alterations with DFS through Cox regression models. Concordance probability estimate (CPE) was used to discriminate performance between the existing TNM model and the developed prediction models.

Results: The median age was 70 years (range, 55-84), one-third were female (n=35), 55% (N=52) were pathologic stage I, and 98% (N=93) were ever-smokers. Median follow-up was 2 years. Recurrence occurred in 20% (19/95) and DFS was 74% (95% CI, 84-96%) at two years. Clinicopathologic features associated with DFS were lymphovascular invasion, visceral-pleural invasion, and pathologic stage. Tumor genomic analysis revealed alterations in the transcription factor BCL6 were independently associated with worse DFS (HR 5.23, 95% CI 1.73, 15.9, p=0.009), while mutations in the tumor suppressor ARID1A were associated with a worse OS (HR 2.98, 95% CI 0.91, 9.77, p=0.07).

Pathway-centric analyses revealed no associations with our primary or secondary endpoints. Our prognostic clinicopathologic model outperformed the internally validated TNM model (CPE, 0.74 vs. 0.70) for prediction of DFS and our clinicopathologic model, adjusted for BCL6 genomic alterations, further improved discrimination (CPE=0.77).

Conclusions: We show that integration of high-risk clinicopathologic and tumor genomic profiling better predicts DFS than TNM classification alone in early-stage, surgically resected LUSC -- an observation that may facilitate enrichment in future adjuvant therapy clinical trials. This exploratory genomic analysis also suggests future studies investigate putative therapeutic vulnerabilities in LUSC tumors harboring BCL6 and ARID1A genomic alterations.

A24
The Genome-Wide Mutational Landscape of Lung Cancer in Never-Smokers: The Women’s Health Initiative (WHI) Cohort
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Lung cancer is the leading cause of cancer deaths worldwide, and a positive history of smoking remains one of the most significant risk factors for lung cancer development. However, about 20% of lung cancer diagnoses are reported in individuals with no smoking history. Lung cancer in never-smokers (LCNS) is clinically distinct from tobacco-induced lung cancer, with a greater proportion of LCNS occurring in women and having adenocarcinoma histology. One of the key challenges in identifying the cancer-promoting genetic events that drive LCNS is the relatively small number of tumors that have been sequenced using genome- or exome-wide approaches. We address this gap through the collection and exome sequencing of lung tumor tissue and matched blood-derived normal DNA from 77 women who participated in the Women’s Health Initiative (WHI), the majority of whom have light or no smoking history. Samples were sequenced with a custom exome approach at the Center for Cancer Genome Discovery (CCGD), Dana-Farber Cancer Institute, with baits for all protein coding regions of the genome and noncoding regions that are frequently rearranged/translocated in lung cancer, such as introns within the ALK gene. Somatic mutations were identified using MuTect2 and mutational significance was determined using MutSigCV2. Preliminary analysis involving 18 tumor/normal pairs identified an enrichment of somatic alterations in genes such as EGFR and TP53. Additionally, tumor purity and copy number alterations were estimated using ichorCNA. Translocation analysis was performed using BreaKmer identifying a CD74-ROS1 fusion. 72% of the cases harbored previously known oncogenic drivers of lung adenocarcinoma such as mutations in EGFR, KRAS, RIT1, and MET with mutations that are clinically targetable using FDA-approved or investigational agents. Overall this project will double the number of exome profiles from never-smokers and, importantly, leverage the extensive metadata curated under the WHI to evaluate secondary/environmental factors such as second-hand smoke and radon exposure and their potential role in LCNS.

A25
PTPRH Mutations in NSCLC Regulate EGFR Phosphorylation
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The dysregulation of receptor tyrosine kinases (RTK) has garnered plenty of interest within the cancer field, and attention has begun to turn to phosphatases regulating RTK behavior. Under normal cellular conditions, protein tyrosine phosphatases remove phosphate groups from tyrosine residues, thus maintaining signaling homeostasis. In whole-genome sequencing of primary mouse mammary tumors from the polymavirus middle T antigen (PyMT) mouse model, we found a mutation in the protein tyrosine phosphatase receptor type H (Ptprh) gene. Targeted resequencing of 45 mouse tumors showed a conserved heterozygous or homozygous mutation present in 80% of tumors. This C>T mutation results in a valine-to-methionine shift within one of the fibronectin domains of PTPRH. Previous literature has shown interactions between PTPRH and epidermal growth factor receptor (EGFR). To determine the relevancy of PTPRH mutations in human cancer, data from The Cancer Genome Atlas (TCGA) were analyzed and revealed PTPRH mutations in 5% of non-small cell lung cancer (NSCLC) patients. Moreover, patients with a mutation in PTPRH were mutually exclusive from those with mutation or amplification of EGFR. We hypothesize a mutation in PTPRH results in a failure of PTPRH to dephosphorylate EGFR, resulting in inappropriate maintenance of downstream signaling pathways important for proliferation and evading apoptosis. Since NSCLC patients with EGFR mutations are successfully treated with tyrosine kinase inhibitors (TKI), we also hypothesize tumors with a mutation in PTPRH will be sensitive to TKIs. In support of this, we demonstrated mouse tumors with a mutation in Ptprh had increased phosphorylated EGFR (pEGFR). Furthermore, CRISPR-mediated knockout of PTPRH in H23 NSCLC cells leads to increased pEGFR. Pathway signature analysis applied to microarray gene expression data from the Breast TCGA dataset (due to low sample size in the NSCLC dataset), and single sample gene set enrichment analysis applied to RNA sequencing data from the NSCLC TCGA dataset both predicted an increase in PI3K and AKT activity. This suggested the EGFR residue targeted by PTPRH was tyrosine 1197. Western blots on Ptprh mutant mouse tumors confirmed increased levels of pAKT. Additionally, immunohistochemistry for pEGFR 1197 revealed increased staining in mouse tumors with a mutation in Ptprh, with subcellular location in the nucleus rather than the membrane. To determine whether TKIs may be an effective treatment for NSCLC patients who harbor a PTPRH mutation, H1155 and H2228 NSCLC cell lines with PTPRH mutations in the fibronectin and phosphatase domains, respectively, were subjected to a dose response curve with the TKI osimertinib. These lines show significant growth differences as compared to the negative control cell line A427. While more work is needed to elucidate the role of mutant PTPRH in NSCLC, preliminary data suggest mutant PTPRH fails to dephosphorylate EGFR, and patients with a mutation in PTPRH may benefit from TKI therapy.

A26
Deciphering the Functional Redundancy of USP4 and USP15
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Ubiquitin-specific proteasomes (USPs) are a class of deubiquitinating enzymes that catalyze the removal of ubiquitin from various proteins and are involved in many cancers. Previous work established the USP paralogs USP4 and USP15 emerged from an ancestral USP about 500 million years ago from a whole genome duplication, and the majority of
known vertebrate genomes retain a functional copy of both. USP4 was found to be consistently overexpressed in primary tumor tissue from small-cell carcinomas and adenocarcinomas of the lung. Despite their similarity, high expression of USP4 is correlated with decreased overall survival in lung adenocarcinoma, whereas high expression of USP15 is correlated with increased survival. Both USPs are known to be involved in some of the same signaling pathways such as Wnt/β-catenin; however, subfunctionalization has occurred such that they each regulate the stability of distinct substrates. To better understand each USP’s role, we are analyzing mice in which one or both genes have been inactivated and have found that the absence of both USPs results in a lethal phenotype. Although USP4 and USP15 have diverged over evolutionary time, we hypothesize that there may still be some level of functional redundancy. We found that embryos null for both genes die at midgestation and are physically smaller than embryos heterozygous for both genes. They have underdeveloped livers, indicating a possible defect in hematopoiesis. Proper fetal hematopoiesis requires signaling through Wnt/β-catenin pathway, and a systematic analysis of the components of this pathway has been undertaken by Western blot and qPCR. Current data indicate that there are deficiencies in at least some USP4 substrates, and that the TCF transcriptional complex is greatly reduced. Published reports assert a role for USP4 in metastatic spread of lung cancer to the brain, mediated by its effects on the Wnt/β-catenin pathway. Potential functional compensation by USP15 must be evaluated before targeted therapies can be considered. Our studies will establish the extent and mechanism of such compensation.

### A27

**Stage I Lung Adenocarcinoma Gene Expression Associated with Aggressive Histologic Features for Guiding Precision Surgery and Therapy**


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**Background:** Stage I lung adenocarcinomas (LUADs) show heterogeneity in histologic patterns that correlate with malignant behavior. Solid, micropapillary, and cribriform patterns are associated with worse survival whereas lepidic (in situ) predominance has the best prognosis. In this study, we sought to characterize histologic pattern-specific gene expression in resected clinical stage I LUADs. We also aimed to train and evaluate a genomic biomarker predictive of histologic aggressive patterns with the ultimate goal of being able to impact surgical and therapeutic decision making for post-biopsy management.

**Methods:** A training cohort of 56 tumors from patients with stage I LUAD was included for pathologic annotation and whole-exome RNA sequencing. Histologic pattern subtyping in 5% increments including all diagnostic slides was performed. A single representative FFPE block was chosen for RNA sequencing. Negative binomial models were used to identify gene expression differences associated with percent solid, cribriform, or micropapillary histology, and EnrichR was used for pathway enrichment analysis. A random-forest classifier predicting aggressive histologic patterns was trained using 5-fold cross validation. An independent set of 21 surgically resected NSCLC (7 EGFR mutant and 14 EGFR wild) were used for pathway enrichment analysis. A random-forest classifier predictive of aggressive histologic features using either lepidic (in situ) or nonlepidic components. This biomarker has the potential to predict histologic aggressiveness even from presurgical tumor biopsies where all histologic patterns may not be represented. Such a biomarker may be useful in guiding clinical decision making, including extent of surgical resection.

### A28

**Investigating Antitumor T-Cell Responses Using NIJNA: An Inducible Genetic Model for Creating Neoantigens**

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Historically, attempts to generate inducible neoantigens in mouse models have been hindered by leaky expression of the antigen in the thymus, leading to central tolerance in developing CD8 and CD4 T cells. We have developed the iNversion Inducible neoAntigen (NIJNA) model to resolve the existing problems of tolerance and leakiness using RNA splicing, DNA recombination, and three levels of regulation to control induction of neoantigen. Furthermore, this inducible model system is compatible with existing Cre-driven models of cancer, and we have generated a NIJNA-antigen-inducible tumor cell line from a KrasG12D/P53-/- mouse lung tumor. Antigen expression in this model is temporally controlled via systemic drug delivery, and generates responses in both transgenic and endogenous CD8 T cells. We will use this model to investigate specific T-cell responses to tumors and to assess how therapies such as checkpoint blockade impact T-cell response.

### A29

**Immune-Suppressive Microenvironment Induced by Increased Treg During EGFR-TKI Mediated IP-10 and TGF-β**

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**Background:** Studies on the immune microenvironment of EGFR mutant lung cancer have been limited. We analyzed the effect of immune microenvironments on the development of EGFR-TKI resistance in EGFR-mutated lung cancer. **Methods:** The EGFR mutant lung cancer cell lines (HCC827 and H4406) were cocultured with activated PBMC for 72 hours with EGFR-TKI. Changes of cytokines/chemokines in the media, PD-1 expression of CD8+ T cells, regulatory T cells fraction, and transcriptome analysis of tumor cells were performed. We also performed immune profile analysis of fresh tissues of 21 surgically resected NSCLC (7 EGFR mutant and 14 EGFR wild) by multicolor FACs. **Results:** IFN-γ, IL-6, VEGF, TGF-β1, and IP-10 were significantly increased after coculture but did not decrease after EGFR-TKI. PD-L1 expression on tumor cells increased after coculture (p = 0.08 in HCC827 and p = 0.09 in H4406) but did not decrease after coculture with activated PBMC and EGFR-TKI treatment (p =