Presenter's Name: Abdo, Rober

Additional Authors: Jackson-Boeters L, Johnston, D, Penuela, S, Zhang, Q

Abstract Title: Pannexin1 expression in lung cancer-brain metastasis

Introduction: Brain metastases (BMs) are the leading cause of cancer-related deaths, comprise the majority of central nervous system malignancies, and widely originate from lung carcinomas. Immunotherapy, and particularly, checkpoint inhibitors have emerged as the standard of care for most patients with advanced lung cancer. However, the variance in brain metastasis progression and tumor infiltrating immune profile among metastatic lung carcinoma patients requires more robust predictive biomarkers. Pannexin1 (PANX1) is a transmembrane glycoprotein that forms large-pore channels and has been reported to promote tumorigenesis in metastatic melanoma. The roles of PANX1 in lung cancer-associated brain metastases and tumor-infiltrating lymphocytes have not been identified.

Methods: Forty-two patient-matched formalin-fixed paraffin embedded tissue samples from lung carcinomas and subsequent brain metastases were constructed into two master-tissue microarrays (TMAs). Each patient was represented by 3 cores from primary lung carcinoma and 3 cores from metastatic lung carcinoma in the brain. PANX1 and tumor-infiltrating immune cells (TIIC) (CD3+, CD8+, CD68+, PDL1+) were assessed using immunohistochemistry and digital image analysis (Qu Path software) followed by investigating the cellular distribution of PANX1 through immunofluorescence in primary lung carcinoma samples and their metastases in the brain.

Results: The expression of PANX1 was significantly higher in the lung carcinoma brain metastases compared to their paired primary lung carcinoma. There is no correlation between the PANX1 expression level and the metastatic time interval. CD8+ and CD68+ TIIC densities were significantly higher in primary lung carcinoma, in comparison to the paired brain metastases. Additionally, the level of PANX1 in lung carcinoma-brain metastases correlated negatively with the density of CD68+ TIICs. PANX1 localization in lung carcinomas predominantly distributed in the cytoplasm in both lung and brain specimens.

Conclusion: In our study, we discovered that PANX1 is expressed in patient-derived lung carcinomas. The expression level is higher in the metastatic lung tumor in the brain compared to primary tumors, and influences (TIIC) profiles. These findings indicate a role of PANX1 in cancer progression and immunological profile of metastatic lung carcinomas.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Al Jawhri, MohdWessam

Additional Authors: Kim SJ, Dick FA

Abstract Title: Effects of p38 Mitogen-Activated Protein Kinase induction on RB phosphorylation at the non-CDK sites, S838 and T841

Introduction: The retinoblastoma tumor suppressor protein (RB) is an important cell cycle regulator. The canonical model of RB regulation states that cyclin-cyclin-dependent kinases (CDK) complexes inactivate RB by phosphorylation and permit cell entry into the S phase. Mono-phosphorylation of RB (phosphorylation of a single phosphorylation site) has been shown to impact RB function. Recently, our lab has investigated two non-CDK phosphorylation sites in Jurkat cells, Serine 838/Threonine 841, and discovered that these phosphorylation events are required for Condensin II release from chromatin, a cell-cycle independent/non-canonical role. We hypothesized that conditions of cellular stress will elicit RB phosphorylation at these sites in a p38-dependent manner.

Methods: Jurkat cells will be cultured in suspension and treated under conditions of osmotic shock, oxidative stress, genotoxic stress, and with nocodazole (microtubule destabilizing agent). Whole-cell lysates were prepared after treatment using RIPA lysis. GST-E7 pulldown was performed on the whole-cell lysates to isolate RB. Isolated RB and p38 activation were assessed using immunoblot. RB phosphorylation at the Serine 838/Threonine 841 sites and the effects p38 inhibition on RB phosphorylation will be assessed.

Results: This work will reveal the breadth of circumstances where p38 regulates Condensin II through RB phosphorylation. We anticipate that phosphorylation at the Serine838/Threonine841 sites is p38-dependent under these circumstances, and inhibition of p38 will result in reduced phosphorylation.

Discussion: The results of this study will aid in gaining insight into the breadth of cellular stresses that activate this regulatory effect on RB and Condensin II. This study may then contribute towards characterizing the relevance of p38 signaling to broader RB functions that could be involved in modulating chemotherapeutic sensitivity.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Gerhardt, Lara

Additional Authors: Gerhardt L, Figueredo R, El Hajjar M, Maleki S

Abstract Title: Mechanism of CD39 induction on tumor-specific T-cells and its role in anti-tumor immunity

Introduction: Stimulating and sustaining anti-tumor immunity is crucial for maintaining long-term remission in cancer patients. Tumor-specific CD8+ T-cell are one of the most critical components of anti-tumor immunity, and we can now use CD39 to identify tumor-specific T-cells, as those specific for cancer unrelated epitopes do not express this molecule. CD39 is an ectonucleotidase that hydrolyzes extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) leading to the generation of adenosine, an immunosuppressive molecule. Given this function of CD39, it is puzzling that tumor-specific CD8+ T-cells commonly express CD39. In this study, we aim to identify the mechanism of CD39 induction on these cells and investigate the functional consequences of its expression. We hypothesize that pro-inflammatory cytokines IL-12 and IL-27 induce the upregulation of CD39 on tumor-specific CD8+ T-cells through the concurrent activation of STAT-1, -3, and -4, resulting in dynamic anti-tumor immunity.

Methods: In testing our hypothesis, we vaccinated immunocompetent, syngeneic A/J mice by injecting them with neuro-2a, mouse neuroblastoma tumor cell lysates. Blood was collected from mice over time to measure the levels of IL-12 and IL-27 in serum using ELISAs. Mice were euthanized at different timepoints to measure the levels of CD39+CD8+ T-cells within the spleen.
In studies currently underway, we are investigating the same properties in neuro-2a tumor-bearing mice. In doing so, we will be able to compare the levels of CD39+CD8+ T-cells in the tumor compared to the spleen, and the spleen of tumor-bearing mice compared to those vaccinated. Future experiments will assess the specificity and functionality of CD39+CD8+ T-cells using MHC tetramer staining and ex vivo cytotoxicity assays, respectively. Cytokine depletion studies in vivo will allow us to determine whether IL-12 and IL-27 induce CD39 expression on tumor-specific T-cells in tumor-bearing mice and animals vaccinated with tumor lysates.

Results: Our preliminary data indicate that CD39+CD8+ T-cells peak within the spleen of mice 14 days after vaccination with a tumor lysate, and IL-12 levels peak as early as 6 hours after vaccination in mice.

Discussion: These initial findings provide a timeline for detection of our desired cells and molecules, which we will use in subsequent experiments. Data collected from this study will reveal the mechanism of induction of CD39 on CD8+ T-cells, in addition to the the function of tumor-specific CD39+CD8+ T-cells. Data generated in this study will allow us to better understand how tumor-specific immune response is formed and how it can be exploited in designing future immune-based therapies for cancer.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Krishnamoorthy, Mithunah

Additional Authors: Lenehan J, Silverman M, Parvathy SN, Burton JP, Maleki S

Abstract Title: Combination of Fecal Microbiota Transplantation in with Anti-PD1 Immunotherapy In Treatment-Naïve Metastatic Melanoma Patients

Introduction: Immunotherapy with checkpoint inhibitors have drastically improved the survival of patients with advanced melanoma. However, many patients are still unresponsive to these drugs. Recent studies have identified host factors including gut microbiota to affect anti-tumor immunity and ultimately patient's response to immunotherapy. We hypothesize that modifying the melanoma patient's gut microbiome via Fecal Microbiota Transplant (FMT) from healthy donors will enhance anti-tumor immunity and improve patient's response to checkpoint inhibitors.

Methods: 80 to 100 grams of fresh stool was collected from 2 healthy pre-screened donors and was processed per standardized procedure into capsules. FMT recipients were melanoma patients (n=5) with unresectable or metastatic disease who were anti-PD1 naïve for their advanced disease. Bowel preparation was completed using a prescribed polyethylene glycol solution and FMT was performed using oral administration of 40 capsules the day after. Pembrolizumab (anti-PD1 treatment) was started at least 1 week after FMT to allow for microbiome engraftment. Immune cells in blood and the gut microbiome were analyzed at baseline (preFMT), before initial Pembrolizumab infusion, three weeks after and three months after Pembrolizumab infusion using flow cytometry of peripheral blood mononuclear cells (PBMCs) and 16S rRNA sequencing of stool samples.

Results: Five patients were diagnosed with stage IV metastatic melanoma at the start of the treatment. The most serious adverse effects after FMT included grade I diarrhea or flatulence. All patients had a vigorous immune response to FMT measured by changes in the immune subpopulations in peripheral blood one week after FMT, including an increase in CD28+ CD8+ T cells. This was despite some patients not showing evidence of complete donor microbiota engraftment after receiving FMT. The PD1+ CD38+ CD8+ dysfunctional T cell levels also decreased in all patients post-FMT and anti-PD1 therapy. To date, 4 out of 5 patients had either a partial or complete response.

Conclusions: FMT combined with anti-PD1 therapy in patients with advanced melanoma appears to be safe. More strikingly, a measurable immune response was observed one week after FMT in all patients. This suggests that FMT has the potential of increasing efficacy of anti-PD1 treatments by increasing activation of CD8+ T cells. This trial is ongoing with 10 patients currently accrued from three different sites.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Khosravifar, Ojan

Additional Authors: Shin A, Zhang L, Asfaha S

Abstract Title: Single-Cell Analyses of Mouse Models of Colitis

Introduction: Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disease of the gastrointestinal tract. IBD is associated with an increased risk of colitis-associated colorectal cancer (CAC). Animal models of colitis provide a useful system for examining the factors that contribute to inflammation and for drug testing. Although there several commonly used mouse models of colitis, it is unclear how well, or which model best resembles IBD in patients. Recent experiments using different models of colitis has revealed the surprising finding that only the dextran sulfate sodium (DSS) model of colitis leads to CAC in mice. We identified that a subset of macrophages (F4/80+Ly6Chigh) are significantly increased in the DSS model, and that these macrophages play an important role in tumorigenesis. In this study, we aim to better characterize macrophages and various immune and epithelial cell populations using single-cell RNA sequencing (scRNA-seq). ScRNA-seq provides a powerful tool to study the transcriptional expression profiles at a single cell resolution, thereby revealing the cellular heterogeneity amongst broader cell types.

Hypothesis: scRNA-seq will reveal important differences in gene expression and identify the cellular heterogeneity amongst the immune and epithelial cell populations in different mouse models of colitis.

Methods: Colitis induction will be induced in 6-week old C57BL/6 mice using DSS, oxazolone, TNBS or Citrobacter rodentium. Mice will be sacrificed at the peak timepoint of colitis induction. Immune and epithelial cell isolation from the colon was completed in parallel. Epithelial cells and intra-epithelial immune cells were dissociated from the mesenchymal tissue and processed into a single-cell solution. Mesenchymal tissue was dissociated, and immune cells extracted using Percoll gradient density centrifugation. Cell barcoding, library preparation and scRNA-seq was performed using the 10x Genomics Chromium platform. Quality control was done using FastQC software, and cell clustering and differential expression data analysis completed using the Seurat pipeline on R.

Project Significance: Recent investigations using scRNA-seq has already been performed on patients with UC and CD. This project will enable direct comparison between the transcriptional expression profiles of mouse models of colitis to these existing human transcriptional expression profiles. This will shed light on which of these mouse models is most similar to UC and CD, thereby guiding their use in future IBD research.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Larsen, Frederikke

Additional Authors: Good H, Shin A, Zhang L, Asfaha S

Abstract Title: Hypomethylation of Dclk1+ tuft cells inhibits colitis-associated colorectal cancer

Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract. A major complication of IBD is colitis-associated cancer (CAC) that results in death in up to 10-15% of patients. We have been studying the role of tuft cells, a relatively rare quiescent epithelial cell type, that is long-lived and marked by the expression of Dclk1. In previous studies we showed that in the presence of colitis, tuft cells that have undergone deletion of the Apc gene can give rise to CAC. Interestingly, IBD and CAC both display epigenetic changes that modulate gene expression during colitis. However, the contribution of these epigenetic changes to the transformation of tuft cells is not known. DNA methylation is one form of epigenetic changes that affects gene expression, with Dnmt1, a DNA methyltransferase enzyme, being important for maintenance of DNA methylation pattern in newly synthesized DNA. DNA methylation can also be modulated pharmacologically by the drug 5-AZA-2'-deoxycytidine (5-AZA-CdR). 5-AZA-CdR is a DNA demethylating drug that inhibits DNA methylation and has previously been shown to have antitumor effects. Thus, in this study we investigated the role of DNA methylation in colitis-associated colorectal cancer by inhibiting methylation through genetic and pharmacologic means.

Hypothesis: We hypothesize that DNA hypomethylation of DCLK1+ tuft cells inhibits CAC.

Materials and methods: As previously described, we crossed our Dclk1CreERT2 mice to Apcf/f mice to generate an inducible model of colitis-associated cancer. Mice were then treated with tamoxifen to activate Cre-recombinase and delete Apc in DCLK1+ cells, and 2.5% dextran sodium sulfate (DSS) to induce colitis. Next, mice were treated with either vehicle or 5-AZA-CdR once a week for 6 weeks to examine the effect of pharmacological inhibition of DNA methylation on colitis-associated tumor formation. 16 weeks post tamoxifen induction, the colonic tumors were quantified and examined. In vitro intestinal organoids were used to test if DNA methylation affects DCLK1+ cell number and viability.

Results: Mice receiving 5-AZA-CdR had significantly reduced colonic tumor number and tumor size when compared to vehicle-treated mice. Intestinal organoids derived from Dclk1CreERT2;Apcf/f;Dnmt1f/f mice were treated with 4-hydroxytamoxifen (4-HT) to induce deletion of Apc and Dnmt1 in DCLK1+ cells. We observed no change in DCLK1+ cell number or organoid size upon Dnmt1 deletion. In separate experiments, organoids derived from Dclk1CreERT2;Apcf/f mice were treated with 4-HT followed by either vehicle or 5-AZA-CdR.

Discussion and Conclusions: Our findings demonstrate that inhibition of DNA methylation by pharmacologic or genetic means reduces colonic tumor formation. These data suggest that DNA methylation plays an important role in inflammation-associated tumorigenesis and may provide a novel strategy to the inhibition of colitis-associated cancer.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Liu, Qi

Additional Authors: Hu XT, Diao H, Joshi R, Min WP

Abstract Title: DLC1-β regulates Akt1/ROCK1-mediated cell migration by a NTR-SAM domain interaction mechanism

Deleted in liver cancer 1(DLC1) is a RhoGAP protein whose dysfunction is implicated in aberrant actin cytoskeleton processes, including cell migration, in development and cancer. Of the 4 DLC1 isoforms identified in humans and rodents, little is known about DLC1 isoform (beta/isoform1) which is highly expressed in the brain, placenta and heart. Intriguingly, it has been proven to associate with sporadic congenital heart disease (CHD).

Our study first analyses the isoform-specific N-terminal region (NTR) of DLC1β, which a hotspot for mutations and kinase targets, by bioinformatics and validation with an invitro Akt1 phosphorylation assay. Next, cell migration regulation by DLC1 derivatives, in human HEK293 fibroblasts and rat H9c2 cardiomyoblasts, under Akt1 modulation, was assayed by wound healing, protein expression and phosphorylation analyses, and by the transcript profiling of the RhoGAP downstream effector, ROCK1.Lastly, to test the hypothesis that NTR specific Akt1 phosphorylation is a putative trigger for the binding of the DLC1 SAM domain, in vitro peptide-binding assays were performed.

Our results indicate that a motif harboring S354 on the DLC1β NTR is an Akt1 target and mutations associated with CHD on this motif disrupts cell migration inhibition in HEK293 cells. Moreover, DLC1β regulated cell migration is more susceptible to insulin-mediated Akt1 modulation than DLC1α. Similarly, both DLC1α and DLC1β inhibit migration in H9c2 cells and an S354A substitution on DLC1β increases cell migration through a ROCK1-involved mechanism at the 16h timepoint. However, by the 24hr DLC1 derivative transfection causes massive cell morphological changes in the form of myotubule formation and fusion. Moreover, Akt1 modulation by insulin in DLC1-transfected H9c2 cells does not increase cell migration but triggers cell death and myotubule formation. Finally, the SAM domain was shown to bind the phosphorylated NTR S354 motif exclusively, binding that was lost upon non-phosphorylation or by a CHD-associated mutation.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Lovell, Jeff

Additional Authors: Jackson-Boeters L, Armstrong J, Hamilton D, Darling, M

Abstract Title: S100A7 as a biomarker for predicting transformation in a potentially malignant lesion: lichen planus.

Introduction: Oral potentially malignant disorders (OPMD) are changes in the oral mucosa that are clinically recognizable. Oral lichen planus (OLP) is a PMD with a malignant transformation rate of approximately 1.09%. Current diagnosis and management of lichen planus and other OPMD includes incisional biopsy, grading of dysplasia, and clinical follow up or lesion excision depending on the presence and severity of dysplasia. Current literature reports that incisional biopsy and grading of dysplasia are not reliable diagnostic or predictive tools for malignant transformation. As a result, novel and more accurate methods for predictive risk of malignant transformation in these lesions should be examined. Tissue biomarkers, such as S100A7, may provide a more accurate method of risk determination.

Hypothesis: We hypothesize that S100A7 is increased in the epithelium of Lichen Planus lesions and other OPMD. The proposed mechanism is through phosphorylation of proteins such as P38, ERK1/2, and JNK of the MAPK signalling pathway.

Objectives:
1. To show that there is greater expression of S100A7 in Lichen Planus and other OPMD than in normal epithelial control tissues.
2. To show that there is a greater expression of S100A7 in Lichen Planus and other OPMD that progress to dysplastic lesions and frank OSCC than in lesions that do not progress.
3. To evaluate the expression of P38, ERK ½, and JNK in Lichen planus lesions that progress vs. lesions that do not progress.
4. To test the utility of an image-based algorithm (Straticyte) utilizing S100A7 in Lichen Planus and other OPMD in accurately predicting progression.

Methods: Tissue samples of cases of OLP, lichenoid mucositis, oral epithelial dysplasia and normal epithelial controls will be obtained from the department of Pathology and Laboratory Medicine at Western University and University Hospital.These will be stained via immunohistochemical methods. The staining will be quantified by semi-quantitative means using an immunoreactivity score based on the proportion of epithelial cells staining, and the intensity of staining. The area of staining will also be measured using image analysis, and an algorithm applied to determine low and high risk levels in the non-cancerous tissues. Tissue samples will also be analyzed by RT-PCR to determine the presence of S100A7 mRNA. Patient samples will be anonymized and the demographic information such as gender, age, smoking status, alcohol consumption, site of lesion, and histopathological diagnosis will be acquired. Follow up data with regard to prognosis and cases that have transformed into malignancy will be obtained; and will be correlated with S100A7 and MAPK protein expression.

Results: Undetermined

Significance: Demonstrating that risk stratification is more accurate through quantification of biomarker S100A7 over traditional methods of evaluation may improve clinical management of OLP.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Lu, Haitao

Additional Authors: Not Applicable

Abstract Title: The mechanism of the mitochondrial fission protein, Dynamin related protein 1 (Drp1)-regulated mitochondrial dyefunction and cell death

Background: Cell death play a critic role in organ injury and transplant rejection. Mitochondria not only play a key role for cell metabolic activities but also regulate cell death programs. The mitochondrial fission protein, Dynamin related protein 1 (Drp1) plays a critical role in mitochondrial dyefunction. The role of Drp1 in necroptosis is not clear and remains controversial. We wanted to study if the inhibition of Drp1 could prevent necroptotic cell death. We tried to examine the expression of the phosphorylated forms of Drp1, and its upstream protein calcium/calmodulin–dependent protein kinases family (CaMK) to construct their pathways in preventing mitochondrial dysfunction and necroptosis in organ transplantion.

Methods: Endothelial cells were treated with TNF-alfa and Z-IETD to induce necroptosis. Cell death was monitored by Incucyte Cell Image System. Western Blotting was applied to detect the expression of phosphorylated-Drp1Ser616 , phosphorylated-Drp1Ser637 and CaMKII. In addition, the roles of Drp1 and CaMKII in necroptosis could be confirmed after treatemtns with specific inhibitors. siRNA was used to inhibit targted genes. Drp1 or CaMK silencing was confirmed using real time PCR and western blot.

Results: The number of dead cells was decreased significantly after inhibitions of Drp1 or CaMKII, from average 13,000 counts in necroprosis group to 3,000 and 4,000 counts respectively. Drp1 and CaMKII were successfully silenced by siRNAs. Furthermore, necroptotic cell death was significantly inhibited by the silence of CaMKII and Drp1, decreasing from 13,000 of WT group to 2,000 counts of siRNA groups. Especially, expression level of p-Drp1Ser616 showed significant decreases ($p<0.05$) in si-CaMKII and si-Drp1 groups compared to wild type group and control siRNA group.

Conclusions: Drp1 and CaMKII are potential candidates for the inhibition and treatment of mitochdrial dysfunction caused by necroptosis and as such further studies should be performed to explore their effects in organ transplantaion.

POSTER PRESENTATIONS 3
3B: CANCER BIOLOGY 2

Presenter's Name: Tan, Djarren

Additional Authors: Lin S, Bikash B, Keow S, Martinez Acevedo J, Loggie J, Cecchini M

Abstract Title: A feasibility study on quantifying tumor density using a semi-automated approach

Introduction: Lung cancer is the most common cause of cancer-related death in Canada. These cancers are staged based on their size and involvement of other structures. The overall tumor size is likely a surrogate measure for the number of cells present in the tumor, however, this is variable based on the density of the tumor. Counting individual tumor cells to generate measurements of tumor density would be essentially impossible for a pathologist using a light microscope due to the large number of cells on a slide. However, with image analysis software and computational pathology, it is possible to calculate tumor density. Developing these image analysis tools requires large datasets of annotated tumors. This relatively simple image recognition task is typically completed by pathologists, but can also be performed by non-pathologists with training. Simple image analysis tools can then be applied to the annotated images in order to quantify the tumor density.

Methods: Utilizing publicly available lung adenocarcinoma digital slides from the Cancer Genome Atlas (TCGA) database, non-pathologists were provided a brief introduction in lung histology to properly identify areas of tumor from non-tumor areas. Three lung adenocarcinoma digital pathology slides were selected and were randomly distributed. All annotations were carried out using QuPath, an open source software for digital histology and image analysis. Each slide was divided into 1260 x 1260 µm boxes. These slides were peer-reviewed weekly and rotated between reviewers bi-weekly. Once the reviewers contoured a significant portion of each slide, the annotations were reviewed by an anatomical pathologist, who edited the contours such that all tumor cells were highlighted and other cell types were excluded. Tumor surface area was compared between the original and revised contours to determine the percentage of the contour that had been revised, and the software's cell detection feature was used to quantify the number of tumor cells per box. Tumor density was modeled using a heat map to detail the variance of tumor density across each case.

Results: The annotations by non-pathologists were highly accurate and required very minimal revisions. Of the three cases contoured, 19% of the boxes required minor revisions and the contours from the group of non-pathologists differed by 0.88% compared to a trained pathologist. Common errors included contouring airways, missing small areas of tumor, and incorrect tumor margins. Each case had a different mean tumor density ranging from 1576 to 2327 cells/mm2 with a standard deviation up to 1264 cells/mm2. In general, tumor density tends to be denser in the periphery with the central space more likely to be occupied with necrosis and mucin.

Discussion: This feasibility study shows that non-pathologists can perform the image recognition task of identifying tumor areas in lung adenocarcinoma slides, and can create robust contours of these tumors with minimal training and supervision using publicly available software. We show that these measurements can be used to highlight how tumor density varies across each slide. These annotated images can then serve as the foundation to develop automated tools that can distinguish tumor cells from non-tumor cells on digital pathology slides. This may be useful in helping future pathologists more accurately prognosticate lung cancer, with the aim of providing better patient care.