Abstract #7399

Development of a Reproducible AI-Based Spatial Biomarker of the Tumor Immune Infiltrate on H&E Slides

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Background

- A comprehensive assessment of the **tumor microenvironment** (TME) **composition** is not performed in routine practice due to the lack of standardized methods and tools for quantification.
- The **immune infiltrate** in the TME has been shown to be associated with prognosis [7].
- Immune-related biomarkers are often based on **IHC slides** and/or require intensive **manual annotations** [5,8].
- Numerous studies highlight that **spatial patterns** of immune cells are related to overall survival and response to immunotherapy [3, 9].
- A comprehensive examination is necessary to assess the **significance of spatial information** within immune-related biomarkers and its **value for clinical outcome** prediction [7]. This point is not fully addressed in the literature.

Objectives

- Develop **reproducible, artificial intelligence (AI)-based spatial biomarkers** of the tumor related immune response to predict patients outcome on routine Hematoxylin & Eosin (H&E) slides.
- Implement a **robust pipeline to assess**, across multiple cohorts, **the prognostic power** and the added-value of spatial immune biomarkers in comparison to the overall lymphocyte density and clinical variables.

Methods

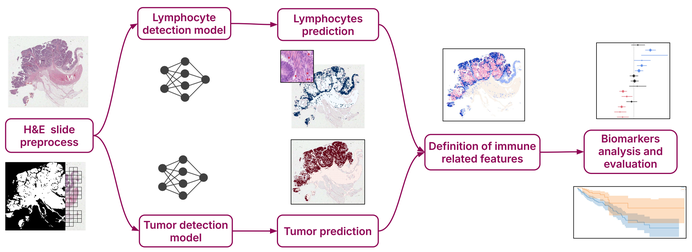

Figure 1: End-to-end pipeline: from slide preprocess to biomarkers analysis.

- We trained deep learning models on publicly available datasets (BCSS [1], CRC-VAI-HE-7K [4], NCT-CRC-HE-100K [4], NuCLS [2], Lizard [6]) to detect tumor and lymphocyte cells in H&E slides.
- We used the trained models to detect tumor and lymphocytes on slides of non-advanced patients from TCGA-BRCA cohort (breast cancer, 892 patients) and TCGA-COAD cohort (colon adenocarcinoma, 422 patients).
- We computed several immune related features.
- We evaluated the prognostic value of each feature, taking into account the spatial information encoded and the added value with respect to the clinical variables.

References

- [1] Amgad M. et al. (2019) Structured crowdsourcing enables convolutional segmentation of histology images, *Bioinformatics*, Volume 35, Issue 18, September 2019, Pages 3461–3467.
- [2] Amgad M. et al. (2022), NuCLS: A scalable crowdsourcing approach and dataset for nucleus classification and segmentation in breast cancer, GigaScience, Volume 11, giac037.
- [3] Ding R. et al. (2022), "Image analysis reveals molecularly distinct patterns of TILs in NSCLC associated with treatment outcome," *Npj Precis. Oncol.*, vol. 6, no. 1, p. 33.
- [4] Kather J. N. et al. (2018). 100,000 histological images of human colorectal cancer and healthy tissue, Zenodo, Apr. 07.
- [5] Galon J. et al. (2006) Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. *Science* 313,1960-1964.
- [6] Graham S. et al. (2021) "Lizard: A Large-Scale Dataset for Colonic Nuclear Instance Segmentation and Classification." Proceedings of the IEEE/CVF International Conference on Computer Vision., Montreal, BC, Canada, pp. 684-693.
- [7] Page D.B. et al. (2023) Spatial analyses of immune cell infiltration in cancer: current methods and future directions: A report of the International Immuno-Oncology Biomarker Working Group on Breast *Cancer. J. Pathol.*, 260: 514-532.
- [8] Salgado R. et al. (2015) International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015 Feb;26(2):259-71.
- [9] Saltz J. et al. (2018), "Spatial Organization and Molecular Correlation of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images," Cell Rep., vol. 23, no. 1, pp. 181-193.

Design of spatial biomarkers

- We design a **Tumor Infiltrating Lymphocytes (TILs) diffusivity score**. It is computed by counting TILs **located next to tumor cells**, normalized by the tumor size. A high score shows a **diffuse immune infiltration**, and a low score shows a **localized** immune infiltration.
- Other spatial scores quantifying **immune density in specific tumor areas** (tumor core, tumor invasive margin, intratumoral stroma) are computed.

Automatic assessment of **immune infiltration pattern (TILs diffusivity score)**: localized vs diffuse

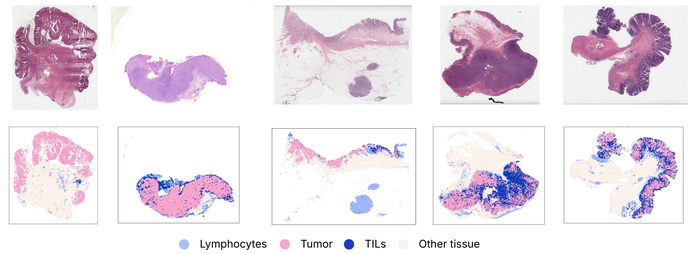

Figure 2: Example of different slides from TCGA-COAD and relative maps with increasing TILs diffusivity score: from localized (left) to diffuse (right).

Robust evaluation of spatial biomarkers

- All spatial biomarkers developed are compared to the non-spatial baseline given by the **overall lymphocytes density** on the whole slide.

Univariate analysis

- The prognostic power of each score is assessed using **Harrell's Concordance Index**
- **Kaplan Meier estimates** of survival are plotted for high and low score groups, and a log-rank test is performed to assess the statistical significance of the difference in survival.

Multivariate analysis

- To correct for potential clinical confounders such as disease stage, age or weight, we fit **Cox Proportional Hazards** models with these covariates, defining our baseline.
- For each spatial biomarker, we consider a Cox model that includes **the specific biomarker in addition to the baseline clinical variables.**
- We compare the performance of these augmented models to identify the effective biomarkers.
- To avoid overfitting and to compare the stability of the performance across subsets of the dataset, we perform **repeated cross validation** and compare performances on several validation folds.
- Improvements in performance are considered significant if they are **consistent across a majority validation folds** (>75%).

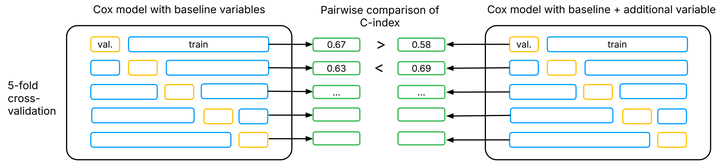

Figure 3: (Repeated) Cross validation process for model benchmark. Models are assessed by comparing their performance metrics (C-index) through pairwise evaluations across (repeated) cross-validation folds.

Results

Stratification of high and low risk patients according to different histological markers

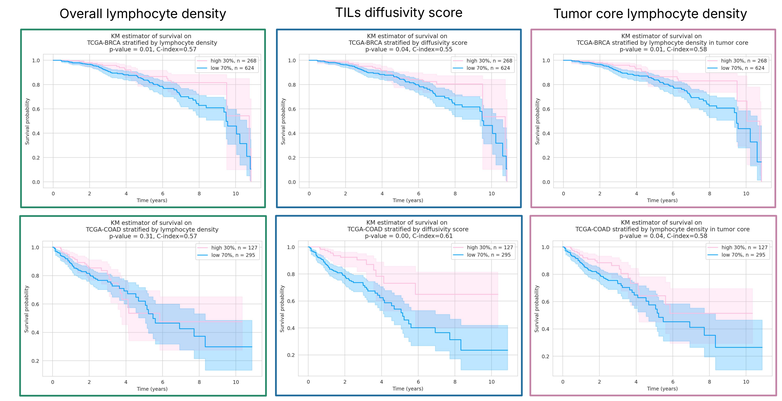

Figure 4: Kaplan-Meier curves of three different biomarkers on TCGA-BRCA (top) and TCGA-COAD (bottom): overall lymphocyte density (left), TILs diffusivity score (center) and lymphocyte density in tumor core (right).

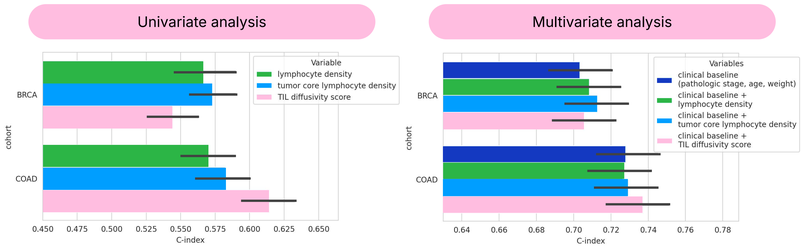

Figure 5: Comparison of c-indexes in univariate (left) and multivariate (right) analysis, on TCGA-BRCA and TCGA-COAD.

TCGA-BRCA

- The overall lymphocytes density is prognostic, and restricting the density computation to the tumor core yields better results.
- TILs diffusivity score is less prognostic than overall lymphocytes density.

TCGA-COAD

- TILs diffusivity score is prognostic: patients with diffuse tumor immunity have a significantly better prognosis.
- TILs diffusivity score is more prognostic than overall lymphocytes density, proving that the spatial distribution brings information to the prognosis.
- Both spatial and non-spatial biomarkers bring marginal improvements compared to clinical variables.
- Compared to overall lymphocyte density, spatial markers improve survival prediction by a small but consistent margin: comparing them on the same cross-validation folds result in an improvement in more than 75% of cases.

Conclusions

In colon cancer, we discovered a reproducible spatial biomarker, based entirely on H&E, which exhibits superior prognostic value compared to overall lymphocyte density.

In breast cancer, we validated the prognostic significance of lymphocyte infiltration with a fully automated assessment.

Selection of spatial biomarkers should be tailored to the indication, offering incremental improvements in prognosis prediction over standard pathology assessment.

These results hold promise for future clinical applications of AI-based spatial biomarkers.