UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

Drug Identification and Repurposing in Lung Adenocarcinoma: Differences in Male and Female Subjects

Oliver Dunn[1*], Ria Khatri[1*], Riddhima Singh[1*], Ray Sun[1*], Erica Wang[1*], Robert E. McCullumsmith[1,2], John M. Vergis[1], Jacob F. Wood[1*#]

[1]Department of Neurosciences and Psychiatry, University of Toledo, College of Medicine and Life Sciences, 3000 Arlington Avenue, Toledo OH 43614
[2]Promedica, 2130 W. Central Ave., Toledo, Ohio 43606 Toledo, Ohio, USA
*Authors contributed equally

Email: Jacob.Wood@rockets.utoledo.edu
Received: 11/2/2025
Accepted: 11/29/2025
Published: 12/10/2025

Abstract
Lung adenocarcinoma (LACA) presents with a range of debilitating symptoms, including chronic cough, hemoptysis, and shortness of breath. In this study, we reanalyzed a published transcriptomic dataset to compare male and female LACA samples with healthy lung tissue and identify potential therapeutic targets capable of reversing disease-associated gene expression signatures. Utilizing cutting-edge bioinformatics approaches, including differential expression analysis, pathway analyses, and analyses of signature-reversing drug profiles, we assessed male and female signatures separately. Using Gene Set Enrichment Analysis, we identified sex-specific differences in tumor-associated pathways, including the downregulation of blood angiogenesis in female lung adenocarcinoma subjects. Leading-edge gene analysis further revealed that angiogenesis-related genes were downregulated in both sexes, though the specific genes contributing to these pathways differed between males and females. Integrative drug repurposing analysis using the LINCs database, based on our transcriptomic profiles, uncovered potential therapeutic candidates for male and female LACA compared with healthy controls. Notably, a VEGFR inhibitor showed the highest discordance score in males, while CLK2 inhibitors demonstrated one of the highest discordance scores in females and males. Together, these findings highlight distinct molecular and pharmacologic signatures between sexes and suggest potential sex-specific therapeutic strategies for lung adenocarcinoma.

Keywords: Lung Adenocarcinoma, Tumors, Pathways, Discordant, MOA, VEGFR, EGFR, Drug Repurposing, Signatures, Transcriptomic Profile, DEGs, Blood Angiogenesis, Anti-angiogenesis.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

1. Introduction

Lung adenocarcinoma (LACA) is a common type of non-small cell lung cancer (NSCLC) that originates in the mucus-producing glandular cells of the lungs (1). LACA often develops in the outer parts of the lungs and is frequently diagnosed in non-smokers despite a common disease etiology being frequent exposure to carcinogens present in tobacco smoke (2). Other risk factors include exposure to environmental toxins like radon and silica, as well as having a family history of lung cancer (3). LACA is the most common subtype of lung cancer in males and females; however, there is a higher incidence in females who have never smoked compared to other lung cancer types, also with the incidence being higher for women than for males for most age groups (4, 5). For example, in the 70–74-year-old age group, the ratio of female-to-nonsmoking males was 1.38 with a 95% confidence interval from 1.3-1.5 (5).

Common treatments for LACA differ based on the staging and progression of the cancer. In early stages, specifically, from stage I to stage IIIa, surgical operations, such as lobectomies and pneumonectomies, can be utilized as treatment options, as well as various forms of radiotherapy and chemotherapy, if surgery is not possible (6). In later stages, such as stage IIIb and stage IV, which are advanced tumor states and potentially metastatic, tumors are more commonly treated through chemoradiation and targeted therapies (7). Personalized treatments depend on the somatic mutations present in the affected tissue (8, 9). EGFR and ALK mutations are typically assessed, and a positive indication for an EGFR mutation could prove a need for a targeted tyrosine kinase treatment that would be most useful to the subtype of LACA. For example, EGFR mutations may include but are not limited to the use of gefitinib and erlotinib, while a positive test for an ALK mutation may lead to an ALK inhibitor targeted treatment (10). If neither of these mutations is present, then the tumor may be treated with a platinum-based doublet and the drug bevacizumab (10). Bevacizumab is an angiogenic inhibitor that targets VEGF-A (11). An additional method that can aid in the prognosis of LACA patients is the total lung capacity (TLC), which is the amount of air that the lungs can hold after maximal inhalation, and the residual volume (RV) ratio, which is the amount of air remaining in the lungs after maximal exhalation. According to a study that utilized the RV/TLC ratio with 2348 NSLC patients, subjects with a higher RV% and a lower TLC% had a higher mortality risk regardless of diagnosed cancer stage (12).

Previously, other LACA RNA-seq datasets showed 3 major gene hubs that have higher levels of expression in LACA than normal lung tissue, including cellular retinoic acid binding protein 2 (CRABP2), matrix metallopeptidase 12 (MMP12), and DNA topoisomerase II alpha (TOP2A) (13). High levels of expression for these 3 genes (CRABP2, MMP12, and TOP2A) are associated with a poorer LACA prognosis (13). A previous study showed that the top five pathways with the highest association with LACA prognosis are as follows: Purine metabolism, MAPK signaling pathway, Cytokine-cytokine receptor interaction, Ubiquitin-mediated proteolysis, and Lysosome (14). Although general pathways and genes that are prevalent regarding LACA were reported, specific details regarding the differences between males and females are rarely examined in the literature. Thus, we sought to identify changes in LACA using a cutting-edge bioinformatics toolkit, which includes DEG analysis, pathway analysis, targeted pathway analysis, and drug repurposing candidate identification (Figure 1).

2. Methods

Data analysis Summary:

We re-analyzed a previously published GEO dataset (GSE229705) examining LACA, using an integrated R package, 3PodR for a robust and cutting-edge transcriptomic analysis (15). RNA-seq count data were obtained from GSE229705 and included 246 LACA and matched healthy control tissue (male and female) samples (16). Our analyses included differential gene expression, pathway enrichment using Gene Set Enrichment Analysis (GSEA), EnrichR, and the Library of Integrated Network-based Cellular Signatures (iLINCS), compiled as the integrated 3PodR code package (15).

Differential Expression Analysis:

Using a limma-voom R script with count normalization for differential gene expression analysis, we compared male LACA samples vs matched healthy control tissue (N=35 patients) with female LACA samples to matched healthy control tissue (N=88 patients)(17, 18). Genes with adjusted p-values ≤ 0.05 (Benjamini-Hochberg

correction) were considered significant. Volcano plots were generated, which illustrated log2 fold change vs. adjusted p-value, with thresholds marked for significance (padj ≤ 0.05, log2FC ≥ 1)(19). A heatmap was also generated showing the top 50 shared genes between males and females, and their corresponding log2FC expression values (19).

GSEA Pathway/Leading Edge Gene Analysis:
Biological pathways were analyzed using GSEA; GSEA pathways were sorted by Normalized Enrichment Scores (NES), with positive scoring being "upregulated" and negative NES scores being "downregulated." To identify leading-edge (LE) genes, or genes most contributing to pathway enrichment, we used the GSEA leading-edge gene output. Shared leading-edge genes across multiple pathways were prioritized and summarized. Pathways Analysis Visualization with Embedding Representations (PAVER) was used to cluster identified pathways, with results shown as a PAVER heatmap (20).

EnrichR Pathway Analysis:
GSEA identified global pathway enrichment across ranked gene lists, while EnrichR was used to analyze the top 10% of up- and down-regulated genes, identifying enriched pathways through over-representation analysis (21).

Perturbagen Analysis (iLINCS):
We queried the iLINCS database to identify drugs with transcriptomic signatures either concordant (i.e., "simulate") or discordant (i.e., "reverse") with LACA mRNA expression in male LACA vs. healthy control tissue and female LACA vs. healthy control tissue (22). This provided candidate molecules that may mimic or reverse LACA transcriptomic profiles. These candidate molecules were then sorted via their listed mechanism of action (MOA), and outputs are displayed as bar charts. All potential drug repurposing candidates were sorted with an iLINCs threshold of <-0.3 and >0.3.

3. Results

Differential expression analysis identified 12,041 differentially expressed genes in female LACA vs healthy control samples, with 6,043 being downregulated and 5,998 being upregulated (padj ≤ 0.05, log2FC ≥ 1)(Figure 2B). In male LACA vs healthy control tissue, 9,749 differentially expressed genes were identified, with 5,188 downregulated and 4,561 upregulated (padj ≤ 0.05, log2FC ≥ 1)(Figure 2C). Among the most common upregulated transcripts that were shared between male and female LACA vs. healthy control, SPINK1, CXCL13, and HS6ST2 each increased more than 60-fold. In contrast, SERTM1, ITLN2, and SLC6A4 were downregulated by approximately 16-fold in male and female LACA vs healthy controls.

Sex-stratified analyses revealed unique gene expression profiles. In female LACA vs healthy control, 1,978 genes were uniquely upregulated, and 1,956 were uniquely downregulated in female samples (Figure 2D). In male LACA vs. healthy control, 540 genes were uniquely upregulated, and 1,103 were uniquely downregulated (Figure 2D). Across sexes, 4,023 upregulated and 4,087 downregulated genes were shared between male and female LACA vs healthy control (Figure 2D).

We used Gene Set Enrichment Analysis (GSEA) to perform pathway-level analysis on the LACA transcriptomes of male LACA vs. male healthy control and female LACA vs. female healthy control samples. The GSEA pathway normalized enrichment score (NES) ranged from -2.2 to +3.86, reflecting the strength and direction of pathway regulation (Figure 3). Blood vessel morphogenesis was the most downregulated pathway in female tumors (NES ≈ -2.2), suggesting reduced angiogenic activity and signaling. In contrast, male tumors showed more variable responses within this pathway, with upregulation of blood vessel morphogenesis pathways observed. Nucleotide-sugar metabolic processes, which are essential for biosynthesis and cell growth, were upregulated in the LACA vs. healthy control comparison for both sexes, reaching an NES as high as +3.86 in male samples. Ribosome-related pathways, necessary for protein production, exhibited complex (both up- and down-regulated) pathway regulation in both the male LACA vs. healthy control and the female LACA vs. healthy control comparison. Finally, sister chromatid segregation, a pathway involved in cell division, was broadly upregulated in male LACA vs. healthy control and female LACA vs. healthy control, with log2 fold-change values of +1.5 and +2.5.

PAVER analysis highlighted sex-specific angiogenic differences. In female LACA vs. healthy control, blood vessel morphogenesis (NES = -1.9), endothelial cell differentiation (NES = -1.9), and regulation of sprouting angiogenesis (NES = -1.9) were all significantly decreased, while these pathways were not significantly enriched in male LACA vs. healthy control (Figure 3).

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

iLINCS connectivity analysis identified compounds with discordant signatures relative to the LACA transcriptomes (FDR ≤ 0.05). In female LACA vs healthy control tissue, the most discordant perturbagen was SCHEMBL3954849, a protein kinase inhibitor (-0.39)(Table I). Epothilone A, a microtubule-stabilizing agent, was in the top ten (-0.36)(Table I). WZ3105 also ranked among the top perturbagens in females, with a discordance score of -0.39. In male LACA vs. healthy control, 3,096 discordant signatures were identified, while 1,722 were detected in females. The top perturbagen in males was WZ3105, a non-FDA-approved CLK2 inhibitor (discordance score = -0.44). Gefitinib, an FDA-approved EGFR inhibitor, was also among the most discordant (-0.42)(Table II).

Comparison of mechanisms of action (MOA) of perturbagens with an iLINCs threshold of <-0.3 and >0.3 revealed overlap across sexes. Dopamine receptor antagonists, VEGFR inhibitors, PDGFR PTK inhibitors, and CDK inhibitors were among the top discordant MOAs in male and female subjects (Figure 4A-B). VEGFR inhibition was represented by 129 signatures in males and 92 in females. Certain MOAs were sex-specific: HDAC inhibition ranked highly (fifth) in male LACA vs. healthy control but was absent in female LACA vs. healthy control, while NF-κB pathway inhibition was observed in female LACA vs. healthy control but not male LACA vs. healthy control. The most discordant MOA was dopamine receptor antagonism in males and CDK inhibition in females. In males, VEGFR-pathway inhibitors appeared among the most discordant perturbagens, indicating a strong predicted reversal of the male cancer signature. In females, although VEGFR signaling was more broadly downregulated at the pathway level, VEGFR inhibitors did not rank among the top discordant perturbagens.

4. Discussion

Our study reveals that while male and female LACA tumors appear similar when considering only the top differentially expressed genes vs. healthy control samples, deeper pathway-based analyses uncovered notable biological differences. Thousands of genes were commonly up- or downregulated across both sexes, suggesting a shared transcriptional foundation. However, Gene Set Enrichment Analysis (GSEA) and pathway-focused visualizations using PAVER uncovered sex-specific patterns in key biological processes, including blood vessel morphogenesis pathways. Female LACA vs. healthy control showed a consistent suppression of angiogenic pathways, while male tumors demonstrated a more variable and sometimes activated profile, suggesting different tumor microenvironments and potentially distinct mechanisms of vascular regulation. These findings suggest that LACA should not be treated as a uniform disease, with sex being a meaningful biological variable influencing tumor behavior and therapeutic response. By looking beyond typical “greatest hits” analysis and into pathway regulation and perturbagen responses, this study highlights the potential of precision oncology approaches tailored by sex-specific biology.

Similarities of DEGs and Number of Discordant MOA Annotations in Both Sexes

We identified the top DEGs and their signatures in pathways in heatmaps and volcano plots through GSEA pathway analysis in male and female samples (Figure 2A and Figure 2B). We found notable similarities between male and female DEG signatures, such as upregulation of SPINK1 and downregulation of ITLN2 (Figure 2A, 5). Interestingly, a study that compared LACA tissues to normal, histological lung tissue in male and female patients identified that upregulation of SPINK1 was more positively associated in males than females, indicating significant cell proliferation and tumor progression in male subjects (23). Given that SPINK1 was the top upregulated DEG, this is an indication that while there are similarities in our GSEA analysis of tumor tissue and healthy control tissue samples in male and female signatures for this gene, there is a stronger association of SPINK1 gene expression levels in male LACA subjects than female LACA subjects (Figure 2A). Also, according to a study that analyzed lung cancer transcriptomic signatures, the ITLN2 signature was significantly downregulated compared to normal tissue, consistent with our findings (24).

In addition, we identified the drug transcriptomic profile of discordant signatures in MOAs through iLINCS analysis, and we analyzed the top 10 discordant MOAs in each sex (Figure 4). In male and female LACA samples, VEGFR inhibitors had the most discordant signatures across both sexes (Figure 4A and Figure 4B). Interestingly, according to a previous study, females with non-small cell lung cancer had a higher overall survival (OS) rate than males due to VEGF blockade by bevacizumab, an angiogenic inhibitor in anti-angiogenic therapies (11, 25). This

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

implication highlights that while a high number of discordant signatures in MOAs in male and female LACA transcriptional profiles are similar, there may be gender differences in survival rates depending on the efficacy of the drugs in the respective sex.

Differences in Drug Transcriptomic Profiles

We looked at the top 10 discordant perturbagens associated with LACA in males and females, as well as their respective MOAs. Both males and females had high discordance scores for the CLK2 inhibitor MOA and for the perturbagen WZ3105 (Tables I and II). However, except for WZ3105, the top 10 discordant drugs for males and females differed significantly (Tables I and II). Consistent with our findings, a study investigating the influence of sex on gene regulatory networks in lung adenocarcinoma (LACA) identified sex-specific differences in response to targeted therapies. Specifically, trametinib, vorinostat, and dactinomycin were effective only in female LACA, whereas panobinostat demonstrated efficacy exclusively in male LACA (26). Of the four drugs identified (trametinib, vorinostat, dactinomycin, and panobinostat), three showed consistency with our findings and identification of these perturbagens (26). Currently, trametinib, vorinostat, and dactinomycin are all FDA-approved drugs. These results, as well as our own, suggest sex-specific drug repurposing candidates based on transcriptomic profiles associated with LACA. Interestingly, a study focusing on pregnancy-specific glycoproteins (PSGs) found an association between poor survival in female LACA patients and DEG signatures in these PSG-related genes, with little to no effect in males (27). Through GSEA pathway analysis, the study identified that the DEG levels of PSG3, PSG7, and PSG8 were linked to poor prognosis in females and possible downregulation of the KRAS signaling pathway (27). Interestingly, the study identifies that combinations of PSG genes could regulate tumor microenvironments of LACA, and PSGs can serve as potential therapeutic targets for LACA in female subjects (27). This finding aligns with our results, which indicate that possible perturbagens that reverse the transcriptomic profile of LACA can differ greatly in males and females. When looking at the drug transcriptomic profiles, a targeted treatment for one sex may have a very discordant signature, while one for the other sex can be almost unaffected.

Blood Angiogenesis and Its Relation to the Drug Transcriptomic Profiles

One pathway of interest we identified was blood vessel morphogenesis. Angiogenesis or blood vessel morphogenesis is the development of new blood vessels, which are composed of endothelial tissues, from pre-existing blood vasculatures through processes such as sprouting angiogenesis (28, 29). During sprouting angiogenesis, the stimulation of an angiogenetic factor called vascular endothelial growth factor (VEGF) causes activation of surrounding endothelial cells in pre-existing blood vasculatures to migrate to the surrounding tissue and proliferate to form solid sprouts (30). The proliferated cells are then reorganized to create a hollow space or a lumen to allow blood flow in newly formed capillaries (30, 31). However, tumors can disrupt nutrient flow in the bloodstream by secreting VEGF, and endothelial cells of an existing blood vessel respond to this chemical signaling by promoting the development of additional blood vessels (32). After sprouting occurs, tumor progression in cancerous tissue, such as epithelial tissue in LACA, occurs by growing beyond its initial size (33).
In addition to the disruption of nutrient flow in the blood, the tumor mass can become metastatic, or the spread of cancer to sites outside of the original, affected tissue, by shedding its cancer cells in the bloodstream (34). After one is diagnosed with LACA, doctors use the same diagnostics to determine the cancer stage by looking at tumor size, the location of the tumor mass, such as lymph nodes and organs, and the number of locations that have been affected by LACA (35). If LACA is in stages 2A-2B, stages 3A-3C, or stages 4A-4B, where 2B, 3C, and 4B are the most detrimental due to the tumor size and the number of locations it has affected, LACA is metastatic and can be addressed accordingly with effective treatments depending on the stage (35, 36).

To examine blood vessel morphogenesis as a potential therapeutic target for LACA, we performed two separate analyses with tumor-adjacent tissue and normal lung tissue in male and female subjects: GSEA and PAVER. We observed similarities in clusters of tumor-associated pathways, such as upregulation of nucleotide metabolic process (NMP) in females and males (Figure 3). In contrast, we found notable differences between male and female signatures for the blood angiogenesis cluster, with this pathway being more downregulated in females versus male subjects (Figure 3). To understand the

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

different mechanisms that are associated with significant tumor suppression in blood angiogenesis for female LACA samples, we found evidence of genes and pathways related to angiogenesis through LE analysis. Within this cluster, the leading-edge genes with the most downregulated annotations in females were NOTCH1, EDN1, and VEGFA, and the leading-edge genes with the most downregulated signatures in males were KCNQ1, KCNA5, and KCNJ8. One clinical study on primary breast cancer analyzed transcriptomic profiles of specific cell signatures in tumor-affected samples, non-cancerous samples, and normal breast tissue samples in endothelial and lymph-endothelial cell markers for females. Interestingly, reduced expression levels of endothelial cell markers and significant suppression of angiogenetic factors were reported in tumor samples compared to other sample types for females, which is consistent with our findings for LACA in female subjects (37). The study also discussed different therapeutic techniques of maintaining the balance of angiogenic microenvironments in female patients diagnosed with primary breast cancer by identifying different proteins that can promote angiogenic processes, such as VEGF-A-E receptors, svVEGF, and placental growth factor (PGF)(37).

These findings suggest that the VEGFR family and other related receptors are potential therapeutic targets for angiogenesis in female LACA samples, though they are downregulated. However, we also found evidence of a different drug class that reverses the male LACA transcriptional profile, including inhibitors of angiogenesis such as VEGFR and EGFR. Also, a CLK2 inhibitor had the most discordant signature in male and female subjects (Figure 5)(Table I and Table II). However, one study reported that combining anti-angiogenic therapies, such as VEGF receptor and epidermal growth factor receptor (EGFR) inhibitors, can effectively reduce blood vessel morphogenesis in solid tumors of non-small cell lung cancer. Notably, the study did not address potential sex-based differences in treatment response (38). Given that VEGFR and EGFR inhibitors ranked among the top ten discordant drugs in male LACA compared with male healthy controls and considering that gefitinib is an FDA-approved EGFR inhibitor, these findings suggest that angiogenic inhibitors potentially reverse the transcriptomic profile of LACA in males. However, there is limited evidence supporting angiogenic inhibitors as therapeutic targets for anti-angiogenesis in females (Table II).

Acknowledging that females are more frequently diagnosed with LACA than males is crucial to examining sex specific results. A study that analyzed patients of all subgroups who were diagnosed with NSCLC identified that females have a higher prevalence of EGFR mutations than males, suggesting a more prominent role for angiogenic signaling in females (39, 40). Further, according to a study that analyzed the epidemiology of women with lung cancer, women had an odds ratio of 1.9 in prevalence compared to men with similar smoking histories (41). Another factor that contributes to women being more susceptible to LACA is the estrogen level status in women: pre-menopausal women have estrogen levels ranging from 30-400 picograms per milliliter (pg/mL), but post-menopausal women have estrogen levels ranging from 0-30 pg/mL (42, 43). Young pre-menopausal women in an experimental group had more aggressive LACA tumors at diagnosis compared to men and post-menopausal women, suggesting a potent impact of estrogen on tumor progression in women (43). These studies underscore the importance of incorporating sex-based stratification when examining LACA, as biological differences between male and female subjects may influence tumor behavior, molecular signatures, and treatment responses.

5. Limitations

Several limitations should be acknowledged in this study. First, mRNA expression does not necessarily correlate with protein abundance or activity; therefore, the transcriptomic changes observed cannot be directly interpreted as functional or phenotypic outcomes (44). Second, the iLINCs database includes cell lines with varying and often undocumented treatment histories, meaning that prior drug exposures may influence their current transcriptional responses. A key limitation of using L1000 data for drug repurposing is that the transcriptional signatures are derived from immortalized cell lines and may not fully recapitulate the biology of primary human tissues, particularly in specialized cell types such as brain endothelial cells. Finally, cancer staging information was not available in this analysis, and the transcriptomic and pharmacologic profiles of lung adenocarcinoma (LACA) may differ substantially between early and late disease stages.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

6. Conclusion

We found similarities in male and female LACA tissue at the DEG level, but sex-specific differences when looking at the different pathways and drug transcriptomic profiles, suggesting that LACA is not a uniform disease. Through GSEA analysis, similarities in DEG signatures were observed in both sexes; differences in male vs. female LACA were discovered using GSEA, PAVER, and iLINCS analysis (Figure 2A,3,4). Thus, sex differences in LACA with respect to biological pathways and drug repurposing candidates may have significant implications for sex-specific targeted angiogenic therapies. Further clinical trials considering male and female subjects separately should also be conducted to see whether tumor progression has a relationship to male and female signatures in LACA-associated pathways; this could provide insight into why tumor suppression of blood angiogenesis occurred in females compared to males.

References

1. Ge, L., L. Wang, and D. Pei. *Pulmonary mucinous adenocarcinoma: An overview of pathophysiology and advancements in treatment.* Heliyon, 2024. **10**(9): p. e28881.
2. Myers, D.J. and J.M. Wallen, *Lung Adenocarcinoma*, in *StatPearls*. 2025, StatPearls Publishing Copyright © 2025, StatPearls Publishing LLC.: Treasure Island (FL).
3. Urrutia-Pereira, M., J.M. Chatkin, H.J. Chong-Neto, and D. Solé. *Radon exposure: a major cause of lung cancer in nonsmokers.* J Bras Pneumol, 2023. **49**(6): p. e20230210.
4. May, L., K. Shows, P. Nana-Sinkam, H. Li, and J.W. Landry. *Sex Differences in Lung Cancer.* Cancers (Basel), 2023. **15**(12).
5. Chien, L.H., H.F. Jiang, F.Y. Tsai, H.Y. Chang, N.D. Freedman, N. Rothman, Q. Lan, C.A. Hsiung, and I.S. Chang. *Incidence of Lung Adenocarcinoma by Age, Sex, and Smoking Status in Taiwan.* JAMA Netw Open, 2023. **6**(11): p. e2340704.
6. Indini, A., E. Rijavec, C. Bareggi, and F. Grossi. *Novel treatment strategies for early-stage lung cancer: the oncologist's perspective.* J Thorac Dis, 2020. **12**(6): p. 3390-3398.
7. Petrella, F., S. Rizzo, I. Attili, A. Passaro, T. Zilli, F. Martucci, L. Bonomo, F. Del Grande, M. Casiraghi, F. De Marinis, and L. Spaggiari. *Stage III Non-Small-Cell Lung Cancer: An Overview of Treatment Options.* Curr Oncol, 2023. **30**(3): p. 3160-3175.
8. Ramos, R., C.S. Moura, M. Costa, N.J. Lamas, R. Correia, D. Garcez, J.M. Pereira, T. Lindahl, C. Sousa, and N. Vale. *Lung Cancer Therapy: The Role of Personalized Medicine.* Cancers (Basel), 2025. **17**(5).
9. Hensing, T., A. Chawla, R. Batra, and R. Salgia. *A personalized treatment for lung cancer: molecular pathways, targeted therapies, and genomic characterization.* Adv Exp Med Biol, 2014. **799**: p. 85-117.
10. de Castro, J., P. Tagliaferri, V.C.C. de Lima, S. Ng, M. Thomas, A. Arunachalam, X. Cao, S. Kothari, T. Burke, H. Myeong, A. Grattan, and D.H. Lee. *Systemic therapy treatment patterns in patients with advanced non-small cell lung cancer (NSCLC): PIvOTAL study.* Eur J Cancer Care (Engl), 2017. **26**(6).
11. Brahmer, J.R., S.E. Dahlberg, R.J. Gray, J.H. Schiller, M.C. Perry, A. Sandler, and D.H. Johnson. *Sex differences in outcome with bevacizumab therapy: analysis of patients with advanced-stage non-small cell lung cancer treated with or without bevacizumab in combination with paclitaxel and carboplatin in the Eastern Cooperative Oncology Group Trial 4599.* J Thorac Oncol, 2011. **6**(1): p. 103-8.
12. Zhai, T., Y. Li, R. Brown, M. Lanuti, J.F. Gainor, and D.C. Christiani. *Residual Volume and Total Lung Capacity at Diagnosis Predict Overall Survival in Non-Small Cell Lung Cancer Patients.* Cancer Med, 2025. **14**(10): p. e70962.
13. Zhang, Y., Q. Chen, T. Huang, D. Zhu, and Y. Lu. *Bioinformatics-based screening of key genes for transformation of tyrosine kinase inhibitor-resistant lung adenocarcinoma to small cell lung cancer.* Frontiers in Medicine, 2023. Volume 10
14. Zhang, D., Q. Jiang, X. Ge, Y. Shi, T. Ye, Y. Mi, T. Xie, Q. Li, and Q. Ye. *RHOV promotes lung adenocarcinoma cell growth and metastasis through JNK/c-Jun pathway.* International Journal of Biological Sciences, 2021. **17**(10): p. 2622-2632.

15. Ryan, W. *3PodR Bookdown*. Accessed From: [cited last updated on 2025-06-04]; Available from: https://github.com/willgryan/3PodR_bookdown.git.
16. Dolgalev, I., H. Zhou, N. Murrell, H. Le, T. Sakellaropoulos, N. Coudray, K. Zhu, V. Vasudevaraja, A. Yeaton, C. Goparaju, Y. Li, I. Sulaiman, J.J. Tsay, P. Meyn, H. Mohamed, I. Sydney, T. Shiomi, S. Ramaswami, N. Narula, R. Kulicke, F.P. Davis, N. Stransky, G.A. Smolen, W.Y. Cheng, J. Cai, S. Punekar, V. Velcheti, D.H. Sterman, J.T. Poirier, B. Neel, K.K. Wong, L. Chiriboga, A. Heguy, T. Papagiannakopoulos, B. Nadorp, M. Snuderl, L.N. Segal, A.L. Moreira, H.I. Pass, and A. Tsirigos. *Inflammation in the tumor-adjacent lung as a predictor of clinical outcome in lung adenocarcinoma.* Nat Commun, 2023. **14**(1): p. 6764.
17. Law, C.W., Y. Chen, W. Shi, and G.K. Smyth. *voom: precision weights unlock linear model analysis tools for RNA-seq read counts.* Genome Biology, 2014. **15**(2): p. R29.
18. Ritchie, M.E., B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, and G.K. Smyth. *limma powers differential expression analyses for RNA-sequencing and microarray studies.* Nucleic Acids Res, 2015. **43**(7): p. e47.
19. Kolde, R. *pheatmap: Pretty Heatmaps.* 2018. Accessed From: [cited last updated on 2025-06-04]; Available from: https://github.com/raivokolde/pheatmap.git
20. Ryan, V.W., A.S. Imami, H. Ali Sajid, J. Vergis, X. Zhang, J. Meller, R. Shukla, and R. McCullumsmith. *Interpreting and visualizing pathway analyses using embedding representations with PAVER.* Bioinformation, 2024. **20**(7): p. 700-704.
21. Kuleshov, M.V., M.R. Jones, A.D. Rouillard, N.F. Fernandez, Q. Duan, Z. Wang, S. Koplev, S.L. Jenkins, K.M. Jagodnik, A. Lachmann, M.G. McDermott, C.D. Monteiro, G.W. Gundersen, and A. Ma'ayan. *Enrichr: a comprehensive gene set enrichment analysis web server 2016 update.* Nucleic Acids Res, 2016. **44**(W1): p. W90-7.
22. Pilarczyk, M., M. Fazel-Najafabadi, M. Kouril, B. Shamsaei, J. Vasiliauskas, W. Niu, N. Mahi, L. Zhang, N.A. Clark, Y. Ren, S. White, R. Karim, H. Xu, J. Biesiada, M.F. Bennett, S.E. Davidson, J.F. Reichard, K. Roberts, V. Stathias, A. Koleti, D. Vidovic, D.J.B. Clarke, S.C. Schurer, A. Ma'ayan, J. Meller, and M. Medvedovic. *Connecting omics signatures and revealing biological mechanisms with iLINCS.* Nat Commun, 2022. **13**(1): p. 4678.
23. Xu, L., C. Lu, Y. Huang, J. Zhou, X. Wang, C. Liu, J. Chen, and H. Le. *SPINK1 promotes cell growth and metastasis of lung adenocarcinoma and acts as a novel prognostic biomarker.* BMB Rep, 2018. **51**(12): p. 648-653.
24. Parida, S., S. Siddharth, and D. Sharma. *Role of Omentin in Obesity Paradox in Lung Cancer.* Cancers (Basel), 2021. **13**(2).
25. Baiu, I., A.L. Titan, L.W. Martin, A. Wolf, and L. Backhus. *The role of gender in non-small cell lung cancer: a narrative review.* J Thorac Dis, 2021. **13**(6): p. 3816-3826.
26. Saha, E., M.B. Guebila, V. Fanfani, J. Fischer, K.H. Shutta, P. Mandros, D.L. DeMeo, J. Quackenbush, and C.M. Lopes-Ramos. *Gene regulatory Networks Reveal Sex Difference in Lung Adenocarcinoma.* bioRxiv, 2023.
27. Oh, J.H., G. Rizzuto, R. Elkin, C. Weistuch, L. Norton, G. Dveksler, and J.O. Deasy. *Pregnancy-specific glycoproteins as potential drug targets for female lung adenocarcinoma patients.* Brief Funct Genomics, 2025. Volume 24.
28. Ngaha, T.Y.S., A.V. Zhilenkova, F.E. Essogmo, I.K. Uchendu, M.O. Abah, L.T. Fossa, Z.D. Sangadzhieva, D.S. V, S.R. A, N.P. Y, A. Boroda, A. Rozhkov, J.D. Kemfang Ngowa, N.B. L, and I.S. M. *Angiogenesis in Lung Cancer: Understanding the Roles of Growth Factors.* Cancers (Basel), 2023. **15**(18).
29. Birau, A., R.A. Ceausu, A.M. Cimpean, P. Gaje, M. Raica, and T. Olariu. *Assessement of angiogenesis reveals blood vessel heterogeneity in lung carcinoma.* Oncol Lett, 2012. **4**(6): p. 1183-1186.
30. Gerhardt, H. *VEGF and endothelial guidance in angiogenic sprouting.* Organogenesis, 2008. **4**(4): p. 241-6.
31. Zhang, R., Y. Yao, H. Gao, and X. Hu. *Mechanisms of angiogenesis in tumour.* Frontiers in Oncology, 2024. Volume 14
32. Prager, G.W., M. Poettler, M. Unseld, and C.C. Zielinski. *Angiogenesis in cancer: anti-VEGF escape mechanisms.* Translational

Lung Cancer Research, 2011. **1**(1): p. 14-25.
33. Daly, S., J.C. Kubasiak, D. Rinewalt, R. Pithadia, S. Basu, C. Fhied, G.C. Lobato, C.W. Seder, E. Hong, W.H. Warren, G. Chmielewski, M.J. Liptay, P. Bonomi, and J.A. Borgia. *Circulating angiogenesis biomarkers are associated with disease progression in lung adenocarcinoma.* Ann Thorac Surg, 2014. **98**(6): p. 1968-75; discussion 1975.
34. Popper, H.H. *Progression and metastasis of lung cancer.* Cancer Metastasis Rev, 2016. **35**(1): p. 75-91.
35. Zhu, T., X. Bao, M. Chen, R. Lin, J. Zhuyan, T. Zhen, K. Xing, W. Zhou, and S. Zhu. *Mechanisms and Future of Non-Small Cell Lung Cancer Metastasis.* Front Oncol, 2020. **10**: p. 585284.
36. Duda, D.G., A.M.M.J. Duyverman, M. Kohno, M. Snuderl, E.J.A. Steller, D. Fukumura, and R.K. Jain. *Malignant cells facilitate lung metastasis by bringing their own soil.* Proceedings of the National Academy of Sciences, 2010. **107**(50): p. 21677-21682.
37. Boneberg, E.M., D.F. Legler, M.M. Hoefer, C. Ohlschlegel, H. Steininger, L. Füzesi, G.M. Beer, V. Dupont-Lampert, F. Otto, H.J. Senn, and G. Fürstenberger. *Angiogenesis and lymphangiogenesis are downregulated in primary breast cancer.* Br J Cancer, 2009. **101**(4): p. 605-14.
38. Tian, W., C. Cao, L. Shu, and F. Wu. *Anti-Angiogenic Therapy in the Treatment of Non-Small Cell Lung Cancer.* Onco Targets Ther, 2020. **13**: p. 12113-12129.
39. Hsu, R., D. Chen, B. Xia, R. Feldman, W. Cozen, L.E. Raez, H. Borghaei, C. Kim, M. Nagasaka, H. Mamdani, A.M. Vanderwalde, G. Lopes, M.A. Socinski, A.J. Wozniak, A.I. Spira, S.V. Liu, and J.J. Nieva. *Impact of gender and mutational differences in hormone receptor expressing non-small cell lung cancer.* Front Oncol, 2023. **13**: p. 1215524.
40. Huang, Y., H.J. Cho, B.E. Stranger, and R.S. Huang. *Sex dimorphism in response to targeted therapy and immunotherapy in non-small cell lung cancer patients: a narrative review.* Translational Lung Cancer Research, 2022. **11**(5): p. 920-934.
41. Anwar, M.S. *Lung cancer is more common but less often fatal in women.* Thorax, 2006. **61**(10): p. 885.
42. Chakraborty, S., A.K. Ganti, A. Marr, and S.K. Batra. *Lung cancer in women: role of estrogens.* Expert Rev Respir Med, 2010. **4**(4): p. 509-18.
43. Rodriguez-Lara, V. and M.R. Avila-Costa. *An Overview of Lung Cancer in Women and the Impact of Estrogen in Lung Carcinogenesis and Lung Cancer Treatment.* Frontiers in Medicine, 2021. **Volume 8 - 2021**.
44. Fukao, Y. *Discordance between protein and transcript levels detected by selected reaction monitoring.* Plant Signal Behav, 2015. **10**(5): p. e1017697.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

Figure Legends

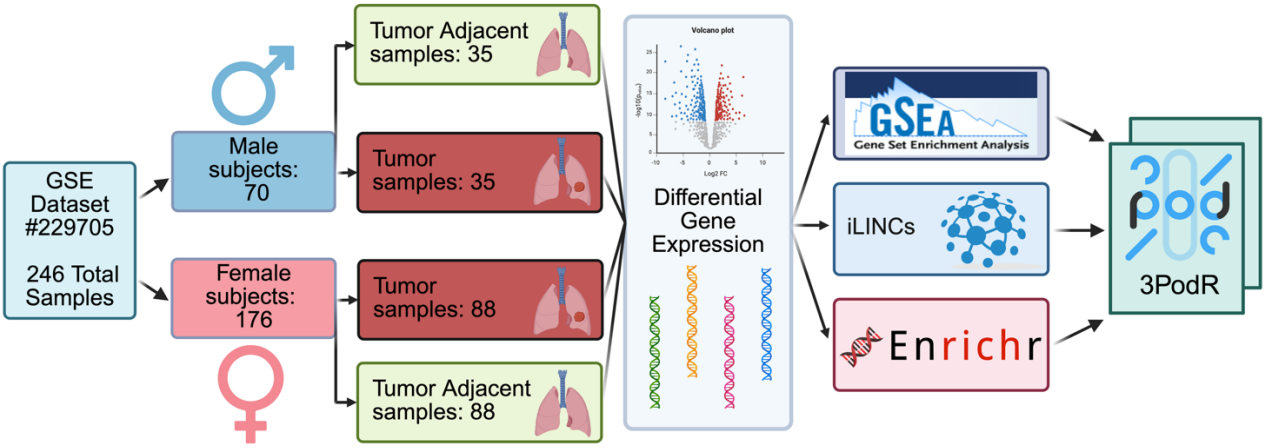

Figure 1: Overview figure. We re-analyzed GSE229705, an RNAseq dataset containing stage 1 LACA vs. healthy control samples, with a total of 246 subjects. We separated subjects based on sex, further dividing them into whether the sample was a tumor or a tumor-adjacent sample. This resulted in 4 subdivided groups: male LACA, male healthy control, female LACA, and female healthy control. Next, all four sample groups were analyzed using limma-voom for differential gene expression (DGE), demonstrating how the GEO data were reanalyzed to produce transcriptomic data. Finally, we used different analysis methods in the integrated 3PodR code package: GSEA, Enrichr, and iLINCS.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

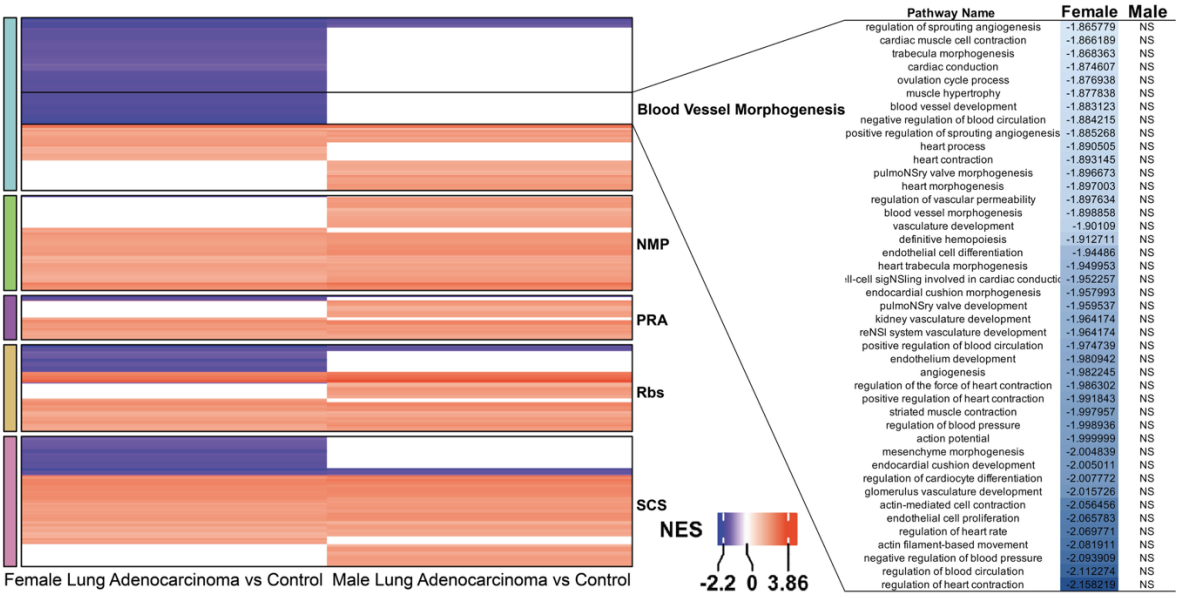

Figure 2: (A) The heat map illustrates the top 50 shared genes between males and females, with the two columns representing expression patterns in each sex. Gene names are listed along the right side, and each is associated with a specific color reflecting its expression level. Notably, many genes appear to exhibit similar regulation in both sexes. Gene expression is shown by Log2Fold Change, with red indicating upregulation and blue indicating downregulation. Volcano plots, male (B) and female (C), representing differential gene expression in LACA tissue versus adjacent healthy control tissue. (D) Venn diagram showing counts of unique and shared differentially expressed genes across male and female analysis (padj ≤ 0.05, log2FC ≥ 1).

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

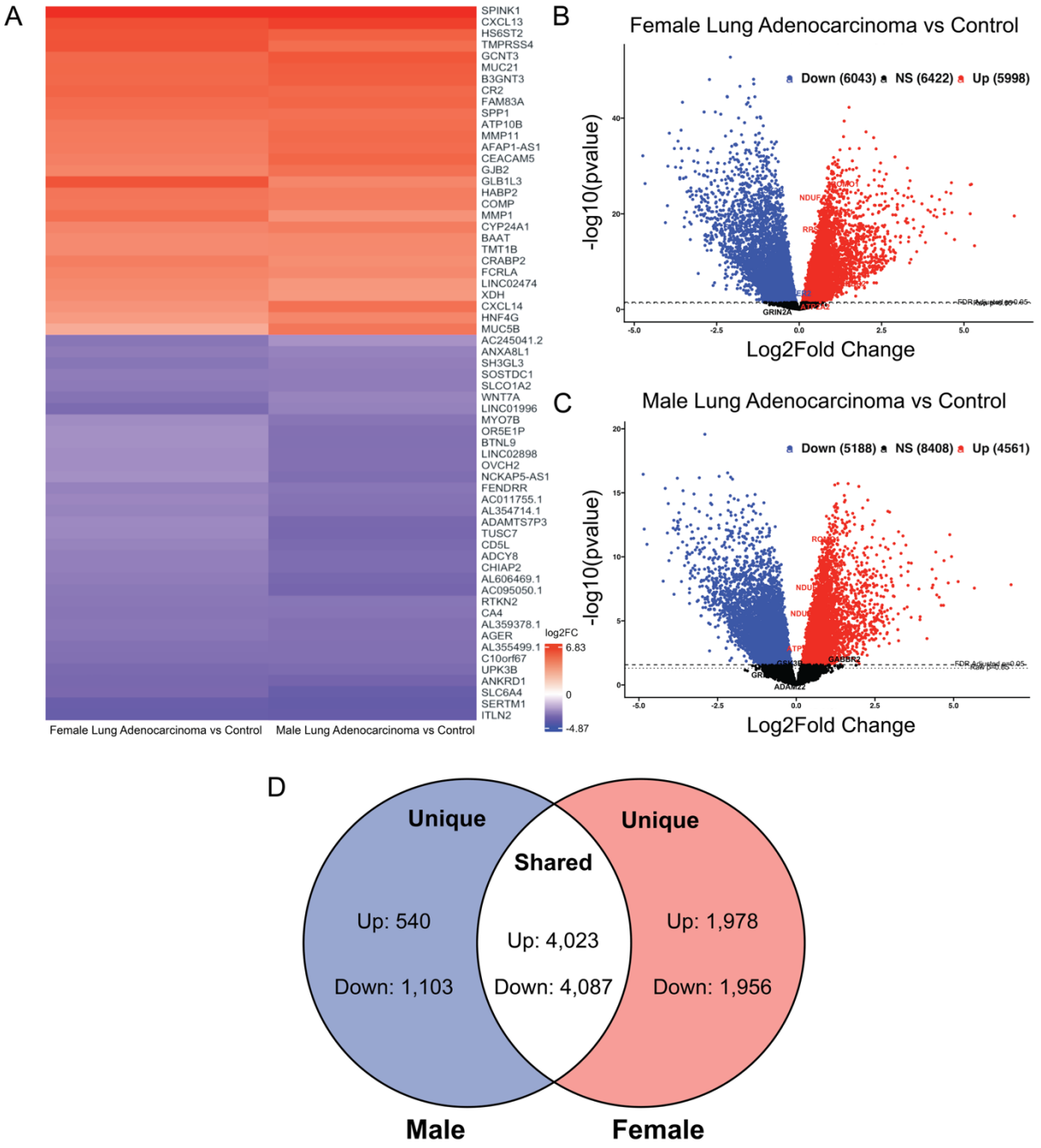

Figure 3: The GSEA PAVER Plot shows male and female samples represented in two separate columns as a heatmap. The heatmap consists of pathways identified through Gene Set Enrichment Analysis (GSEA), clustering those pathways into related groups as described in the methods. Pathways that are upregulated are shown in red, and pathways that are downregulated are shown in blue, based on PAVER scoring. Magnification of specific male and female pathways is shown to the right. Abbreviations: NMP: Nucleotide Metabolic Process, PRA: Peptidase Regulator Activity, Rbs: Ribosomes, SCS: Sister Chromatid Segregation, PAVER: Pathway Analysis Visualization with Embedding Representations.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

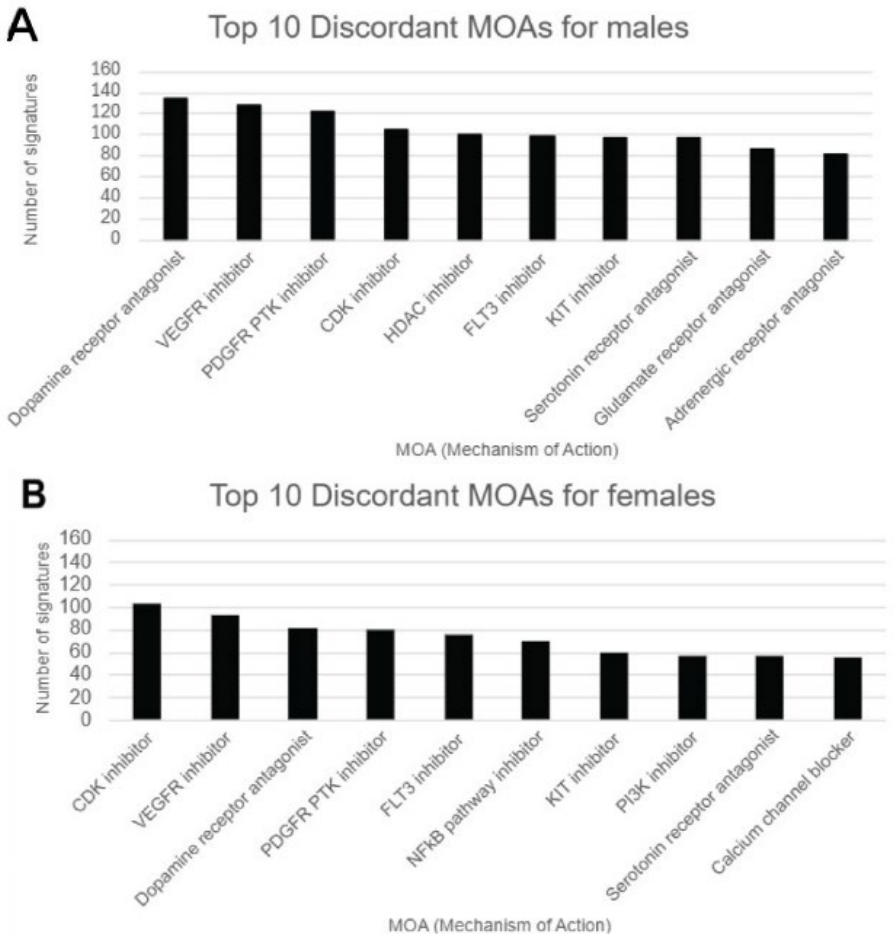

Figure 4: The bar chart shows the top ten mechanisms of action for discordant perturbagens for male LACA samples. Drug level data for these charts comes from the iLINCS database. A larger "N" indicates the MOA listed appears more often in the discordant perturbagen list. On the graph, the X-axis displays the mechanism of actions, while the Y-axis displays the number of signatures or patients it affects. All MOAs listed pass a concordant and discordant iLINCs threshold of >0.3 and <-0.3, respectively.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

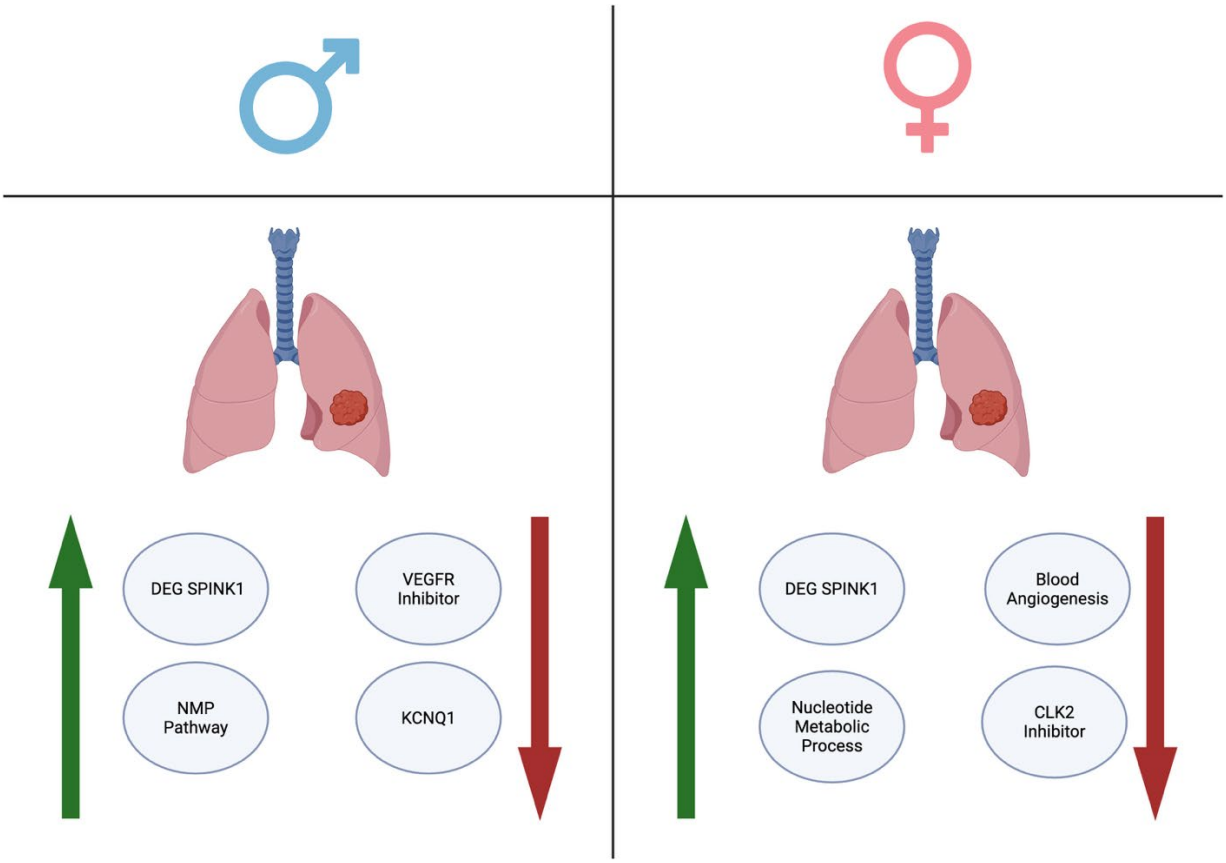

Figure 5: Summary figure showing male and female signatures in pathways, highlighting pathways and genes that are modulated in LACA. While blood angiogenesis was significantly downregulated in females, there was no significance for male signatures in this tumor-suppressed pathway. iLINCS' top discordant MOAs are also listed, such as the CLK2 inhibitor and the VEGFR inhibitor (Figure 5).

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

Perturbagen	Discordance Score	Mechanism of Action
SCHEMBL3954849	-0.39	Protein kinase inhibitor
WZ3105	-0.39	CLK2 inhibitor
BRD-A35207501	-0.38	Unknown
Wortmannin	-0.38	PI3K inhibitor
PIK 75	-0.37	DNA protein kinase |PI3K inhibitor
Sirolimus	-0.37	FK506-binding protein 1A inhibitor
BS-181	-0.37	Unknown
Epothilone A	-0.36	Microtubule stabilizing agent
AS-605240	-0.36	PI3K inhibitor
ZG-10	-0.36	JNK inhibitor

Table 1. Top 10 Discordant Perturbagens for Female Lung Adenocarcinoma

Legend: The top 10 discordant perturbagens or drugs were given a similarity score from the database iLINCS based on the transcriptome of all the differentially expressed genes (DEGs) (FDR) ≤ 0.05) that were detected in female LACA vs female healthy control tissue through RNA-sequencing. Negative scores indicate a reversal of the LACA transcriptomic signature, while positive scores indicate a simulation effect of the signature. The mechanism of action and perturbagens are also noted, with the bolded discordant perturbagens being FDA (Food Drug and Administration)-approved, including the drug, sirolimus.

UTJMS 2025 December 10, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1897

Perturbagen	Discordance Score	Mechanism of Action
WZ3105	-0.44	CLK2 inhibitor
Linifinab	-0.44	VEGFR inhibitor
Ruxolitinib	-0.43	JAK inhibitor
Alaproclate	-0.43	Serotonin receptor antagonist
Entinostat	-0.43	HDAC inhibitor
N-Desmethylclozapine	-0.43	Acetylcholine receptor agonist
Doramapimod	-0.42	P38 MAPK inhibitor
BI 78D3	-0.42	JNK inhibitor
Momelotinib	-0.42	Tyrosine-protein kinase JAK2 inhibitor
Gefitinib	-0.42	EGFR inhibitor

Table 2. Top 10 Discordant Perturbagens for Male Lung Adenocarcinoma

Legend: The top 10 discordant perturbagens or drugs were given a similarity score from the database iLINCS based on the transcriptome of all the differentially expressed genes (DEGs) (FDR) ≤ 0.05) that were detected in male LACA tissue vs male healthy control through RNA-sequencing. Negative scores indicate a reversal of the LACA transcriptomic profile, while positive scores indicate a simulation effect of the LACA transcriptomic signature. The mechanism of action is also noted. The bold discordant perturbagens are FDA (Food and Drug Administration)-approved, which include ruxolitinib, momelotinib, and gefitinib.