Feasibility of Virtual Spatial RNA Inference from Histopathology to Identify Gene Signatures of Response to Chemoimmunotherapy in Early Stage Triple Negative Breast Cancer

Yuan Yuan[1], Vivek Pujara[1], Joseph Lownik[1], Murray Graeme[1], Jin Sun Bitar[1], Luxi Chen[1], Parisa Najafzadeh[1], Keeyon Dabirian[1], David Lin[1], Fred Kolling IV[2], Jonathan Marotti[3], Louis Vaickus[2], Minh-Khang Le[1], Zarif Azher[1], Keluo Yao[1] , Joshua Levy[1]. 1. Cedars-Sinai Medical Center, 2. Dartmouth Geisel School of Medicine, 3. Dartmouth-Hitchcock Medical Center

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Background

- Triple negative breast cancer (TNBC) remains a biologically aggressive breast cancer subtype with molecular heterogeneity. Immune checkpoint inhibitor (ICI) has become standard of care for treatment of TNBC with considerable immune toxicities. Currently there is limited biomarkers for prediction of pathological complete response (pCR) from neoadjuvant chemo-immunotherapy, therefore an unmet need in identifying novel biomarkers for optimal treatment selection.
- Visium Spatial transcriptomics (Visium ST) enables the spatial mapping of whole transcriptome gene expression across tissue sections at near single cell resolution, hence better characterize biological features of the tumor microenvironment (TME) which associates with ICI response. Routine use of ST is limited by cost and scalability.
- Virtual RNA Inference (VRI) can infer spatial gene expression from unseen histopathology slides in large study cohorts, with the potential to facilitate discovery of ICI response biomarkers.
- The current study was designed to link histologic features of the tumor microenvironment(TME) for response prediction to chemo-immunotherapy in early stage TNBC, facilitated through inference of ST from routinely stained whole slide images (WSI).

Methods

- This study was conducted through an institutional IRB approved protocol with inclusion of 2 cohorts of WSI.
  - Development Cohort (Dartmouth): 25 TNBC cases with matching tissue WSI, ST (Visium) measured at 50-micron resolution, representing 125,000 paired image patches and ST gene expression profiles.
  - Validation Cohort (Cedars Sinai): 58 retrospective TNBC resection specimens, neoadjuvant chemo+/- pembrolizumab: Neoadjuvant chemo-only: 20 pCR, 18 non-pCR; Neoadjuvant chemo+pembrolizumab: 8 pCR, 12 non-pCR
- Development Cohort: Pathology foundation deep learning models (e.g., Virchow2) trained to spatially infer 1000 genes, 8 cell-types
- Validation Cohort:
  - Infer expression in segmented tumor regions (DeepLabV3)
  - Differential expression: Identify genes associated with pCR
  - Composite markers: Identified using classifier + pathway analysis

Results

Figure 1. Methods Overview: A) Development: Deep learning model infers gene expression spatially from H&E WSI, B) Validation: Inference in tumor regions from external cohort to identify pCR biomarkers

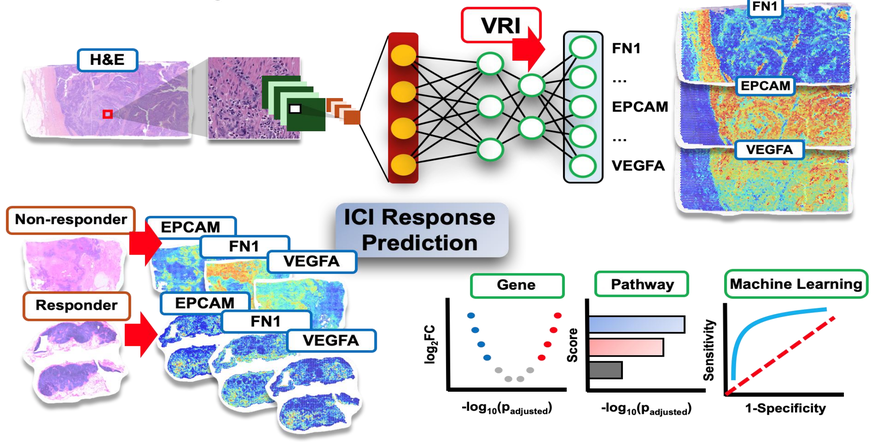


Figure 2. Inference on Held-Out Slides: Tumor regions segmented using DeepLabV3, inferred expression is averaged within these regions

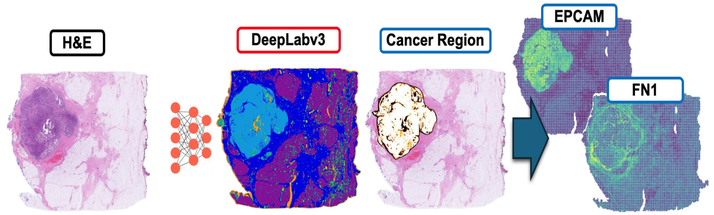


Figure 4. Validation Cohort: Association of 10 predictive genes to pCR.

Gene	AUC	p-value	Gene	AUC	p-value
MKI67	0.690	0.005	*GOLM1*	0.686	0.006
SSR4	0.694	0.005	*COL5A1*	0.669	0.007
MDK	0.700	0.005	*TXNDC5*	0.685	0.007
SRM	0.680	0.006	*CTHRC1*	0.669	0.007
CKAP4	0.696	0.006	*S100A9*	0.694	0.007

Figure 5. Receiver operating characteristic analysis of composite marker, produced by integrating 10 predictive VRI genes.

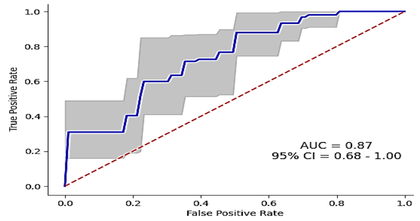
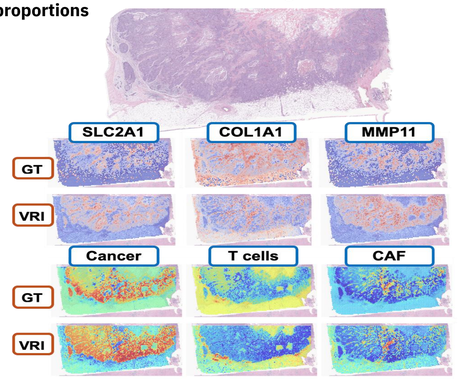


Figure 3. Visual Comparison of VRI-Inferred ST versus Visium ST (ground truth) for select: A) Genes, B) Deconvolved cell proportions



Conclusion

- Our findings demonstrate a proof-of-concept that deep learning models applied to H&E WSI, through incorporating ST, can help uncover clinically-relevant, TME-related ICI response biomarker in early stage TNBC
- By enabling large-scale molecular analysis of archived slides, VRI offers a path toward faster, more accessible biomarker discovery for TNBC, with future work to expand use in various indications.
- Our limited sample size may affect confounding adjustment and performance, especially in varied clinical settings and histologies.

Future Direction

- We will refine ICI-related VRI metrics by incorporating interpretable metrics from VRI gene maps to stratify ICI response in TNBC, e.g., stromal variation.
- We will confirm the current finding through external validation: 1) Finalize expanded ICI-only cohort, 2) apply VRI to public + industry cohorts, e.g., TCGA-BRCA, Tempus.

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Study Contact: Yuan Yuan MD PhD, Cedars-Sinai Medical Center,
Email: Yuan.Yuan@cshs.org