quickly to these drugs. There is, therefore, an urgent need for novel treatment strategies or combinations to improve efficacy and durability of response. We sought to identify effective combinations to enhance the efficacy of KRAS G12C inhibitors using in vitro and in vivo models of KRAS G12C-positive NSCLC. We employed a high-throughput in vitro drug screening effort using KRAS mutant NSCLC cell lines and observed that EGFR/HER2 tyrosine kinase inhibitors (TKIs) produced a synergistic effect when combined with KRAS G12C inhibitors adagrasib or sotorasib. We investigated the role of HER family members in the adaptive RAS pathway feedback reactivation in KRAS G12C mutant NSCLC cells and determined that activation of EGFR, HER2 and HER3 induced resistance to KRAS G12C inhibitors. Moreover, the inhibition of these HER family members improved the sensitivity to KRAS G12C inhibitors by preventing re-activation of KRAS. We observed that in combination with KRAS G12C inhibitors pan-HER TKIs such as poziotinib yielded a synergistic effect compared to HER2 TKI treatment. However, the addition of an EGFR or HER2 antibody to KRAS G12C inhibitors did not enhance its efficacy. The combination of pan-HER TKIs with KRAS G12C inhibitor suppressed tumor growth significantly as compared to KRAS G12C inhibitor alone with a tolerable toxicity profile in KRAS G12C mutant patients derived xenograft models. Collectively, our findings indicate that the adaptive feedback activation of HER family members, including not only EGFR but also HER2 and HER3, diminishes the efficacy of KRAS G12C inhibitors against KRAS G12C-positive NSCLC tumor cells, and that the combination of pan-HER TKIs with KRAS G12C inhibitors may be more effective than KRAS G12C inhibitors alone.

Unraveling the Role of SWI/SNF Complexes in Acquired Resistance to Osimertinib in EGFR-Mutant Lung Adenocarcinoma

Shannon Silva, Fernando de Miguel, Claudia Gentile, William Feng, Akshay Sankar, Francisco Exposito, Wesley Cai, Keita Maemura, Mary Ann Melnick, Camila Robles-Oteiza, Madeline Hinkley, Jeanelle Tsai, Antja-Voy Hartley, Jin Wei, Anna Wurtz, Fangyong Li, Maria Toki, David Rimm, Robert Homer, Craig Wilen, Andrew Xiao, Jun Qi, Qin Yan, Don Nguyen, Pasi Jänne, Cigall Kadoch, Katerina Politi

Yale University, Dana-Farber/Harvard

Presented by: Shannon Silva

Osimertinib is a first-line therapy for patients diagnosed with EGFR mutant lung adenocarcinoma (LUAD). However, acquired resistance to osimertinib and other tyrosine kinase inhibitors (TKIs), is inevitable and poses a significant clinical challenge. Notably, the mechanisms that underlie acquired resistance to first-line osimertinib are unknown in 40-50% of tumors. Therefore, it is critically important to investigate processes that promote osimertinib resistance.

We used isogenic models of acquired osimertinib resistance and patient-derived models to identify novel mechanisms that drive osimertinib resistance. We found that SMARCA4, an ATPase subunit in the SWI/SNF chromatin remodeling complex family, mediates resistance in a subset of EGFR-driven tumors. RNA-Seq, ATAC-Seq, and Cut&Run revealed that SMARCA4 causes global chromatin accessibility changes in genes involved in epithelial-to-mesenchymal transition, cell migration, proliferation, and ROS-attenuation; that collectively lead to TKI resistance. Importantly, genetic knock-down and pharmacologic inhibition of SMARCA4 (and SMARCA2) can reverse this resistance-promoting, chromatin accessibility signature and resensitize a subset of resistant models to osimertinib. In summary, we have identified a new epigenetic mechanism of acquired osimertinib resistance that is driven by SMARCA4. Tumors that depend on SMARCA4 are vulnerable to clinical-stage SMARCA4/2 inhibitors and may be leveraged in the clinic as a potential therapy in conjunction with osimertinib.

Inhibition Of hTERT/Telomerase/Telomere as an Important Mechanism Mediating Therapeutic Efficacy of Third Generation EGFR Inhibitors in EGFR Mutant Lung Cancer

Zhen Chen, Karin Vallega, Dongsheng Wang, Zhihan Quan, Songqing Fan, Qiming Wang, Ticiana Leal, Ramalingam Suresh

Emory University, Xiangya Second Hospital, Henan Cancer Hospital

Presented by: Shi-Yong Sun

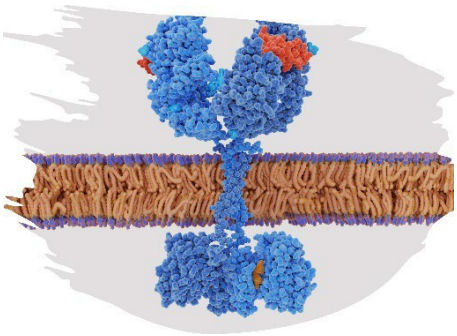
Telomere maintenance via telomerase reactivation is linked to uncontrolled cell growth and thus is a cancer hallmark and an attractive cancer therapeutic target. The inevitable development of acquired resistance to osimertinib (AZD9291), an FDA-approved 3rd generation EGFR tyrosine kinase inhibitor (EGFR-TKI) for the treatment of patients with advanced non-small cell lung cancer (NSCLC) harboring EGFR activating or T790M resistant mutations, limits its long-term clinical benefit. To understand the underlying resistance mechanisms and develop mechanism-driven strategies to manage acquired resistance, we identified downregulation of the expression of *hTERT* gene, which encodes a catalytic subunit of telomerase, in osimertinib-treated human EGFR-mutant (EGFRm) NSCLC cells in a c-Myc-dependent manner accompanied with inhibition of telomerase and telomere and induction of telomere dysfunction. In various osimertinib-resistant EGFRm NSCLC cell lines, basal hTERT levels were elevated with increased telomerase activity and telomere length and cells were insensitive to osimertinib modulation. hTERT elevation was also detected in the majority of EGFRm NSCLC tissues relapsed from EGFR-TKI treatment. Knockdown of hTERT or chemical inhibition of telomerase or telomere in osimertinib-resistant cell lines enhanced cell sensitivity to osimertinib, whereas enforced overexpression of ectopic *hTERT* gene in sensitive cells conferred resistance to osimertinib, suggesting a critical role of hTERT/telomerase or telomere in modulating the responses of EGFRm NSCLC cells to osimertinib. Osimertinib combined with the telomere inhibitor, 6-Thio-dG, effectively inhibited the growth of osimertinib-resistant tumors, regressed EGFRm NSCLC patient-derived xenografts, and delayed the emergence of acquired resistance to osimertinib, warranting clinical validation of this strategy to manage osimertinib acquired resistance.

Understanding the Role of Alternative RNA Splicing in Osimertinib Resistance in Lung Adenocarcinoma

Matthew Wang, Robert Tesng, Li-Ting Ku, Xinning Shan, Joanne Chen, Katerina Politi, Luisa Escobar-Hoyos
Yale University

Presented by: Matthew Wang

The third generation of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, especially Osimertinib, has achieved remarkable clinical outcomes in the treatment of lung adenocarcinomas with EGFR mutations. However, resistance eventually emerges in most patients and the underlying molecular mechanisms are not yet fully understood. Using matched-pair Osimertinib-sensitive and -resistant previously published patient data and isogenic cell lines, we found that that Osimertinib-resistant (OR) lung adenocarcinomas have altered splicing of cassette exons, which are retained or excluded in Osimertinib-resistant conditions based on nucleotide sequence. In particular, these altered exons localize to specific chromosomal hotspots. We next sought to discover if alternative splicing was having a biological impact on gene expression in OR cells by inducing nonsense-mediated decay (NMD) of select transcripts. We found that alternative splicing-mediated NMD (AS-NMD) targets 82 genes in Osimertinib-resistant vs. -sensitive cells, and that these genes are enriched for double-strand DNA damage repair pathways. We also found increased DNA damage signal and increased NMD signal, as well as decreasing expression of specific AS-NMD targets as a function of Osimertinib resistance. These results suggest a possible mechanism in which OR cells bypass DNA repair and/or apoptosis by crippling the DNA damage repair response through altered pre-mRNA splicing and NMD. Current experiments continue to explore the cause of this resistance-correlated AS-NMD behavior. These findings shed new light on the mechanisms of Osimertinib resistance with regard to DNA repair and provide a rationale for targeting altered RNA splicing and NMD as therapeutic strategies to overcome acquired Osimertinib resistance in lung adenocarcinomas.



Session 2:

Health Disparities in Lung Cancer

Disparities in Stereotactic Radiosurgery Receipt in Patients with Brain Metastases from Non-Small Cell Lung Cancer

Kelly Becht, Meghan Lindsay, Pamela Soulos, James Hansen, Charu Singh, Yi An, Sanjay Aneja, Sarah Goldberg, Veronica Chiang, Henry Park

Yale University

Presented by: Kelly Becht

Purpose/Objectives
Stereotactic radiosurgery (SRS) is a commonly used alternative to whole-brain radiotherapy for patients with a limited number and volume of brain metastases from non-small cell lung cancer (NSCLC). However, the choice of modality may be influenced by not only clinical factors but also sociodemographic factors. We hypothesize that there are disparities by age, sex, race, ethnicity, and urban/rural status associated with SRS receipt among older patients with brain metastasis in the U.S.

Materials/Methods
We used the Surveillance, Epidemiology, and End Results (SEER)-Medicare database to characterize disparities in SRS receipt for patients ≥66 years who were diagnosed with brain metastasis at the time of NSCLC diagnosis between 1/1/10 and 12/31/17. All patients in our cohort received brain-directed radiotherapy. We defined SRS as having a billing code for SRS and receiving five or fewer fractions of radiation. Our outcome was receipt of SRS vs. non-SRS radiotherapy. We developed a hierarchical multivariable logistic regression model using hospital referral region as a clustering level to identify predictors of SRS receipt.

Results
In our cohort of 5,038 patients, 28% underwent SRS and 72% underwent non-SRS radiotherapy. Most patients were Non-Hispanic White (80%), while a minority had dual Medicare/Medicaid eligibility (18%) and resided in a rural location (18%). Residence in rural vs. urban locations (odds ratio [OR] 0.79, 95% confidence interval [CI] 0.64-0.97) and severe vs. no comorbidities (OR 0.78, 95% CI 0.65-0.94) were associated with lower odds of SRS receipt. Conversely, age, sex, race, ethnicity, and Medicare/Medicaid dual eligibility were not associated with SRS receipt.

Conclusions
Using the SEER-Medicare data, sociodemographic factors like age, sex, race, ethnicity, and Medicaid eligibility were not associated with SRS receipt for NSCLC brain metastases. However, we observed disparities in patients with comorbidities and those living in rural areas, indicating that more work needs to be done to ensure optimal accessibility of SRS for sicker and more geographically isolated patients.

Communication Issues in Patient/Provider Discussions of Immunotherapy

Kersten Pierre, Ayannah Lang, Rebecca Pentz

Emory

Presented by: Kersten Pierre

Although only approved by the FDA in 2015, Immunotherapy significantly improves survival of Non-Small Cell Lung Cancer patients, with fewer side effects. As with treatments, it is important for patients to understand their treatment and its potential side effects. In a prior study we demonstrated that many patients did not fully understand what Immunotherapy was or its side effects, even after having conversations about it with their physician. These results prompted our team to create culturally appropriate educational videos: one explaining the Immune System and another Immunotherapy, to improve patient understanding. The aim of the study reported here is to test the efficacy of these videos and to develop and test a third video specifically describing a combination therapy (FAK inhibitor plus immunotherapy) which will be tested in a lung P01 funded study. A pre and post methodology will be used by asking participants to define the concepts presented in the video before and after viewing. To reach statistical significance we will test the videos with 50 cancer patients, with 25 of these patients to be enrolled in the P01 funded lung cancer trial. While waiting for the Lung trial to open, we tested the videos with 25 patients: 8 breast cancer patients, 4 GI cancer patients, and 13 GU cancer patients. Before viewing the Immunotherapy video, 16 patients successfully described Immunotherapy and 9 did not. For the Immune System video, 20 patients successfully defined the Immune System and 5 did not. After viewing the videos, 5 of the 9 patients who incorrectly defined Immunotherapy were able to provide a correct definition and 3 of the 5 patients who incorrectly defined the Immune System were able to provide a correct definition. Although results of this study suggest improvement in Immunotherapy understanding, further research with a more diverse patient population, including those not being treated at a Comprehensive Cancer Center in an urban area is needed. Overall, short animated educational videos can be a useful tool to increase patient understanding of their lung cancer treatments.

The Impact of Diverse Clinical Trials Personal on the Diversity of Clinical Trials Participants

Victoria L. Seewaldt, Robert Winn, Augusto Ochoa

City of Hope, Massey Cancer Center, LSU Cancer Center

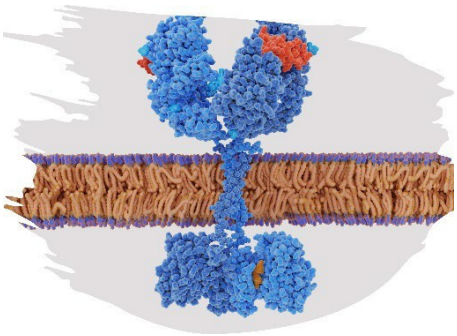
Presented by: Victoria L. Seewaldt

Introduction: Cancer Centers struggle to recruit individuals to clinical trials that represent the diversity in their catchment areas. While the National Cancer Institute (NCI) has focused on the diversity of subjects recruited to clinical trials, there has been little attention paid to the diversity of individuals who recruit the subjects. Accordingly, we designed a prospective population-based mixed-methods investigation to examine the consenter-participant relationship and factors contributing to successful recruitment of diverse research subjects to clinical trials at City of Hope Comprehensive Cancer Center (CoHCCC). We hypothesized individuals from diverse racial/ethnic backgrounds will be more likely to enroll in a clinical trial when consented by someone who looked like them, spoke their language, and understood their culture.

Methods: Between 2018 and February 2020, 205 women were approached for participation in one of three non-therapeutic BCTs and were eligible for the survey questionnaire assessing factors related to clinical trial enrollment. The investigation comprised of two components, a survey questionnaire and in-depth in-person interviews. Surveys were conducted by clinical research associates of diverse race, ethnicity, gender, and language.

Results: Of 205 women, 24 (11.7%) women declined to participate in this survey. Of the 181 participants who completed the survey questionnaire, 94 (51%) were self-identified as non-Hispanic White (NHW) and 87 (48%) self-identified as Latina/Hispanic, Asian, Pacific Islander, Indigenous, or African American/Black (Women-of-Color; WoC). For WoC, like NHW participants, there was a statistically significant difference though in the feeling that the consenter created an atmosphere of trust and support. Ninety-four percent (n=77) of the enrollers but only 60% of the decliners (n=3) agreed with that statement (p=0.05). The consenter's gender was considered "Not important" across racial groups (93% among NHW enrollers, 85% in WoC enrollers, p=0.08). However, there were statistically significant differences according to the importance of the consenter's characteristics in the decision to enroll or decline participation in the trial. Among NHW enrollers, none (0%, n=0) reported that consenter race was important in influencing their decision to enroll while 22% (n=18) of the WOC enrollers stated the consenter race was important (p=0.0009). Similarly, none of the NHW enrollers rated the consenter "looks like people in my community" as important while 24% (n=20) of the WoC enrollers rated this as an important factor influencing their participation in a clinical study (p=0.0004). Surprisingly, consenter language was equally important to both NHW enrollers (69%) and WoC (56%) (p=0.07). This reflected the diversity of individuals in our catchment area who were classified as NHW but had recently immigrated from another region of the world (e.g. Armenian, Middle Eastern, Eastern European).

Conclusions: These results highlight the importance of diversity of the individuals who consent for clinical trials in enrolling clinical trial subjects that reflect the diversity of cancer center catchment areas.



Session 3:

Immuno-oncology

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In Situ Vaccination With *Flt3l* Gene Modified CD103$^+$ Type 1 Conventional Dendritic Cells Synergizes with Anti-PD-1 Checkpoint Blockade in Murine Models Of Non-Small Cell Lung Cancer

Jensen Abascal, Raymond Lim, Ramin Salehi Rad, Zhe Jing, Michael Oh, William Crosson, Bitta Kahangi, Edgar Reyes, Camelia Dumitras, Diana Reyimjan, Linh Tran, Manash Paul, Kostyantyn Krysan, Bin Liu, Steven Dubinett

University of California, Los Angeles

Presented by: Jensen Abascal

A major hurdle in treatment of Non-Small Cell Lung Cancer (NSCLC) with anti-PD-1 immune checkpoint blockade (ICB) therapy is a lack of response (primary resistance) and relapse after an initial response (acquired resistance). Recent studies reveal that responses to PD-1/PD-L1 blockade are associated with high tumor mutational burden (TMB), increased CD8$^+$ T cell infiltration and high baseline PD-L1 expression within the tumor microenvironment (TME), while impaired tumor antigen presentation and the immunosuppressive TME have been associated with resistance to ICB. One approach to overcome anti-PD-1 resistance is to intratumorally vaccinate NSCLC tumors with gene modified conventional dendritic cells (cDC), specifically the type I conventional DC (cDC1) lineage. Recent studies have established that generation of an anti-tumor immune response driven by CD8$^+$ T cells requires the cross presentation of tumor associated antigens and that cDC1s are the primary cross presenting APC subtype *in vivo,* which can license CD8$^+$ T cells to initiate an adaptive anti-tumor immune response. In addition, previous studies have shown that intratumoral administration of the FMS-like tyrosine kinase 3 ligand (FLT3L) protein can expand endogenous CD103$^+$ cDC1s in the TME and augment anti-tumor immune responses to ICB therapy. Here, we engineered murine CD103$^+$ cDC1s to constitutively secrete soluble FLT3L (FLT3L_cDC1) and performed *in situ* vaccination studies on anti-PD1 resistant murine models of NSCLC with LKB1-deficiency and elevated TMB that better represents human disease. *In situ* vaccination with FLT3L_cDC1 enhances anti-tumor efficacy compared to non-modified cDC1 vaccination and synergizes with anti-PD-1 ICB to inhibit tumor growth. FLT3L_cDC1 + anti-PD-1 combination therapy induces significant activation and expansion T cells and cDC1s within the TME. Furthermore, combination therapy significantly increases DC progenitor numbers within the tumor draining lymph node, including DC progenitors that are committed to the cDC1 lineage. Our data suggests *in situ* vaccination with FLT3L_cDC1 may represent a promising strategy to potentiate the efficacy of ICB and improve outcomes for patients with primary resistance to PD-1/PD-L1 monotherapy.

The combination of modified IL-18 with anti-PD1 elicits a CD4+ T cell-mediated antitumor response in MHC Class I-deficient models of non-small cell lung cancer

Jordan Cardenas, Camila Robles-Oteiza, Ting Zhou, John Huck, Paula Kavathas, Aaron Ring, Katerina Politi

Yale University, Fred Hutchinson Cancer Center

Presented by: Jordan Cardenas

Immune checkpoint inhibitors (ICIs), such as anti-PD1 and anti-CTLA4, provide a survival benefit compared to chemotherapies and are FDA-approved for first line use in advanced non-small cell lung cancer (NSCLC) alone or in combination with chemotherapy. However, acquired resistance to ICIs is common. Loss of MHC Class I antigen presentation by tumor cells which is essential for recognition of tumor cells by $CD8^+$ T cells is a well-established mechanism of acquired resistance. Therefore, there is a need to investigate therapies that can eliminate ICI-resistant tumors via alternative, MHC Class I-independent mechanisms. Our lab has previously developed a β2M-deficient syngeneic model of lung squamous cell carcinoma (using UN-SCC680 cells) rendering it unable to present antigens on MHC Class I. When tumors are implanted subcutaneously, they are not cleared with an anti-PD1 monoclonal antibody in this β2M-KO model. To overcome this ICI resistance, we tested a novel cytokine therapy, decoy-resistant IL-18 (DR-18), alone and in combination with anti-PD1 for its ability to eliminate UN-SCC680 β2M-KO tumors. We found that ~40% of tumors completely regress when DR-18 is used in combination with anti-PD1. This effect is reversed with $CD4^+$ T cell depletion as well as IFNγ inhibition, and flow cytometry reveals an increase of tumor infiltrating $CD4^+$ T cells. Employing single-cell RNA sequencing on treated tumors, we found enrichment of lymphocytes and myeloid cell subsets that coordinate a pro-inflammatory, anti-tumor immune microenvironment, optimally in the presence of both DR-18 and αPD-1. These results reveal a mechanism by which $CD4^+$ T-helper cells coordinate remodeling of an ICI-resistant immune microenvironment, enabling MHC Class I-independent killing of tumor cells when a cytokine therapy is combined with immune checkpoint blockade. Overall, this work highlights a strategy of overcoming ICI-resistance by targeting tumor-infiltrating $CD4^+$ T cells and innate immune cells.

The Antigen Specificity of Tumor-Targeting T Cells in Non-Small Cell Lung Cancer

Berkay Yahsi, Benjamin Lu, Jianlei Gu, Kalyn Whitehead, Heather Lazowski, Yalai Bai, David Rimm, Hongyu Zhao, Veronica Chiang, David Hafler, Kurt Schalper, Mark Lee

Yale University

Presented by: Mark Lee

Immune checkpoint blockade (ICB) can mediate remarkable clinical responses in patients with lung cancer, but responses are highly variable with the majority of patients failing to respond. In ICB non-responders, the demonstration of durable remissions after treatment with T cell receptor (TCR)-based therapies is a major driver of clinical interest in realization of these therapies. However, the currently limited set of well-defined tumor-reactive TCRs – and the technical difficulty of identifying tumor antigen/TCR pairs – currently restricts the number of patients with lung cancer that can be treated with TCR-based therapies such as engineered cellular therapies and TCR bispecific proteins. We recently developed a novel technology, called “APC (antigen-presenting cell) cytokine capture”, that allows many thousands of encoded peptides to be synthesized at low cost on DNA oligonucleotide arrays, and efficiently enables T cell-activating peptides to be separated from non-immunogenic ones. Here we demonstrate use of this technology to systematically test T cells from patients with non-small cell lung cancer (NSCLC) for tumor antigen reactivity.

We performed single-cell RNA and TCR sequencing of NSCLC patient TILs (n=6). For each patient, we selected clonally-expanded T cells (up to 96) that express established markers of tumor reactivity, and expressed these TCRs in TCR knockout T cells. To generate libraries of putative antigens, for each patient we performed whole exome sequencing of paired tumor and normal tissue, and generated encoded peptides containing all patient somatic mutations. We also cloned >150 tumor-associated antigen (TAA) genes curated from the literature. To identify tumor antigen-reactive T cells, we performed library-on-library screening (i.e. all T cells co-cultured with APCs expressing candidate antigen sets), sorted activated T cells, and sequenced their TCRs. We identified the target antigens of these TCRs using APC cytokine capture. We are now working to expand our candidate antigen sets to discover the targets of the orphan T cells. Taken together, our study seeks to provide the most complete understanding of the targets of intratumoral T cells in individual NSCLC patient specimens to date, with the goal to identify new “public” tumor-reactive TCRs for development of novel TCR-based therapies.

Hyper-Interferon Sensitive Influenza Induces Adaptive Immune Responses and Overcomes Resistance To Anti-PD-1 In Murine NSCLC

Ramin Salehi-Rad, Yushen Du, Tian-Hao Zhang, Dongdong Chen, William Crosson, Abascal Jensen, Yuan Shi, Jiang Hong, Tseng Yenwen, Liu Bin, Sun Ren,
Steven Dubinet

UCLA, Key Laboratory of Cancer Prevention and Intervention, Westlake Laboratory of Life Sciences

Presented by: Ramin Salehi-Rad

Despite recent advances in immunotherapy, many patients with NSCLC fail to respond to immune checkpoint inhibitors (ICI) or acquire resistance after an initial response. 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 ICI. 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.

In situ vaccination (ISV) with immune stimulating viruses has emerged as a potential strategy to overcome immune resistance by directly ameliorating the immunosuppressive TME and promoting host anti-tumor immune activation. Utilizing a high-throughput, genome-wide approach, we recently engineered a hyper–interferon-sensitive (HIS) virus as a vaccine candidate by incorporating multiple interferon (IFN)-sensitive mutations into the influenza A 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. ISV with HIS demonstrated superior efficacy compared to WT virus in multiple syngeneic murine models of NSCLC with known driver mutations (K, KP, KPL) and varying mutational burden. Flow phenotyping and single cell RNA-sequencing studies revealed that HIS induced host adaptive immune responses. The efficacy of HIS was depended on local IFN signaling and endogenous T lymphocytes. HIS ISV synergized with anti-PD-1 to overcome resistance in murine NSCLCs. Successful combination therapy with HIS ISV and anti-PD-1 resulted in improved overall survival and the establishment of enduring systemic tumor-specific immune memory. These studies present compelling evidence supporting the clinical translation of HIS virotherapy as a novel 'off-the-shelf' strategy to combat resistance to immunotherapy in patients with NSCLC.

Comparison of Spatial Transcriptomic Platforms in Lung Adenocarcinoma Tissue Samples

Nejla Ozirmak, Max Molina, Sharia Hernandez, Alejandra Serrano, Wei Lu, Sean Barnes, Beatriz Sanchez-Espiridion, John Heymach, Jianjun Zhang, Boris Sepesi, Tina Cascone, Don Gibbons, Khaja Khan, Ignacio Wistuba, Cara Haymaker, Ken Chen, Luisa Solis Soto

UT MD Anderson Cancer Center

Presented by: Luisa Solis Soto

Background: Single cell spatial transcriptome (scST) technologies are evolving rapidly as potential tools that can help to unveil the tumor biology of lung cancer and allows to investigate a large number of targets, gene signaling pathways and cell-cell interactions which are important to understand the lung cancer tumor microenvironment, however the performance of these platforms and comparison with morphological and biological data have not been previously performed.

Experimental design: We used formalin fixed paraffin embedded (FFPE) surgical resected lung adenocarcinoma placed in a tissue microarray. Serial sections of 5um were processed with CosMx, MERFISH and 10X Xenium commercial scST platforms using CosMx universal 1000plex, MERFISH 500plex immuno-oncology and 10X Xenium 289plex lung + 50 custom designed gene panels. We correlated gene expression information with orthogonal ST assay using the GeoMx Digital spatial profiler (DSP). Pathology review of the resulting phenotyping annotations produced against mIF and H&E sections was carried out in parallel. We then evaluated both relative technical and biological performance from a pathology and bioinformatics standpoint as they are essential for downstream analysis and phenotyping.

Results: All three platforms were highly concorded ($R > 0.62$, $p < 0.0001$) with matched orthogonal RNA spatial analysis using the GeoMx DSP, however performance characteristics varied among them including of transcript counts per cell, gene counts per cell, cell area size per cell, cell segmentation false discovery rates and morphological concordance with pathology annotations, which resulted in different phenotyping characteristics. Higher counts of negative control probes increased the false discovery rates of CosMx platform compared to 10X Xenium. Correlation of shared gene expressions between CosMx and Xenium data was better. These findings highlight the need for reproducibility when considering spatial RNA analysis.

Conclusion: ScST is an area that is under rapid development. Our work provides information of the advantages and limitations of these platforms that can be considered to develop workflows for downstream phenotyping and other methods for scST gene expression analysis in lung cancer.

Transmembrane Serine Protease TMPRSS11B Promotes Tumorigenesis in Lung Squamous Cell Carcinoma Through Enhanced Lactate Export and Modulation of the Tumor Microenvironment

Hari Shankar Sunil, Barrett Updegraff, Jingfei Zhu, Lisa Thomas, Anthony Grichuk, Bret Evers, Jean Clemenceau, Isabel Barnfather, John Minna, Ralph Deberardinis, Trudy Oliver, Tae Hyun Hwang, Jinming Gao, Kathryn O'Donnell

UT Southwestern, Seagen, Mayo Clinic, Duke University

Presented by: Hari Shankar Sunil

Lung cancer is the leading cause of cancer-related deaths worldwide. Existing therapeutic options have limited efficacy, underscoring the critical need for the identification of new actionable therapeutic targets. We previously identified the Transmembrane Serine Protease *TMPRSS11B* as a novel gene that promotes the transformation of human bronchial epithelial cells *in vitro* and induces tumorigenesis *in vivo* (PMC6338450). Importantly, *TMPRSS11B* is frequently overexpressed in human lung squamous cell carcinomas (LUSCs), high expression is associated with poor patient survival, and TMPRSS11B inhibition in human LUSCs reduces transformation and tumor growth. We further demonstrated that TMPRSS11B promotes extracellular release of Basigin, an obligate chaperone of the lactate monocarboxylate transporters MCT1/4, enhancing lactate export and glycolytic metabolism, thereby promoting tumorigenesis. Moreover, as a cell surface protein and enzyme, TMPRSS11B represents a tractable target for therapeutic intervention. To determine whether TMPRSS11B activity impacts the host immune system and the tumor microenvironment (TME), we evaluated the effect of *Tmprss11b* depletion in a syngeneic LUSC mouse model, KLN205. *Tmprss11b* loss of function in KLN205 LUSC tumors significantly reduced tumor burden in immunocompetent mice and triggered an accumulation of $CD4^+$ T cells. Additionally, we observed a significant reduction in MAPK/ERK signaling upon *Tmprss11b* depletion. We are currently extending these studies by investigating the effect of *Tmprss11b* loss of function using CRISPR/Cas9 editing in the *Rosa26*$^{\text{LSL-Sox2-IRES-GFP}}$; Nkx2-1 $^{fl/fl}$; *Lkb*1$^{fl/fl}$ (SNL) mouse model of LUSC. Tumor burden analysis and pH-sensitive fluorescent imaging are ongoing to assess changes in lactate accumulation. Moreover, RNA FISH analysis and spatial transcriptomics revealed that *Tmprss11b* expression is highly enriched in lung squamous tumors compared to lung adenocarcinomas and normal lung in SNL mice. Interestingly, we observe an enrichment of immunosuppressive signatures in the *Tmprss11b*-high squamous tumors as well as the adjacent low pH (lactate high) regions within the lung. Further validation and immune deconvolution analyses are ongoing to assess the effects of *Tmprss11b* expression on the TME. Collectively, these studies will elucidate the molecular mechanisms through which TMPRSS11B promotes tumor growth and alters the tumor microenvironment in LUSC.

Modeling Immune Evasion And Immunotherapy Response in Distant Metastases of Lung Adenocarcinoma

Tang Tang, Kelli Conolly, Nikhil Joshi, Don Nguyen

Yale University

Presented by: Tang Tang

Kirsten rat sarcoma virus (KRAS) mutated non-small cell lung cancers (NSCLCs) are a major lung cancer subset, and the current standard of care for patients is immune checkpoint blockade (ICB), including anti-Programmed cell death protein 1 (PD-1) or Programmed death-ligand 1 (PD-L1) therapies. While immunotherapy have transformed NSCLC management, only some patients respond to it, and the investigation of immune evasion mechanisms has been hindered by the lack of immunogenic animal models. This challenge is particularly pronounced in brain metastases, which sometimes progress after initially responding to ICB, while tumors in other sites are kept under control. The mechanisms underlying immunosuppression during brain metastasis also remain poorly understood, making it difficult to improve the effectiveness of immunotherapy.

The Joshi Lab at Yale Immunobiology has previously generated a neoantigen-expressing KRAS-driven lung adenocarcinoma model (KP-NINJA), which provides an immunogenic setting to study antigen-specific T cell responses. But like other genetically engineered mouse models, they rarely form spontaneous metastases. Here, we further developed metastatic KP-NINJA mouse models to investigate the tumor intrinsic mechanisms of immune evasion during metastasis or following ICB. Two brain metastatic lines, BrM1.1 and BrM1.2, were derived through *in vivo* selection of the KP-NINJA cells, administered via intracardial injections into C57BL/6J mice. When re-injected intracardially into mice, both lines consistently formed distant metastases with increased metastatic frequency and burden in organs such as the lung, liver, and brain. Despite the presence of neoantigen-specific T cells in distant metastases, anti-PD-1 treatment had minimal effect on mice injected with these metastatic lines. RNA sequencing showed that BrM1.1 and BrM1.2 may evade immune responses through different pathways, which are significantly associated with resistance to anti-PD-1/PD-L1 therapy in NSCLC patients. We have also identified some potential targets like the chemokine ligand 2 (CCL2), which could be potentially targeted to enhance ICB response. These results suggested that our syngeneic models recapitulate immune evasion mechanisms observed in patients and enable the study of immune evasion and resistance to ICB in distant metastases.

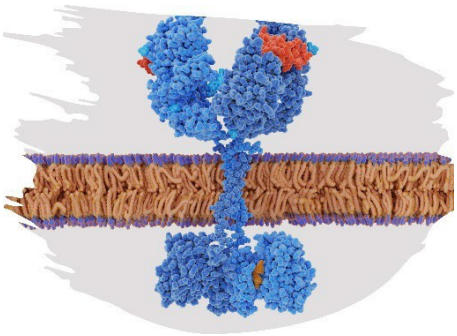
The Integrated Stress Response (ISR) Pathway Coordinately Regulates Multiple Immune Checkpoint Proteins in Lung Cancer

Shayna Thomas-Jardin, Shruthy Suresh, Nicole Novaresi, Lisa Thomas, Cheryl Lewis, Chul Ahn, Bret Evers, Luisa Maris Solis Soto, Ignacio Wistuba, John Minna, Kathryn O'Donnell

UT Southwestern, UT MD Anderson Cancer Center

Presented by: Shayna Thomas-Jardin

Groundbreaking discoveries in identifying the PD-1/PD-L1 (Programmed Death 1/ Programmed Death Ligand 1) immune checkpoint axis have resulted in the approval of monoclonal antibodies that disrupt this interaction as a first-line therapy for non-small cell lung cancer (NSCLC) patients. However, only ~20% of NSCLC patients have sustained benefit from this immune checkpoint blockade (ICB). There is a critical need to identify mechanisms that facilitate resistance to PD-1/PD-L1 therapy and determine whether other immune checkpoints will enhance anti-tumor immune responses in combination with this therapy. The integrated stress response (ISR) pathway represents an emerging therapeutic vulnerability. The ISR is a generalized response to maintain cellular homeostasis and is known to be activated by oncogenic or ER stress, heme deprivation, and amino acid starvation. We discovered that ISR activation potently induces PD-L1 in NSCLC, leading to suppression of anti-tumor immunity (Suresh et al, *Nature Cancer*, 2020). We further demonstrated that ISR pathway activation enhances *PD-L1* translation through the bypass of inhibitory upstream open reading frames (uORFs) in the *PD-L1* 5' UTR, revealing a mechanistic link to the eukaryotic translation initiation factor eIF5B. Our latest studies demonstrate that the immune checkpoint protein, CD155/PVR (Cluster of Differentiation 155, poliovirus receptor), is induced by ISR activation. Importantly, we observed a significant correlation between PD-L1 and CD155 expression in a panel of primary human lung adenocarcinomas. We find that both PD-L1 and CD155 are induced by multiple arms of the ISR pathway, and CD155 harbors inhibitory uORFs in its 5' UTR. We further demonstrated that ISR activation inhibits T cell function *in vitro* and tumorigenesis *in vivo*, promoting tumor growth in the syngeneic mouse lung adenocarcinoma (LUAD) CMT167 model. Analysis of immune cell infiltration using mass cytometry and multiplexing IHC in this *in vivo* model are ongoing. We are also determining the extent to which ISR inhibition suppresses tumorigenesis by promoting anti-tumor immunity and whether this may synergize with ICB. Overall, these studies set the stage for determining whether inhibition of the ISR pathway alone or in combination with ICB will benefit lung cancer patients, trigger anti-cancer immune responses and improve current strategies.



Session 4:

Small Cell Lung Cancer

A Model of Human Small Cell Lung Cancer Metastasis Utilizing Orthotopic Transplantation of Patient-Derived Xenografts

Shreoshi Pal Choudhuri, Kyle May, Thomas Salisbury, Chamey Suchors, Braeden Freitas, Seth Hamilton, Victor Chien, Benjamin Drapkin, James Kim

UT Southwestern

Presented by: James Kim

Small Cell Lung Cancer (SCLC) is a deadly and highly metastatic disease. Approximately 70% of patients are found with distant metastasis at diagnosis. The development of patient-derived xenografts (PDXs) has significantly advanced the study of human SCLC. The PDXs are genetically and functionally faithful to their original tumors including their responses to therapy. Most of the PDXs are grown in the subcutaneous flank of immunocompromised mice. To date, SCLC PDX metastasis have yet to be reported – despite that most of PDXs are derived from biopsies of metastatic sites or circulating tumor cells. Here, we report on a model of orthotopic SCLC PDXs that consistently metastasize to distant organs. We adapted an orthotopic transplantation method in which dissociated SCLC PDXs are directly injected into the left lung of immunocompromised NSG mice. Three PDX lines have been tested – one treatment naïve, and two drug-resistant lines. In addition to the primary left lung tumor, all three lines generate metastases to distant organs including the contralateral right lung, mediastinal lymph nodes, liver, adrenal glands, ovaries and brain. Rates of metastases range from 55 – 92% of injected mice, depending on the PDX line. Mice bearing one particular SCLC PDX line consistently develop hydrocephalus and brain metastases. The metastases express the neuroendocrine markers, synaptophysin and NCAM-1. Brain metastases were identified through their expression of TTF-1, a marker of SCLC and lung adenocarcinoma. In conclusion, we have adapted an orthotopic transplantation method to SCLC PDXs that consistently generates metastases and faithfully mirrors the pattern of human SCLC. Our model can be utilized to elucidate the biology of SCLC metastasis and as a platform for therapeutic strategies against metastatic SCLC.

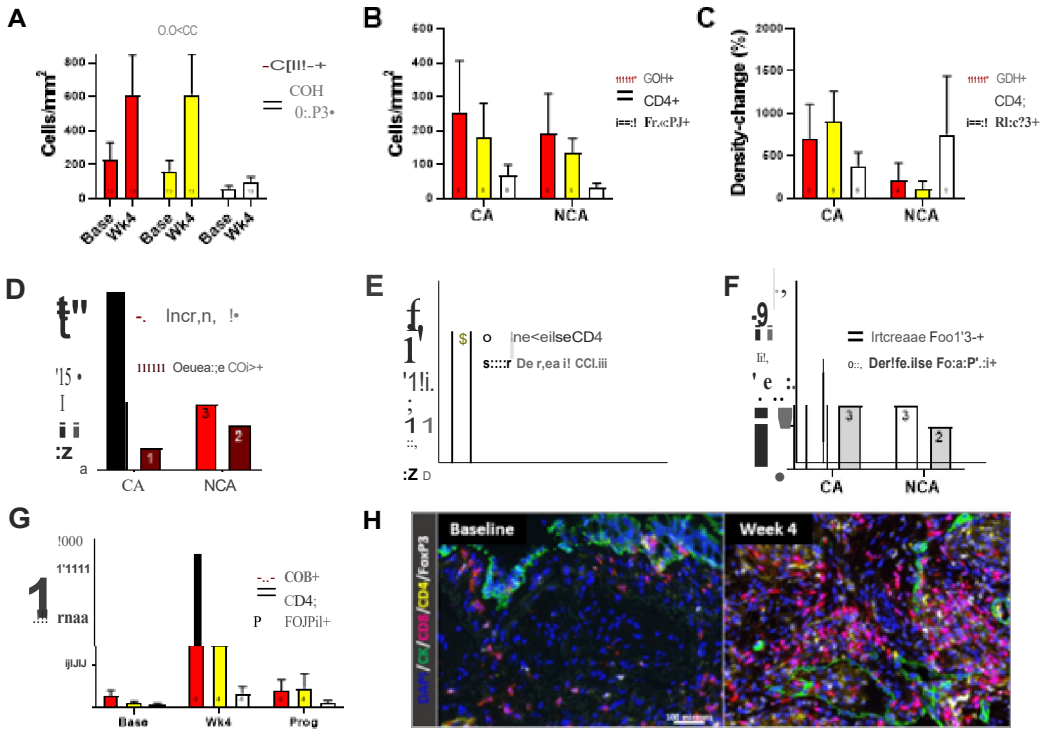
Longitudinal Tumor Microenvironment Analysis In Dual Checkpoint Inhibitor Blockade Using Ipilimumab/Nivolumab In Patients With Advanced Stage Small Cell Lung Cancer

Anne Chiang, Robert Matera, Kerryan Ashley, Barani Kumar, Scott Gettinger, Sarah Goldberg, Roy Herbst, Frederick Wilson, Kurt Schalper
Yale University
Presented by: Robert Matera

Background: In patients with advanced small cell lung cancer (SCLC), the impact of immunotherapy on the tumor microenvironment and clinical implications of these alterations are very poorly understood with no clear predictive biomarkers to guide patient selection.

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 13/22 patients, as well as 4 biopsies at progression. The tumor samples were studied using multiplexed quantitative immunofluorescence (mQIF) in addition to whole exome DNA sequencing (including germline DNA) and RNA-sequencing.

Results: 9/17 evaluable patients had PD; 8 patients showed clinical activity of treatment (2 with partial response). Among the 9 evaluable subjects that were previously treated with immunotherapy, there was 1 PR and 2 SD (DCR 33%). Median PFS was 1.7 months (95% CI: 1.1 – 5.8) and median OS of 7.3 months (95% CI: 4.9 – 10.1). mQIF analysis showed an increase in both CD8+ effector T cells and CD4+ helper T cells at week 4 compared to baseline samples without significant change in FoxP3+ regulatory T cells. CD4+ helper T cells exhibited the greatest expansion among patients with clinical activity, whereas FoxP3+ regulatory T cells were increased among those with no clinical activity. Tumors at progression showed a notable reduction of TILs, comparable to baseline levels. C to A mutation rate was significantly elevated in patients with clinical benefit. Week 4 tumors showed multiple novel DNA mutations and changes in transcriptional regulation.
Conclusions: Dual checkpoint blockade using nivo/ipi has clinical activity in patients with relapsed SCLC, even those previously treated with immunotherapy. Immunotherapy induced changes in TIL number and composition are associated with clinical activity. We identified multiple novel genetic and transcriptional changes in week 4 and progression samples which may be implicated in immunotherapy resistance.


A
B
C
D
E
F
G
H
Cells/mm²
Base
Wk4
CA
NCA
CD4+
Density-change (%)
Baseline
Week 4
Prog

Detecting small cell transformation in patients with advanced EGFR mutant lung adenocarcinoma through epigenomic cfDNA profiling

Catherine Meador, Talal El Zarif, Xintao Qiu, Ji-Heui Seo, Matthew Davidsohn, Hunter Savignano, Gitanjali Lakshminaranan, Heather McClure, John Canniff, Brad Fortunato, Rong Li, Mandeep Banwait, Karl Semaan, Marc Eid, Henry Long, Yin Hung, Navin Mahadevan, David Barbie, Matthew Oser, Zofia Piotrowska, Toni Choueiri, Sylvan Baca, Aaron Hata, Matthew Freedman, Jacob Berchuck,

Massachusetts General Hospital and Harvard Medical School, Dana-Farber Cancer Institute

Presented by: Catherine Meador

Histologic transformation to small cell lung cancer (SCLC) is a mechanism of therapeutic resistance in patients with advanced oncogene-driven lung adenocarcinoma (LUAD), which currently requires invasive biopsy for histologic review of tumor tissue for diagnosis. Prior studies suggest that epigenomic reprogramming is a molecular feature of small cell transformation. We developed an epigenomic cell-free (cf)DNA-based liquid biopsy to non-invasively detect small cell transformation in patients with *EGFR* mutant LUAD. We first performed comprehensive epigenomic profiling of LUAD, *de novo* SCLC, and transformed (t)SCLC tumors, which revealed widespread epigenomic reprogramming between LUAD and tSCLC tumors resulting in a large number of differential H3K27 acetylation, DNA methylation, and chromatin accessibility sites between the histologic subtypes. We then leveraged these divergent epigenomic profiles to develop a cfDNA-based test to detect small cell transformation in patients with *EGFR* mutant LUAD. To do so, we utilized novel methods to perform genome-wide profiling of three distinct epigenomic features – the histone modification H3K27 acetylation, DNA methylation, and chromatin accessibility – from 1 ml of plasma collected from patients with *EGFR* mutant LUAD (n=20) or *EGFR* mutant tSCLC (n=12). We then developed a classifier to detect the presence of small cell transformation based on normalized signal at the SCLC-enriched versus LUAD-enriched sites derived from the tumor analysis above. Tumor-informed cfDNA analysis of individual epigenomic features resulted in accurate discrimination of patients with *EGFR* mutant LUAD versus tSCLC, with AUROCs of 0.87 for cfDNA H3K27ac acetylation, 0.85 for cfDNA methylation, and 0.82 for cfDNA chromatin accessibility. Comparative analysis of the LUAD- and SCLC-enriched genomic loci from the tumor analysis revealed that biologically informative sites for the three epigenomic features were largely non-overlapping. We therefore evaluated the performance of a multi-analyte classifier integrating cfDNA H3K27 acetylation, methylation, and chromatin accessibility data, which achieved an AUROC of 0.94 for accurate discrimination of patients with *EGFR* mutant LUAD versus tSCLC. These data support the potential of epigenomic profiling to detect small cell transformation in patients with *EGFR* mutant LUAD and, more broadly, the ability to non-invasively detect histologic transformation in patients with advanced cancer through epigenomic cfDNA profiling.

DLL3 Expression In Early—Stage SCLC: Comparative Analysis Of IHC and mRNA ISH

May-Lucie Meyer, Hui Yu, Zoltan Lohinai, Grace van Hyfte, Fred Hirsch

Mount Sinai Hospital, University of Colorado, OKTPI-Koranyi National Institute

Presented by: May-Lucie Meyer

Introduction: Small cell lung cancer (SCLC) is highly lethal. Delta Like Canonical Notch Ligand 3 (DLL3) is commonly overexpressed in SCLC and is a target for new therapies. Tarlatamab, targeting DLL3 and CD3, showed a 40% response rate in previously treated patients. 80%-93% of advanced SCLC cases are DLL3 positive, but its expression in early-stage SCLC lacks documentation.

Aims: This study aimed to 1) assess DLL3 expression in early-stage SCLC using immunohistochemistry (IHC) and mRNA in situ hybridization (ISH), 2) correlate these assays and 3) correlate DLL3 expression with outcomes and characteristics.

Methods: This monocentric retrospective study evaluated 248 resected samples from patients with early-stage SCLC from 1978 to 2013. IHC staining used the Ventana Benchmark XT autostainer and SP347 Antibody Assay. For mRNA ISH, a Leica Bond RX autostainer was used. IHC positivity was defined as an H-score ≥1 (0-300), mRNA ISH positivity as a score of ≥1 with the ACD approved method (0-3). Linear correlation between IHC and mRNA ISH was calculated. Univariable and multivariable Cox regression were conducted. PASW Statistics 22.0 package and R versions 3.5.2 and 4.3.1 were used.

Results: From 1978 to 2013, 248 patients with histologically confirmed early-stage SCLC were included. 233 were evaluable with IHC and 58% were positive. 235 samples evaluable with mRNA ISH, 87% were positive. IHC and mRNA ISH were correlated (Spearman's rank, $p<0.001$). DLL3 positivity was not correlated with patients' characteristics. There was a non-significant trend toward better median overall survival (mOS) with IHC score <1, with a mOS of 27.1 months (95% CI: 12.5-59.0) vs 21.0 months (95% CI: 18.2-30.4), $p=0.893$. The same was observed with mRNA ISH score <1: mOS of 38.9 months (95% CI: 26.6-NA) vs 18.9 months (95% CI: 14.9-28.8) $p=0.221$.

Conclusion: In 248 early-stage SCLC, positivity for DLL3 was 58% with IHC and 87% with mRNA ISH. A trend for improved survival in patients with no DLL3 expression was observed. Future studies on DLL3 might improve SCLC treatment SCLC.

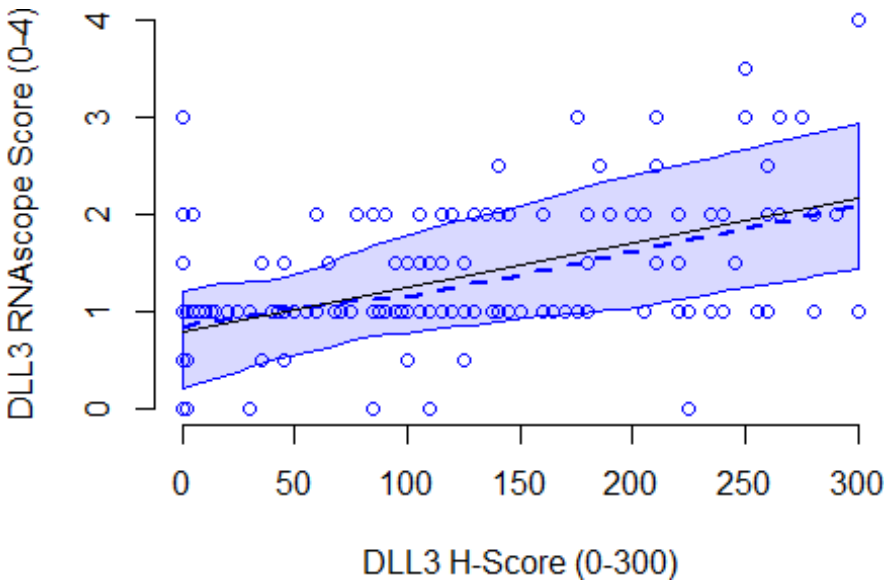
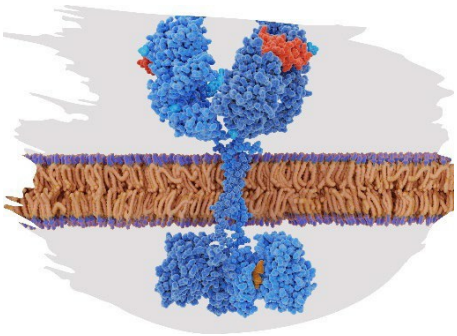


Figure 1. Comparison of DLL3 expression. Linear regression fitted line (black), Loess smoothing line (dashed blue), 95% confidence bands (solid blue). Spearman's rank correlation rho=0.557.



Session 5:

Early Detection and Screening

Schema For The Plasma/CT Matching And Detection of Lung Cancer in a NLST-ACRIN Cohort

Wei Wu, Sudhakar Pipavath, Yuzheng Zhang, Kristin Lastwika, Timothy W. Randolph, Paul Lampe, Viswam Nair, A. McGarry Houghton, Paul Kinahan

University of Washington, Fred Hutchinson Cancer Center

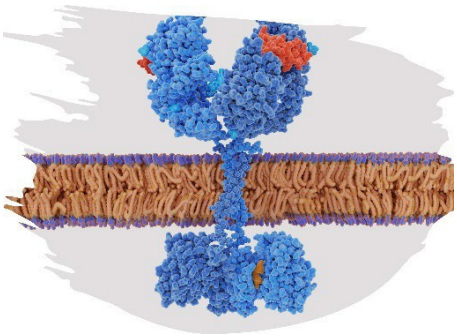
Presented by: Wei Wu

Background
The National Lung Screening Trial (NLST)-American College of Radiology Imaging Network (ACRIN) repository holds plasma samples and CT images for three annual screens, providing a valuable resource for developing lung cancer prediction models using combination of plasma and imaging biomarkers. The matching of plasma samples to CT images and a detailed schema illustrating CT scan results and lung cancer diagnoses remain uncharted.

Methods
We evaluated the alignment of CT images with plasma samples for 681 patients in a NLST-ACRIN cohort, encompassing all available lung cancer cases and 1:2 matched controls based on age, gender, and smoking history. We also examined the outcomes of CT scans (positive defined as one or more non-calcified nodules ≥ 4mm vs. negative defined as no abnormalities or minor abnormalities not suspicious for lung cancer) and lung cancer detection across the initial and subsequent screenings.

Results
From 681 patients, 583 with concurrent plasma samples and CT images across three annual screens were included. We excluded patients with plasma only and ineligible patients.
The patients who have matched plasma samples and CT images at baseline (T0), the first subsequent follow-up (T1), and the second subsequent follow-up (T2) are 559 (95.9%), 323 (55.4%), 191 (32.8%) out of 583 patients respectively. Positive CT scans were defined in 275/583 (47.2%) at T0, 190/583 (32.6%) at T1, and 119/583 (20.4%) at T2. Lung cancer was detected in 54/583 (9.3%) at T0, with additional cases identified at each subsequent screening, including 27/583 (4.6%) at T1 and 39/583 (6.7%) at T2. Post-T2, 40/583 (6.9%) developed lung cancer. Four (0.7%) patients with negative baseline or T1 CT screens developed lung cancer during the intervals between current and subsequent annual screening.

Conclusion
The schema uncovers the pattern of plasma/CT concordance and lung cancer detection across annual screenings, highlighting the decline in sample matching over time. It also suggests a need for enhanced biomarkers to identify cancers between and after scheduled CT screenings, providing insight into the diversity of lung cancer diagnoses in a screening cohort.



Session 6:

Cancer Biology

CDK8/19 Co-inhibition Enhances Osimertinib Efficacy in EGFR Mutant Non-Small Cell Lung Cancers (NSCLC)

Mustafa Al-Dulaimi, Antja-Voy Hartley, Simon Baldacci, Matthew Booker, Nathanael Gray, Michael Tolsturokov, Pasi Jänne

Dana-Farber Cancer Institute, Stanford Cancer Institute

Presented by: Mustafa Al-Dulaimi

The use of Osimertinib in the treatment of EGFR-mutant non-small cell lung cancer (NSCLC) has shown a remarkable effectiveness in improving patient outcomes. However, the sustained effects of these treatments are significantly hindered as patients experience tumor relapse following treatment primarily because of the diversity of mechanisms through which tumors acquire resistance to treatment. The elucidation of adaptive transcriptional programs and important transcription factors facilitating the evasion of cell death by cancer cells remains largely unknown or undruggable and of most interest in clinical settings. Here, we describe a novel role for the Mediator complex kinases CDK8 and its paralog CDK19 in promoting expression of pro-survival transcription-factor dependent gene expression in the presence of Osimertinib. Treatment with Osimertinib and CDK8/19 inhibitor significantly increased apoptotic rates in a selection of NSCLC cells compared to Osimertinib alone treatment. Furthermore, treatment with Osimertinib and CDK8/19 inhibitor significantly repressed tumor regrowth after drug washout. Using RNA-seq methods, we identified several genes related to STAT5, integrin signaling and dysregulation of apoptotic factors to be among the most differentially expressed genes after Osimertinib and CDK8/19 treatment. Interestingly, treatment with Osimertinib and CDK8/19 inhibitor impaired transcriptional expression of the canonical apoptotic protein MCL-1 by upregulating its endogenous inhibitor, NOXA. Based on these findings, we identify CDK8/19 as a potential transcriptional therapeutic target and show that CDK8/19 inhibitor combination treatment enhances the efficacy of Osimertinib treatment and promises potential strategy with clinical relevance in NSCLC.

A Lung Cancer Mouse Model Database

Ling Cai

UT Southwestern

Presented by: Ling Cai

Lung cancer, the leading cause of cancer mortality, exhibits diverse histological subtypes and genetic complexities. Numerous preclinical animal models have been developed to study lung cancer, but data from these models are disparate, siloed, and difficult to compare in a centralized fashion. Here we describe the construction of the Lung Cancer Mouse Model Database (LCMMDB), assembling data from 1,354 samples in 77 transcriptomic datasets, covering genetically engineered mouse models (GEMMs), chemically induced models, and spontaneous models. We performed standardization of sample and genotype curations and engaged data depositors to confirm and correct the datasets. The LCMMDB aligns 859 tumors from GEMMs with human lung cancer mutations, enabling comparative analysis and revealing a pressing need to broaden the diversity of genetic aberrations modeled in GEMMs. Accompanying this resource, we developed a web application at https://lccl.shinyapps.io/LCMMDB/, that offers researchers intuitive tools for in-depth gene expression analysis and fostering potential collaborations. With standardized reprocessing of gene expression data, the LCMMDB serves as a powerful platform for cross-study comparison and lays the groundwork for future research, aiming to bridge the gap between mouse models and human lung cancer for improved translational relevance.

Exploring Targetable Vulnerabilities in Cancer with the AVERON Notebook

Hongyue (Nicole) Chen, Brian Revennaugh, Haian Fu, Andrey A. Ivanov

Emory University

Presented by: Hongyue (Nicole) Chen

Cancer is a global health menace driven by genomic alterations that leads to over 10 million deaths annually. Genetic mutations can change protein structure, functions, and cellular localization. Such changes perturb protein-protein interaction (PPI) networks, leading to the acquisition of cancer hallmarks. The discovery of mutant-directed PPIs may uncover new mechanisms of oncogenic signaling and provide new targets for personalized therapeutic interventions in cancer. The advances in high-throughput screening technologies and computational approaches enabled comprehensive profiling of mutant-dependent PPIs in cancer cells. However, elucidation of functional consequences of mutant-induced changes in PPI networks and their impact on clinical outcomes of cancer patients remains highly challenging. To address this challenge, we develop a new computational platform, termed AVERON, to identify Actionable Vulnerabilities Enabled by Rewired Oncogenic Networks. AVERON is implemented as a Python Jupyter Notebook and serves as a tool for investigating mutant-induced neomorph PPIs (neoPPIs). Based on experimentally determined or computationally predicted networks of mutant-directed neoPPIs, AVERON employs specially designed algorithms and statistical techniques to assess the levels of PPIs in cancer patients in terms of PPI scores. It examines and visualizes neoPPI impact on clinical outcomes and identifies neoPPI-regulated distinctive sets of signature genes and oncogenic pathways. Furthermore, the AVERON can uncover clinically significant and druggable neoPPI-regulated genes to target cancer dependency on mutant-directed PPIs. Together, the AVERON Notebook provides a powerful computational platform to discover molecular mechanisms of neoPPI-dependent tumorigenesis, identify draggable vulnerabilities enabled by mutant-directed PPIs, and inform new target and therapeutic development in cancer.

Brain metastasis Fibrosis Predicts Clinical Outcomes and Response to Treatment in Patients with Non-Small Cell Lung Cancer

Darin Dolezal, Sampada Chande, Anna Arnal Estapé, Sarah Goldberg, Declan McGuone, Veronica Chiang, Don Nguyen

Yale University

Presented by: Darin Dolezal

Lung cancer remains the leading cause of cancer-related deaths worldwide and an estimated 25-30% of patients with non-small cell lung cancer (NSCLC) will develop brain metastasis. Despite advances in treatment, the prognosis of most patients with NSCLC brain metastasis remains dismal. New therapies are limited by their ability to either penetrate or effectively treat central nervous system (CNS) metastases, which is leading to increases in drug-resistant CNS relapse.

We characterized the histopathology of brain metastases from 108 patients with NSCLC who underwent surgical resection at Yale-New Haven Hospital to identify biomarkers which can predict treatment responses. We found that 39% of cases showed the accumulation of fibrosis within the brain-tumor microenvironment (fibrosis-high, FH) whereas 39% showed no fibrosis (fibrosis-low, FL), and 21% showed direct tumor extension into the fibrotic pachymeninges that surrounds the brain (dural-based fibrosis). Patients with fibrosis-high and dural-based metastasis had significantly worse overall survival (OS) and CNS-progression-free survival compared with fibrosis-low metastasis (n=108 patients; median OS: FH=8, D=7, FL=35 months, p<.0001; median cPFS: FH=6, D=7, FL=20 months, p<.0001; Figure 1). Fibrotic brain metastases were seen both at the time of diagnosis (n=50 patients; FH=36%, D=12%, FL=52%) and in the setting of disease recurrence or progression (n=58 patients; FH=41%, D=29%, FL=29%). Interestingly, patients with fibrosis-low brain metastases who received ICB therapy following neurosurgery showed a significantly more durable clinical benefit compared to those with fibrotic brain metastases (n=33 patients; median OS: FL=69, FH=27, D=15 months, p=.001). These data suggest that fibrosis is a strong predictor of CNS disease progression and may confer CNS resistance to therapies, including ICB therapy.

Prior strategies to broadly target fibrosis in cancer (e.g. targeting Hedgehog signaling in pancreatic adenocarcinoma) have largely failed to improve patient outcome. This may be due to our limited understanding of how different fibrotic tumor microenvironments form, whether mechanisms stem directly from tumor cells, and how tumor cell genetics contribute to the process. Our ongoing brain metastasis-to-brain metastasis comparative methylome and transcriptome analyses, combined with functional approaches to study underlying tumor cell biology, aim to provide precise identification of patient subsets for targeted treatments.

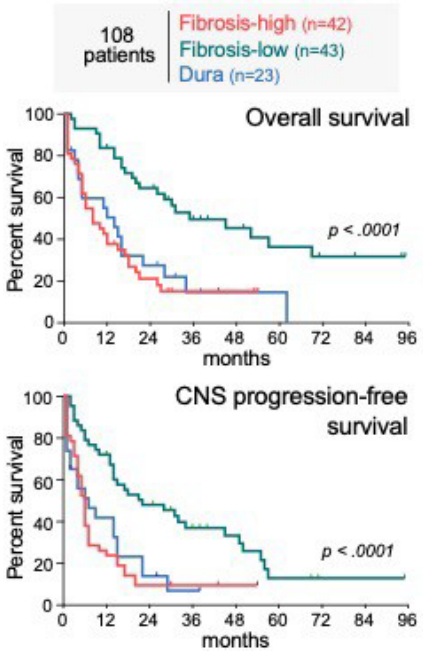


Figure 1. (Top) Overall survival and (**Bottom)** CNS progression-free survival in patients with fibrotic-high, fibrotic-low, and dural-based NSCLC brain metastases.

New Human Cell Line Models for The Study of Pathogenesis of SARS-Cov-2 Infection

Boning Gao, Ian Boys, Sara El Zahed, Kimberley Avila, Long Shan Li, Luc Girard, Hyunsil Park, Victor Stastny, Kenneth Huffman, Shreoshi Pal Choudhuri, Kimberly Batten, Matthew Esparza, Benjamin Drapkin, Lisa Miorin, Jerry Shay, Wendy Maury, Beatriz Fontoura, John Schoggins, Adolfo Garcia-Sastre, John Minna

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There is great inter-individual variation in SARS-CoV-2 (CoV-2) virus infection and response to various anti-viral therapy and the factors that account for this variation which is an important knowledge gap. In addition, in the future it will be important to understand if lung epithelial or tumor cells (or their fields) that have been infected by CoV-2 influence lung cancer risk or biology. Cells of lung origin are relevant for SARS-CoV-2 infection studies, given virus entry through the respiratory tract leading us to evaluate our large panel of molecularly and clinically annotated patient derived lung cancer, normal epithelial cell strains (>200) for their potential use in study of CoV-2 infection studies. We characterized these cells for expression (mRNA and protein) of key known host factors involved in CoV-2 infection (e.g. ACE2, TMPRSS2, interferon responses) and then tested for their ability to be infected with CoV-2 with authentic virus (n=26) or pseudotyped virus (n=54). We found: 1. 10 human cell lines including 8 NSCLC, which all expressed the ACE2 receptor and were susceptible to CoV-2 infection; 2. Infection rates varied among CoV-2 variants with Omicron variants showing the highest infection rate followed by the original CoV-2 virus WA1 and then Delta variants; 3. several patient lung cancer lines that expressed high levels of ACE2 but had a low CoV-2 infection rate suggesting ACE2 is necessary but not sufficient for CoV-2 infection; 4. CoV-2 Delta variants induced the highest while Omicron variants induced the lowest syncytia formation; 5. CoV-2 infection dramatically activated the interferon pathway in some lines but not others; 6. Parallel infection studies of the same lines by the Fontoura lab with influenza virus showed CoV-2 and influenza virus had different replication patterns. Our study identifies multiple new human cell lines to study CoV-2 infection which will be of use to test therapeutics, explore inter-individual differences in viral replication and therapy response, and study host factors involved in viral infection and therapy responses. These models will also be of use in future studies asking if CoV-2 infection of airway epithelial derived cells impacts on subsequent development or behavior of lung cancer.

Co-Occurring Genomic Alterations-Guided in Silico Discovery of Therapeutic Vulnerability in Lung Adenocarcinoma

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Cancer genomic studies have revealed the vast driver mutational landscape in lung adenocarcinoma (LUAD) and diverse molecular subtypes. Single driver mutation models have been widely used to guide clinical molecular subtyping and LUAD patient stratification for targeted therapies. However, accumulating evidence suggests an emerging spectrum of co-occurring genomic mutations as synergistic biomarkers of multiple cancer hallmarks and therapeutic response in LUAD patients. Uncovering the molecular basis of how co-occurring genomic alterations lead to the acquisition of cancer hallmarks and therapeutic response may guide the discovery of additional vulnerabilities to advance clinical intervention strategies. Here, we report the integrated bioinformatics interrogation of co-occurring mutation-mediated cell signaling pathway rewiring and collateral vulnerabilities. First, we capitalized on LUAD patient data to identify and prioritize clinically significant co-occurring genomic alteration events. Then, multi-omics datasets, including transcriptomics, proteomics, gene essentiality, and therapeutic response data from LUAD patient samples and cancer cell lines, were used to correlate the co-occurring genomic alterations with the reprogrammed cell states, gene dependencies, and drug sensitivities. Next, we leveraged our unique oncogenic protein-protein interaction network dataset to impute the physical connectivity and inform the potential molecular mechanisms underlying the observed phenotypic changes. Our study provides a computational framework for predicting the functional consequences of co-occurring genomic alterations and informing new therapeutic hypothesis in LUAD for further experimental validations.

Molecular and Histological Characterization of NSCLC Progression to Leptomeningeal Metastasis with Comorbid Intraparenchymal Disease

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Leptomeningeal disease (LMD) is a rare form of central nervous system (CNS) metastasis wherein tumor cells invade the cerebrospinal fluid (CSF) filled space that surrounds the brain and spinal cord. For patients with LMD, prognosis is extremely poor even with aggressive treatment. Unfortunately, the mechanisms of progression to LMD and the adaptations tumor cells make to enable survival in this metabolically sparse microenvironment are poorly understood, due in part to the limited availability of in vivo models and scarcity of mechanistic studies. As 60% of patients with LMD have concurrent or prior parenchymal metastases, our laboratory examined our established murine models of parenchymal metastases for signs of leptomeningeal infiltration. We identify two xenograft non-small cell lung cancer (NSCLC) models of intraparenchymal metastasis following intra-arterial injection that show progression to LMD in a subset of cases. In the H2030-BrM3 model this occurs de novo, whereas the *EGFR*-mutant PC9-R2 model progresses to LMD only following onset of resistance to tyrosine kinase inhibitor treatment. Subsequent in vivo passaging of the H2030-BrM3 line through the cerebral lateral ventricles resulted in the H2030-LMD2 cell line, which has high affinity for leptomeningeal metastases and shows extensive perivascular invasion within the brain parenchyma. In vitro, the H2030-LMD2 line shows altered clustering behavior and increased survival when cultured in suspension. RNA-sequencing of this cell line across adherent and suspension culture conditions shows multiple biological processes upregulated in the LMD-tropic line, including chemotaxis, apical junction formation, and TGF-β signaling. Targetable pathways emerging from this analysis will be functionally investigated for their role in promoting progression to LMD, including through validation in the syngeneic KPN1-BrM line which has been selected for its CNS affinity and shows progression to LMD. Spatial sequencing and multiplexed immunofluorescence will furthermore investigate the tumor microenvironment in the perivascular niche and subarachnoid spaces. These findings will be clinically corroborated with molecular characterization of the CSF of patients with leptomeningeal metastases in our extensive brain metastasis biorepository, established in collaboration with the Neurosurgery Department at Yale New Haven Hospital.

The Lysine Demethylase KDM2A Regulates Tumor Cell Clustering to Potentiate Metastasis

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Approximately 70% of patients diagnosed with non-small cell lung cancer (NSCLC) are not eligible for curative resection, often due to disseminated disease at diagnosis. Thus, the advent of novel therapeutics, especially those efficacious for metastatic disease, is of primary importance. Metastasis correlates with epigenetic alterations that drive tumor plasticity and adaptive heterogeneity in response to the multiple microenvironments metastatic cells see, yet the molecular mechanisms underlying these epigenetic alterations remain poorly understood.

We performed a functional genomic screen and identified the lysine 36 on histone H3 (H3K36) demethylase KDM2A as a mediator of *in vivo* and *in vitro* metastatic competence in multiple models of NSCLC. KDM2A depletion in highly metastatic NSCLC cell lines reduces their capacity to form multi-tumor cell clusters. *In vivo*, KDM2A depletion does not impact lung orthotopic tumor cell growth or the total number of circulating tumor cells (CTCs) but does reduce their ability to colonize distant metastatic sites. This is consistent with the fact that human CTCs found in clusters are more metastatic than CTCs which disseminate as single-cells.

Because the molecular and biological consequences of H3K36 regulation are context dependent, we performed for the first time an integrated transcriptomic-epigenomic analysis of target gene regulation, KDM2A genomic binding, H3K36 methylation, and H3K27 acetylation, in metastatic cell clusters. KDM2A enhances the expression of cell adhesion and anti-oxidant genes, while suppressing inflammatory gene responses, and this regulation preferentially occurs in tumor cell clusters. Transcriptomic analysis of CTCs from patients confirms that KDM2A regulated transcriptional responses correlate with metastatic relapse across multiple cancer types. Moreover, KDM2A may function both as a transcriptional activator and repressor in metastatic clusters. As KDM2A encodes for several domains which can independently regulate E3-ubiqutin ligation, H3K36me2 demethylation, and DNA methylation, we have also performed a structure function analysis of K2MDA in the context of metastatic phenotypes. Thus, the distinct biochemical functions of KDM2A in relation to its regulation of CTC gene transcription and histone modification will also be presented.

In conclusion, we have identified KDM2A as a novel regulator of NSCLC metastatic spread. The ability to inhibit KDM2A with small molecule inhibitors may be translated into effective therapeutic interventions for NSCLC patients with metastasis disease.

Mechanisms of Epigenetic Sensitization to Lung Cancer Risk

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Epigenetic perturbations in cancer are commonly encountered but poorly understood. We sought to understand how latent epigenetic information contributes to tumorigenesis in lung adenocarcinoma. We developed a mouse model of epigenetic sensitization in which loss of the epigenetic regulator KDM6A (UTX) in the paternal germ line in mice result in elevated lung tumor burden in offspring even when the mutant *Kdm6a* allele itself is not inherited. We hypothesized that these 'epigenetically sensitized' offspring carry silent epigenetic perturbations that predispose them to cancer and can interact with specific driver mutations to enhance lung tumorigenesis. Preliminary data suggests that epigenetic sensitization induced by paternal KDM6A loss enhanced overall lung tumor burden in mice carrying an activated KRAS allele ($Kras^{LA1}$). Further, exome sequencing of epigenetically sensitized tumors revealed frequent mutations in the histone methyltransferase KMT2D (MLL4), a functional interactor of KDM6A. Correspondingly, disruptions in H3K4me1, the histone modification deposited by KMT2D, occur in the KDM6A mutant germline in regulatory regions of lung oncogenes. Ongoing work is aimed at determining if these epigenetic lesions are present in normal lung tissue and tumors of epigenetically sensitized offspring and may lower the threshold to malignant transformation. Future work will evaluate if similar signatures of epigenetic sensitization can be detected in tumors from patients with a history of familial cancer.

CDK8/19 Co-inhibition Enhances Osimertinib Efficacy in EGFR Mutant Non-Small Cell Lung Cancers (NSCLC)

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The use of Osimertinib in the treatment of EGFR-mutant non-small cell lung cancer (NSCLC) has shown a remarkable effectiveness in improving patient outcomes. However, the sustained effects of these treatments are significantly hindered as patients experience tumor relapse following treatment primarily because of the diversity of mechanisms through which tumors acquire resistance to treatment. The elucidation of adaptive transcriptional programs and important transcription factors facilitating the evasion of cell death by cancer cells remains largely unknown or undruggable and of most interest in clinical settings. Here, we describe a novel role for the Mediator complex kinases CDK8 and its paralog CDK19 in promoting expression of pro-survival transcription-factor dependent gene expression in the presence of Osimertinib. Treatment with Osimertinib and CDK8/19 inhibitor significantly increased apoptotic rates in a selection of NSCLC cells compared to Osimertinib alone treatment. Furthermore, treatment with Osimertinib and CDK8/19 inhibitor significantly repressed tumor regrowth after drug washout. Using RNA-seq methods, we identified several genes related to STAT5, integrin signaling and dysregulation of apoptotic factors to be among the most differentially expressed genes after Osimertinib and CDK8/19 treatment. Interestingly, treatment with Osimertinib and CDK8/19 inhibitor impaired transcriptional expression of the canonical apoptotic protein MCL-1 by upregulating its endogenous inhibitor, NOXA. Based on these findings, we identify CDK8/19 as a potential transcriptional therapeutic target and show that CDK8/19 inhibitor combination treatment enhances the efficacy of Osimertinib treatment and promises potential strategy with clinical relevance in NSCLC.

A Genome-Wide Single-Cell 3D Genome Atlas of Lung Cancer Progression

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Presented by: Siyuan Wang

Alterations in three-dimensional (3D) genome structures are associated with cancer. However, how genome folding evolves and diversifies during subclonal cancer progression in the native tissue environment remains unknown. Here, we leveraged a genome-wide chromatin tracing technology to directly visualize 3D genome folding *in situ* in a faithful *Kras*-driven mouse model of lung adenocarcinoma (LUAD), generating the first single-cell 3D genome atlas of any cancer. We discovered stereotypical 3D genome alterations during cancer development, including a striking structural bottleneck in preinvasive adenomas prior to progression to LUAD, indicating a stringent selection on the 3D genome early in cancer progression. We further showed that the 3D genome precisely encodes cancer states in single cells, despite considerable cell-to-cell heterogeneity. Finally, evolutionary changes in 3D genome compartmentalization – partially regulated by polycomb group protein Rnf2 through its ubiquitin ligase-independent activity – reveal novel genetic drivers and suppressors of LUAD progression. Our results demonstrate the importance of mapping the single-cell cancer 3D genome and the potential to identify new diagnostic and therapeutic biomarkers from 3D genomic architectures.

Profiling the Protein Interactome Through Cysteine-Reactive Libraries Has Revealed the Identification of EML4ALK Dimerizers

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Traditionally, targeting so-called 'undruggable' proteins in cancer therapy has been challenging. However, cysteine covalent binders offer a promising solution by facilitating direct interactions with these elusive targets. While previous efforts have focused on designing single-warhead compounds to target specific cysteine residues, crucial aspects such as dimerization and interactome disruption have remained largely unexplored, hampering progress in drug discovery.

In our study, we constructed a library comprised of 32 cysteine-reactive compounds with diverse reactivities, employing a combination of assays including cysteine druggability mapping (CDM) and SDS-PAGE fractionation to assess their effects on protein interactions. Through this approach, we identified Compound 1C9 as a lead compound capable of inducing significant dimerization of EML4, a pivotal fusion gene partner in lung cancer.

Using the EML4ALK fusion gene as a model, we delved into the mechanisms underlying compound-induced dimerization, conducting structure-activity relationship (SAR) studies and screening additional compounds to optimize ALK dimerization while minimizing off-target effects. The optimized compound disrupted key cellular signaling pathways associated with ALK, such as AKT and ERK, upon dimerization of EML4ALK. Notably, we observed that covalent dimerization also triggered EML4ALK degradation, obviating the need for a ubiquitin ligase like a PROTAC.
In summary, our findings unveil a novel class of cysteine covalent binders with the potential to modulate protein-protein interactions in previously 'undruggable' cancer proteins. This discovery represents a significant step forward in cancer therapeutics, offering promising avenues for future drug development endeavors.