Immune phenotypes derived from H&E-stained whole slide images correlate with prognosis and response to checkpoint inhibitors in NSCLC

Nhat Le[1*], Laura Dillon[2*], Ciyue Shen[1], Matthew Bronnimann[1], Jun Zhang[1], Jennifer Hipp[1], Tan Nguyen[1], Michael Griffin[1], Michael Nercessian[1], Jacqueline Brosnan-Cashman[1], Robert Egger[1], Jean-Philippe Guégan[3], Alban Bessede[3], Florent Peyraud[4], Antoine Italiano[4], Xinwei Sher[2], G. Travis Clifton[2*], Bahar Rahsepar[1*]

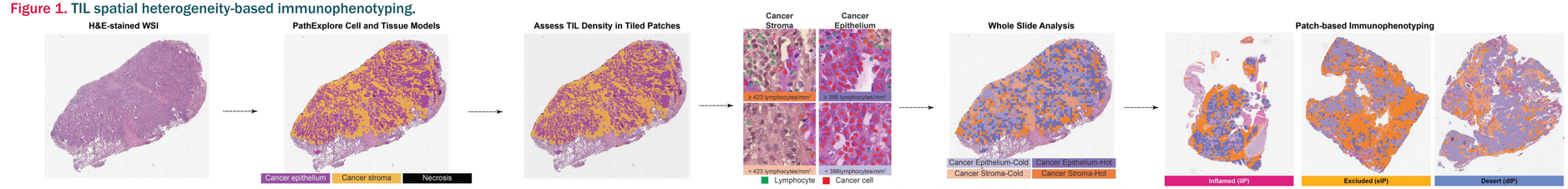
Abstract #8539

STUDY BACKGROUND

- The classification of tumors as inflamed, excluded or desert based on spatial patterns of tumor infiltrating lymphocytes (TILs)[1] is a potential biomarker of patients likely to respond to checkpoint inhibitors (CPI)[2]. However, the subjectivity of manual methods to assess these immune phenotypes (IPs) and poor standardization in the methods and thresholds to define IPs have hampered their clinical adoption[3,4].
- Here, we describe a data-driven approach to inform IP threshold selection based on predicted lymphocyte densities in patches of hematoxylin and eosin (H&E)-stained whole slide images (WSI) by maximizing differences in overall survival (OS) between IPs.

STUDY OVERVIEW

Figure 1. TIL spatial heterogeneity-based immunophenotyping.



1. An H&E-stained WSI is selected for analysis
2. Tissue regions (e.g., cancer epithelium & cancer stroma) and cell types (e.g., cancer cells &TILs) identified by PathExplore™ models*
3. WSI is divided into 0.01 mm² patches tiling entire tumor area
4. Lymphocyte density is calculated in each 0.01 mm² patch designated as cancer epithelium or cancer stroma
5. Each patch is designated "hot" or "cold" based on pre-determined lymphocyte density thresholds for cancer epithelium and cancer stroma
6. Slides are assigned a model-predicted IP based on the percentage of "hot" patches in cancer epithelium and cancer stroma

METHODS

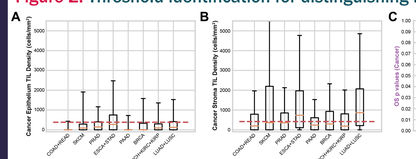
Datasets

- H&E-stained WSI (N=4,082) from multiple datasets from the cancer genome atlas (TCGA; COAD, READ, SKCM, PRAD, ESCA, STAD, PAAD, BRCA, KICH, KIRC, KIRP, LUAD, LUSC)[5] were used to determine thresholds for IPs.
- Two cohorts of patients with NSCLC were used to assess the clinical implications of IPs predicted by our approach: 1) TCGA cohort, consisting of LUAD (N=459) and LUSC (N=424) and 2) a clinical cohort consisting of PD-(L)1 inhibitor-treated NSCLC patients (N=95) enrolled in the BIP precision medicine study (NCT02534649; Institut Bergonié, Bordeaux, France).

Immune phenotype prediction

- A model to classify the IPs of NSCLC samples from H&E images was developed using PathExplore[6] models as described in Fig. 1.
- Lymphocyte densities were extracted for 0.01 mm² patches tiled across WSI. Cut-offs to define cancer epithelium and cancer stroma patches as hot or cold were defined based on the 75th and 50th percentiles, respectively, of lymphocyte densities in cancer epithelium and cancer stroma (Fig. 2A,B).
- Hierarchical fitting yielded optimal thresholds in cancer epithelium and cancer stroma (Fig. 2C) that minimize p-values of OS differences between IPs.

Figure 2. Threshold identification for distinguishing hot and cold patches.



Selected lymphocyte density thresholds in (**A**) cancer epithelium (75th percentile across all sampled patches from all indications) and (**B**) cancer stroma (50th percentile across all sampled patches from all indications) for distinguishing hot and cold patches. **C**) Thresholds (dashed lines) were selected to minimize the p-values of OS differences between IPs.

Exploratory Analyses

- Model-predicted IPs were compared to progression-free survival (PFS) and overall survival (OS) in both the TCGA and clinical cohorts. False discovery rate (FDR) correction was done with Benjamini-Hochberg.
- Survival was also assessed in the clinical cohort using PD-L1 tumor proportion score (TPS), iIP status, and TIL density as covariates.

Table 1. Thresholds chosen for IP prediction in NSCLC.

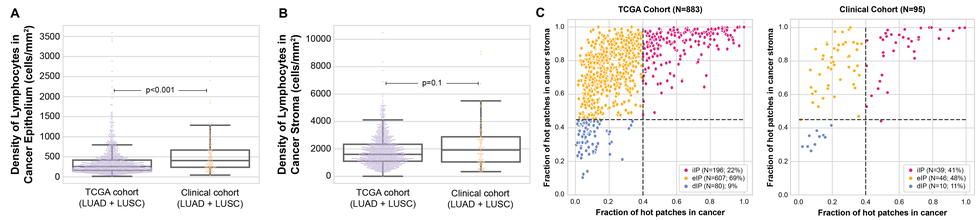
Model Predicted IP	Criteria
Inflamed (iIP)	>40% hot patches in cancer epithelium
Excluded (eIP)	≤40% hot patches in cancer epithelium; >45% hot patches in cancer stroma
Desert (dIP)	≤40% hot patches in cancer epithelium; ≤45% hot patches in cancer stroma

RESULTS

Key Results:

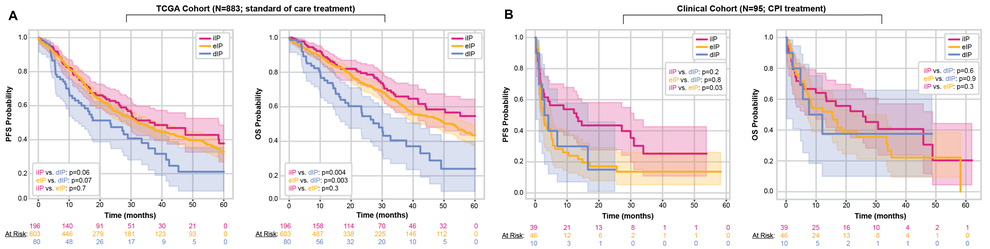
- In the TCGA NSCLC cohort, model-predicted iIP and eIP patients had significantly better OS compared to dIP (HR=0.53, p=0.003 and HR=0.59, p=0.003, respectively; Fig. 4A). In the clinical cohort, PFS was significantly shorter in model-predicted eIP patients compared to iIP (HR=0.54, p=0.045; Fig. 4B).
- Lymphocyte density in cancer epithelium and fraction of hot cancer epithelial patches were significantly associated with PFS (HR=0.64, q=0.04 and HR=0.69, q=0.04, respectively; Table 2).
- Notably, in PD-L1 (-) patients (N=43, TPS ≤1%), iIP patients (orange line) had longer PFS than eIP and dIP patients (blue line; HR=0.35, p=0.02; Fig. 6B, C). No difference in PFS was observed for PD-L1 (+) patients (N=43, TPS >1%).

Figure 3. Distribution of TME-related features and immune phenotypes in NSCLC cohorts.



In the TCGA and clinical cohorts, lymphocyte density was extracted after PathExplore deployment in the cancer epithelium (**A**) and cancer stroma (**B**). **C**) IPs were predicted based on patch-level thresholds of hot patches in cancer and stroma in the TCGA and clinical cohorts.

Figure 4. Association of immune phenotype with PFS and OS.

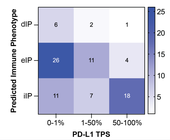


Cox regression using predicted IPs was used to predict PFS and OS in **A**) the TCGA cohort and **B**) the clinical cohort, the latter of which consisted exclusively of CPI-treated patients.

Table 2. PFS regression results with covariates in clinical cohort. Features retaining significance after FDR correction are shown.

Feature	p	q	HR (95% CI)
Number of lymphocytes relative to all predicted cells in cancer epithelium	0.005	0.04	0.64 (0.46, 0.87)
Density of lymphocytes in cancer epithelium	0.006	0.04	0.64 (0.46, 0.88)
Percentage of "hot" patches in cancer epithelium	0.007	0.04	0.69 (0.53, 0.90)

Figure 5. Association of immune phenotype with PD-L1 TPS in the clinical cohort.



Most patients predicted to be non-inflamed (dIP or eIP) show low PD-L1 levels (0-1% TPS). While most patients predicted to be iIP show high PD-L1 TPS (>1%), many still have low PD-L1 levels (0-1%).

Figure 6. Immune inflamed phenotype associates with improved PFS in CPI-treated NSCLC patients independent of PD-L1 status.

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	Covariate	p	PFS HR (95% CI)
Inclusion of lymphocyte density in cancer epithelium as covariate	PD-L1 Low	0.002	2.42 (1.38, 4.23)
	High TIL Density	0.06	0.62 (0.37, 1.03)
	Prior treatment	0.53	0.93 (0.74, 1.17)
	Histology	0.83	0.95 (0.57, 1.59)
	Age	0.91	1.00 (0.97, 1.02)
Inclusion of iIP prediction as covariate	PD-L1 Low	0.002	2.38 (1.36, 4.16)
	iIP prediction	*0.04*	*0.55 (0.32, 0.97)*
	Prior treatment	0.49	0.92 (0.74, 1.16)
	Histology	0.64	0.88 (0.52, 1.49)
	Age	0.92	1.00 (0.98, 1.03)

Multivariable Cox regression using **A**) lymphocyte density binarized at the median cutoff or **B**) IP predictions as covariates was used to predict PFS in the clinical cohort. Lymphocyte density was binarized at the median value, while iIP patients were compared to non-inflamed (eIP and dIP). iIP-inflamed status significantly correlates with better PFS (p=0.04). High lymphocyte density also correlates with better PFS but the effect does not reach statistical significance (p=0.06). **C**) Association between covariates and survival. Similar trends were observed for OS (data not shown).

CONCLUSIONS

- We developed a data-driven approach for predicting IPs using patch-level lymphocyte densities in cancer epithelium and cancer stroma derived from H&E-stained samples.
- Model-predicted IPs associate with OS in the TCGA NSCLC dataset and with PFS in a CPI-treated clinical NSCLC cohort. Association of IP and PFS was independent of PD-L1 status, potentially allowing the identification of PD-L1(-) patients who may derive greater benefit from CPI.

AFFILIATIONS

[1]PathAI, Inc., Boston, MA
[2]Incendia Therapeutics, Boston, MA
[3]Explicyte, Bordeaux, France
[4]Institut Bergonié, Bordeaux, France
*Contributed equally to study

CONTACT

Bahar Rahsepar: bahar.rahsepar@pathai.com
Laura Dillon: laura.dillon@incendiatx.com
publications@pathai.com

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* PathExplore is for research use only. Not for use in diagnostic procedures.

American Society of Clinical Oncology Annual Meeting, May 31-June 4, 2024, Chicago, IL, USA

ACKNOWLEDGMENTS: We thank the members of PathAI Pathology Network who contributed towards this work and GCI Health for assistance with figure design. Results shown here are in whole or in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga. This poster template was developed by SciStories LLC. https://scistories.com/