

P1.248: WNT-Linked Fibrovascular Immune Exclusion Associates with Stromal TRM Loss and Immunotherapy Resistance in EGFR-TKI-Refractory EGFR-Mutant NSCLC

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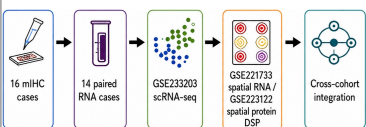
BACKGROUND & OBJECTIVE

- Patients with EGFR-mutant NSCLC often derive limited benefit from immunotherapy after EGFR-TKI failure.
- TRM cells in the stromal compartment are crucial for antitumor immunity, but the basis of stromal TRM loss remains unclear.
- We hypothesized that non-response reflects a fibrovascular immune-excluded stroma driven by WNT-related signaling.
- Objective:** integrate mIHC, targeted RNA profiling, public scRNA-seq, and public spatial RNA/protein datasets.

STUDY DESIGN & METHODS

Study design:

- Discovery cohort (mIHC):** 16 pre-treatment tumor biopsies from EGFR-mutant, EGFR-TKI-resistant NSCLC patients treated with subsequent immunotherapy.
 - Responders (PR): n = 7
 - Non-responders (NPR): n = 9
 - mIHC quantified total, tumor, stromal CD8+ TRM cells and stromal CXCL13.
- Paired transcriptomics:** targeted RNA profiling in 14 patients.
- External validation cohort 1:** GSE232303 single-cell RNA-seq from 7 EGFR-TKI-resistant NSCLC patients before ABCP treatment.
- External validation cohort 2:** GSE221733 GeoMx spatial RNA DSP and GSE223122 GeoMx spatial protein DSP from ICI-treated NSCLC.
- Statistics:** Mann-Whitney tests, Spearman correlation, Kaplan-Meier / Cox analyses, and patient-clustered GEE.

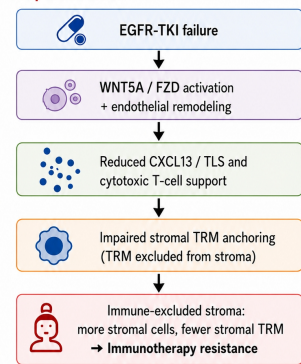


DISCUSSION AND CONCLUSION

Take-home message

- Our discovery mIHC cohort shows loss of stromal TRM density in non-responders.
- Public single-cell and spatial datasets independently show fibrovascular stromal-lineage enrichment and higher WNT signaling in non-responders.
- Together, these findings indicate an immune-excluded stroma characterized by more stromal cells but fewer stromal TRM—linked to WNT5A/FZD signaling and endothelial remodeling.
- WNT5A/FZD and endothelial remodeling associate with reduced CXCL13/TLS and cytokine immune support, and may impair stromal TRM anchoring.

Proposed model



REFERENCES & ACKNOWLEDGMENTS

- Hong S, Park C, Huh J, et al. *MedComm*. 2025; single-cell analysis of EGFR-TKI-resistant NSCLC treated with ABCP (GSE232303).
- Monkman J, Kim H, Mayer A, et al. *Immunology*. 2023; multi-omic and spatial dissection of immunotherapy response in NSCLC. (GSE221733, GSE223122)
- Current discovery cohort: 16 pre-treatment mIHC and 14 paired targeted RNA study.

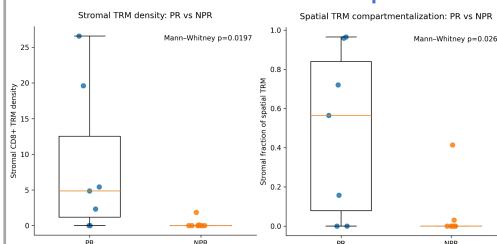
We thank all patients, collaborators, and public data contributors.

DISCLOSURE

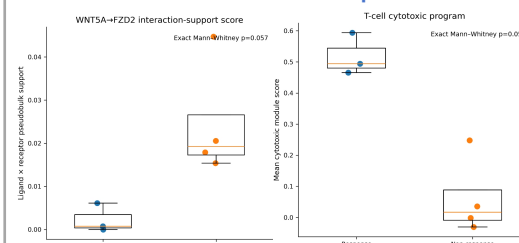
No relevant conflicts of interest declared. AI-assisted language and layout support were used in preparing this poster; all analyses and interpretations were independently reviewed by the authors.

RESULTS

A. Loss of stromal TRM in non-responders.

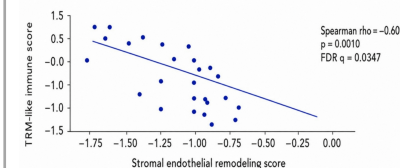


C. EGFR-TKI-resistant scRNA-seq reveals fibrovascular stromal-lineage enrichment and a stromal WNT source in non-responders.

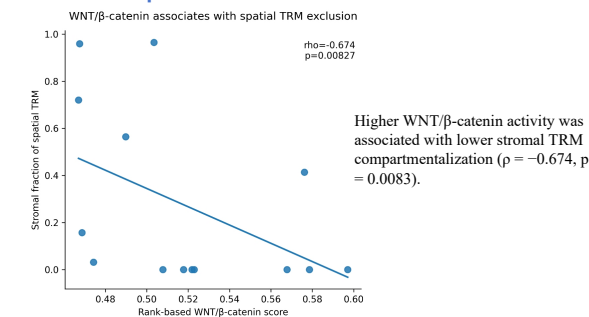


Non-responders in marked by fibrovascular stromal-lineage enrichment (fibroblasts were a major WNT5A source). Fibroblast WNT5A-FZD2 support was higher in non-responders (p = 0.057). T-cell cytotoxic activity was reduced in non-responders.

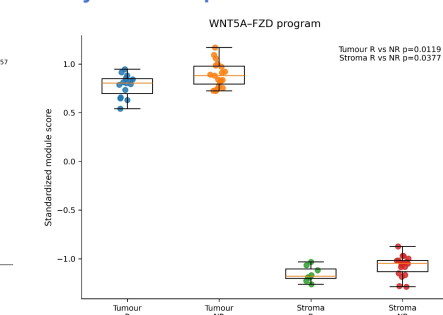
E. Endothelial remodeling is linked to TRM-like immune exclusion.



B. WNT activity inversely associates with stromal TRM compartmentalization.



D. Spatial RNA validation confirms higher WNT5A-FZD activity in non-responders.



Patient-clustered GEE overall non-responder effect: p = 0.0061.

Higher stromal WNT5A-FZD also correlates with TLS/CXCL13 (rho = -0.362; p=0.0065). Higher stromal WNT5A-FZD predicts worse overall survival (HR =2.06; 95% CI 1.19-3.55; p=0.0099).



Integrated interpretation

- The discovery mIHC cohort directly demonstrates loss of stromal TRM density in non-responders (failure of TRM anchoring).
- Independent public datasets reveal fibrovascular stromal-lineage enrichment and higher WNT signaling in non-responders.
- Together, these findings define a WNT-linked fibrovascular/endothelial immune-exclusion state characterized by more stromal cells but fewer stromal TRM, driving immunotherapy resistance.