Predicting response to immune checkpoint inhibitors (ICI) in non-small-cell lung cancer (NSCLC) by combining spatial analysis of cells and RNA sequencing data from biopsies using deep learning (DL)

Ettai Markovits[1], David Soong[2], Becky Arbiv[1], Alon Groisman[1], Maya Lipinsky[1], Yoni Yedidia[1], Yoad Cohen[1], Yuval Gabay[1], Yuval Shachaf[1], Eyal Oren[1], Roman Gluskin[1], Ido Weiss[1], Hisham Hamadeh[2], Kate Sasser[2], Gali Golan[1], Brandon Higgs[2], Suzana Couto[2], Ori Zelichov[1]

[1] Nucleai, Tel Aviv, Israel, [2] Genmab Inc., Princeton, NJ, USA

Contact details: Suzana Couto (scou@genmab.com) and Ori Zelichov (ori@nucleai.ai)

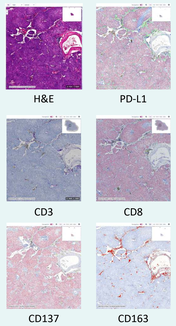
Poster #1289

Introduction

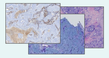
- ICI have transformed treatment of metastatic NSCLC, however, only a small proportion of patients respond.
- Spatial biology, based on information integrated across hematoxylin and eosin (H&E) and multiple immunohistochemistry (IHC) images, plays a significant role in the understanding of drivers of response and unravels the complexities and interactions around the tumor microenvironment.
- Here, we utilized DL to analyze whole slide images (WSI) of sequential biopsies stained for H&E and five IHC stains and combined it with RNA sequencing data to identify features associated with response to ICI.

Materials and Methods

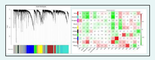
- Samples from 103 NSCLC patients treated with second line ICI (pembrolizumab or nivolumab) were procured.
- For each patient, a total of 12 WSI from pre- and post-treatment biopsies, sequentially stained for H&E, CD3, CD8, CD163, CD137 and PD-L1 IHC were analyzed (six pre- and six post-treatment).
- In addition, RNA sequencing data of pre-treatment biopsies was collected and analyzed for 81 patients.
- Clinical outcomes included overall response (OR), progression-free survival (PFS), and overall survival (OS).
- DL models were trained to classify tumor cells, lymphocytes, fibroblasts, and tumor versus stromal areas from H&E, as well as the positivity of IHC markers per cell.
- Based on biological hypotheses, 362 spatial and 72 RNA features were pre-defined and calculated for each patient. Of the spatial features, the interactions (i.e., average physical proximity) between cell types expressing different biomarkers were also calculated, utilizing virtual multiplexing methodologies in order to accurately overlay multiple images.
- Univariate analysis identified features associated with clinical outcomes and the best performing features were selected to generate a multivariate binary classifier for response prediction. Cross-validation was performed.



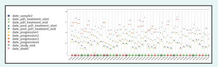
1200+ Images
H&E Pre+post treatment
5 IHCs Pre+post treatment
(PD-L1, CD3, CD8, CD137, CD163)



Gene Expression
RNA-seq
Pre-treatment



Clinical Data
Full clinical data (OR, PFS, OS) for patients treated with advanced line PD-1 inhibitor



Analysis workflow

1200+ Images
H&E Pre+post treatment
5 IHCs Pre+post treatment
(PD-L1, CD3, CD8, CD137, CD163)

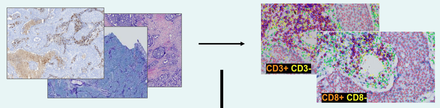
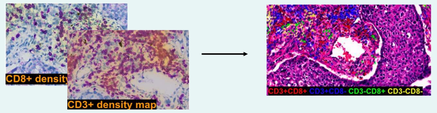
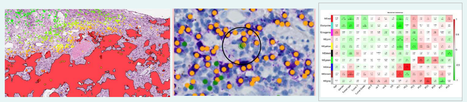


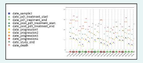
Image registration and projections
Overlay of cell-based density maps
onto H&E cell detections



Features repository

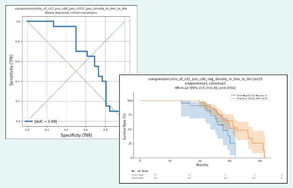
Spatial and genomic features (>450)





Clinical data

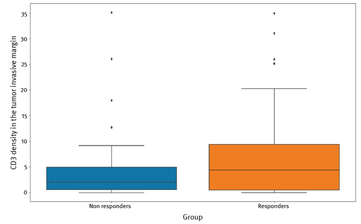
Clinical outcome prediction
OR, PFS, OS
Univariate/multivariate data science to build statistically-valid response prediction models



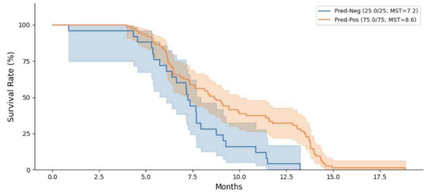
Univariate analysis

- Multiple spatial and RNA features from pretreatment samples were significantly correlated with OR, PFS and OS. Of these features, the most significant features were:
  - Density of CD3 in the tumor invasive margin was greater in responders (Fig. 1a).
  - Closer proximity between PD-L1+ tumor cells and cytotoxic T cells (CD8+ cells) in pre-treatment biopsies in responders compared to non-responders ($p<0.01$) as calculated following virtual multiplexing of consecutive slides stained by both IHCs (Fig. 1b)
  - Gene set enrichment analysis identified upregulation of hallmark TNF-alpha signaling and IFN-gamma response genes in responders to ICI pre-treatment ($p<0.0001$)
- When comparing paired pre- and post-treatment biopsies for the same patients, responders had greater increases in the density of CD8+ cells in the tumor microenvironment following ICI treatment ($p=0.02$).

1a. Distribution of lymphocyte density at the invasive margin in pre-treatment biopsies of responders and non-responders.

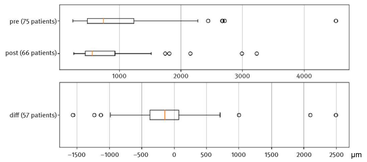


Univariate analysis of the CD3 feature



1b. Patients positive or negative for the CD3 feature (upper 75% of values was considered positive, lower 25% was considered negative).

Distance between PD-L1+ tumor cells and CD8+ T cells



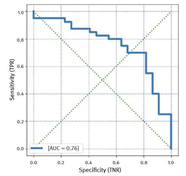
Immune PD-L1+ to CD8+ distance feature differentiates pre- vs. post-treatment ($p=0.0007$).

Multivariate analysis

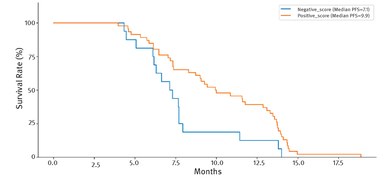
- The multivariate classifier was calculated on 62 patients for which both RNA and spatial features were available.
- Features were correlated with clinical outcome data to train a binary classifier that identifies responders vs. non-responders.
- The resulting classifier combined 7 spatial features and 3 RNA features and reached an AUC=0.76 when correlated with OR features (Fig. 2a).
- The classifier identified patients in the validation set to have either positive (n=46) or negative (n=16) scores.
- In a Kaplan-Meier (KM) analysis, PFS was significantly longer in positive-scored compared to negative-scored patients (HR=0.48, 95% CI 0.26-0.86, p<0.02) (Fig. 2b).
- Features included in the model are T-cell density in the tumor invasive margin compared with nearby stroma.

The combined model outperformed spatial-only model (ROC AUC 0.76 vs. 0.65; Fig. 2a vs 2c) as well as individual known predictive biomarkers such as IFN-gamma signature and PD-L1 which showed lower predictive power on the entire cohort.

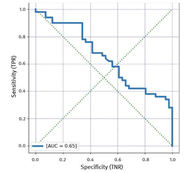
2a. Selected multivariate model (81 patients cohort)



2b. Predictive power PFS:
Cox Hazard Ratio: 0.48 (p=0.014)
HR=0.48 (95% CI 0.26-0.86; p=0.0144)



2c. Spatial-only model (91 patients cohort)



Conclusions

- DL for spatial analysis of cells combined with pathway modulation, as measured by RNA sequencing, can elucidate unique tumor contexture relevant for response to ICI in NSCLC.
- Predictive features found in this work correlate with previously shown results[1], indicating that lymphocytes infiltration had a better outcome and their prognostic value in various cancers. Also, close proximity between tumor PD-L1+ and CD8+ T-cells shows strong prognostic power suggesting that these interactions are potentially more responsive to CPI treatment, as observed in the current study.

References

[1] Li F, Li C, Cai X, Xie Z, Zhou L, Cheng B, Zhong R, Xiong S, Li J, Chen Z, Yu Z, He J, Liang W. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis. EClinicalMedicine. 2021 Sep 16;41:101134. doi: 10.1016/j.eclinm.2021.101134. PMID: 34585125; PMCID: PMC8452798.