Abstract 2633: Effect of elevated expression of *LILRB4* and *TSC22D3* on survival in lung cancer

B. Hrinczenko[1,2*], T. Adeyelu[3], W-Z. Wei[4], A. Elliott[3], G. Bepler[4], A. VanderWalde[3], B. Halmos[5], E. S. Antonarakis[6], M. Lustberg[7], J.B. Jacob[1**]

[1]Michigan State University, East Lansing, Michigan 48824, [2]Karmanos Cancer Institute, Detroit, Michigan 48201, [3]Caris Life Sciences, Phoenix, Arizona 85040, [4]Wayne State University, Detroit, Michigan 48201, [5]Montefiore Einstein, Bronx NY 10467, [6]University of Minnesota, Minneapolis, MN 55455, [7]Yale Cancer Center, New Haven, Connecticut 06510

Background: *LILRB4 and TSC22D3 in Inflammation*

HER2/neu mutations or amplifications can activate STAT3 to promote inflammation and suppressing adaptive immune responses. Elevated systemic inflammation-immune index (SII) in small cell lung cancer (SCLC) correlates with reduced response to PD-1/L1 immune checkpoint inhibitors (ICIs), leading to worse overall survival (OS) and progression-free survival (PFS). We used a model of genetic heterogeneity to identify two genes involved in inflammation; TSC22D3 and LILRB4.

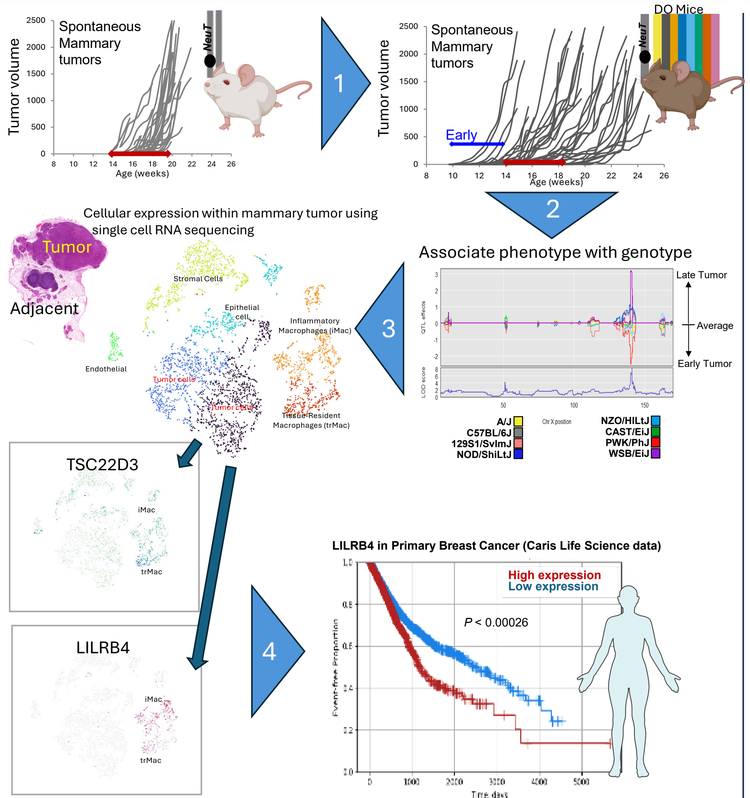
TSC22D3 is a transcription factor and one of the first genes induced by glucocorticoid activation of the glucocorticoid receptor (GR). TSC22D3 is expressed in stroma and macrophages.

LILRB4 is a transmembrane receptor that is regulated by TSC22D3. LILRB4 is expressed exclusively by myeloid cells.

We asked whether LILRB4 expression is associated with survival in lung cancer

- *LILRB4* was identified as a candidate gene involved in inflammation during tumor onset and growth
- Increased expression of *LILRB4* in NSCLC (LUAD and LUSC) and SCLC is correlated with enhanced survival
- Patients with increased expression of *LILRB4* survived longer when treated with PD-1/L1 therapy
- *LILRB4* expression was correlated with higher levels of tumor infiltrating cells in both LUAD and LUSC
- *LILRB4* is expressed by alveolar macrophages and the interaction between *TSC22D3* and *LILRB4* is being explored to understand how glucocorticoids impact expression of *LILRB4* and inflammation in lung cancer

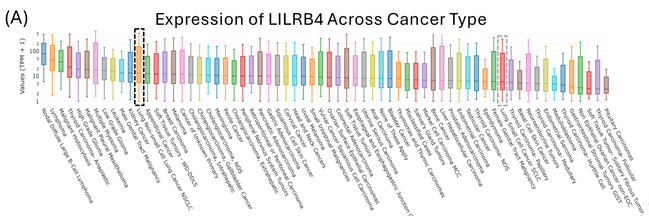
Methods: *Identification of LILRB4 and TSC22D3 in Cancer Onset and Growth*



TSC22D3 and LILRB4 were identified using Diversity Outbred (DO) mice[1]. (1) Mice that generate spontaneous HER2/neu-expressing mammary tumors were bred to generate a cohort of mice that are genetically unique individuals. (2) Association of tumor onset and growth with a mouse's unique genetics allowed us to identify the candidate genes TSC22D3 and LILRB4 (3) We used single cell RNA sequencing to identify that while TSC22D3 is expressed in both stromal fibroblasts and macrophages, LILRB4 is expressed specifically in macrophages.

(4) Caris Life Science data correlated gene expression with survival data in primary and metastatic breast cancer. Survival was calculated as the time from biopsy to final insurance claim. Their data demonstrated that both TSC22D3 and LILRB4 are associated with survival in breast cancer patients. In this study, we wanted to explore whether these genes were significant in Lung Cancer

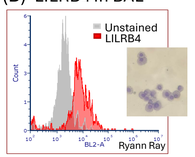
Results: *LILRB4 Expression Correlates with Increased Survival and Response to ICI*



LILRB4 Expression in Cancer

(A) Expression of LILRB4 (transcripts per million/TPM) was analyzed across multiple Caris' cancer types. In this analysis, NSCLC expression of LILRB4 is in the highest quartile across cancer types.

(B) Alveolar macrophages in bronchial alveolar fluid (BAL) were analyzed by flow cytometry for expression of LILRB4 protein. LILRB4 is expressed.



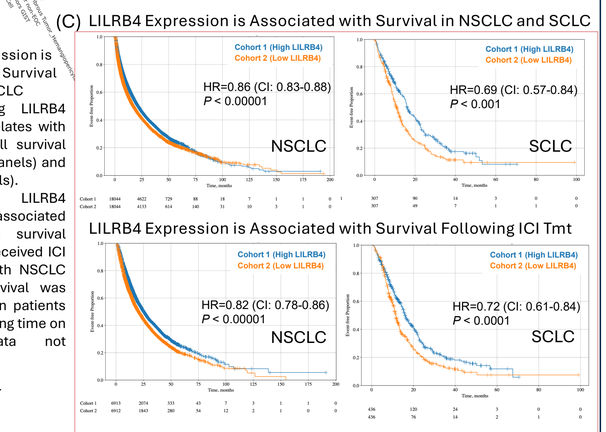


Table 1: Survival in LUAD and LUSC is correlated with LILRB4 expression

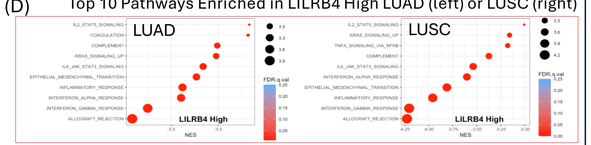
Survival in Months	Q1	Q2	Q3	Q4
LILRB4 expression in LUAD (Q1 = Low; Q4 = High)				
Overall survival (months)	17.0	22.2	21.9	24.8
Survival from start of ICI	17.5	20.8	21.5	24.9
Time on treatment with ICI	5.5	5.6	6.2	6.9
LILRB4 expression in LUSC (Q1 = Low; Q4 = High)				
Overall survival (months)	13.1	15.2	17.6	18.9
Survival from start of ICI	16.1	18.0	16.8	19.0
Time on treatment with ICI	5.1	5.5	5.5	6.7

(C) LILRB4 Expression is Associated with Survival in NSCLC and SCLC
TOP: Increasing LILRB4 expression correlates with enhanced overall survival in NSCLC (left panels) and SCLC (right panels).
BOTTOM: LILRB4 expression also associated with enhanced survival when patients received ICI treatment in both NSCLC and SCLC. Survival was also increased in patients receiving ICI during time on treatment (data not shown).

Table 2: Immune infiltrates in the tumor microenvironment associate with LILRB4 expression

	TME in LUAD (Fold Change)			TME in LUSC (Fold Change)		
Percent	Q1 (LILRB4 low)	Q4 (LILRB4 high)	Δ	Q1 (LILRB4 low)	Q4 (LILRB4 high)	Δ
CD8 T cells	0.19	1.79	1.6	0.01	1.33	1.32
B cells	3.38	5.08	1.7	3.59	5.50	1.91
Myeloid DCs	0.00	0.60	0.6	1.21	1.49	0.28
M1 Macs	3.65	8.34	4.69	1.50	4.97	3.47
M2 Macs	4.00	7.03	3.03	3.66	5.70	2.04
Tregs	1.98	3.85	1.87	1.09	3.55	2.46
NK cells	2.51	2.66	0.15	2.18	2.45	0.27
Neutrophil	5.57	4.84	-0.73	5.65	5.41	-0.24

(D) Top 10 Pathways Enriched in LILRB4 High LUAD (left) or LUSC (right)



(D) Gene Set Enrichment Analysis (GSEA) shows enrichment of interferons and inflammatory gene sets in patients with high LILRB4 expression.

Table 1. Increasing LILRB4 expression correlates with enhanced overall survival and also survival from start of ICI treatment in both LUAD and LUSC subtypes. Expression was analyzed across quadrants in LUAD and LUSC. Time is shown as the difference in months from median LILRB4 expression.

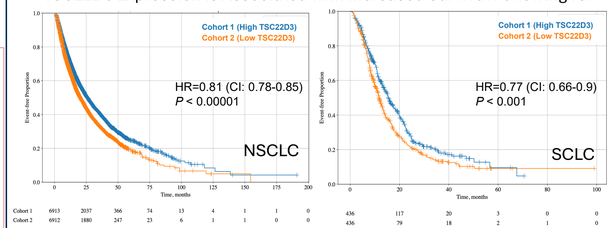
Table 2. In both LUAD and LUSC patient cohorts, tumors with increased levels of LILRB4 had increased infiltration of most immune cells, including CD8 T cells, B cells and NK cells. There were reduced numbers of neutrophils but increased macrophages.

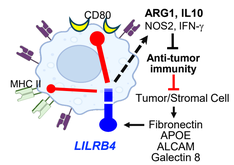
Future Directions: *Elucidating the Link Between Glucocorticoids and Lung Cancer*

LILRB4 expression is associated with response to immune checkpoint inhibitor (ICI) treatment as well as overall survival. TSC22D3 has been hypothesized to directly impact the expression of LILRB4. When we analyzed TSC22D3, we found it is also associated with improved response to ICI. TSC22D3 is induced in response to glucocorticoids, including dexamethasone and then alters expression of LILRB4.

LILRB4 antibodies that activate or inhibit expression are being tested in mice and we are actively seeking drugs that modulate expression of TSC22D3 to test.

TSC22D3 Expression is Associated with Increased Survival Following ICI

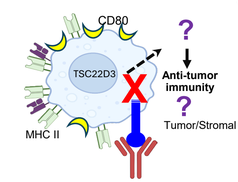


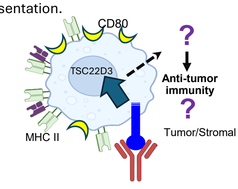


Our working model of LILRB4 function in macrophages

TOP: LILRB4 expression in breast cancer is detrimental. We know that when non-alveolar macrophages lack LILRB4, they express more proteins associated with antigen presentation.

BOTTOM: We don't know how blocking or activating LILRB4 in alveolar macrophages will impact macrophage functions including phagocytosis and antigen presentation.





Acknowledgements and References

- Work on determining expression of *LILRB4* on alveolar macrophages was done by Ryann Ray of the Jacob Lab at MSU. *TSC22D3* work is being done by Devyn Hill, also of the Jacob Lab.
- We acknowledge the Caris Precision Oncology Alliance for the collaboration in validating these genes in their clinical/genomic database

[1]Jacob, J.B. et al., 2023. iScience. PMID: 36968078.