CD8+ T Cells in Tumor Parenchyma and Stroma by Image Analysis and Gene Expression Profiling: Potential Biomarkers for Immuno-oncology Therapy

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*At the time the analysis was conducted

Background

- Inflammation of the tumor microenvironment (TME), marked by infiltration of CD8+ T cells, has been associated with improved clinical outcomes across multiple tumor types¹
- Parenchymal infiltration of CD8+ T cells has been associated with improved survival with immuno-oncology (I-O) treatment, and intratumoral localization also affects outcome, highlighting the importance of spatial analysis of CD8+ T cells within the TME¹⁻⁴
- CD8+ T-cell patterns within tumors, as assessed by immunohistochemistry (IHC), are variable and may be classified as⁵:
- Immune desert (minimal T-cell infiltrate)
- Immune excluded (T cells confined to tumor stroma or invasive margin)
- Immune inflamed (T cells infiltrating tumor parenchyma, positioned in proximity to tumor cells)
- Emerging data suggest that artificial intelligence (AI)-based image analysis can be used to characterize the tumor parenchymal and stromal compartments in the
- Pathology data can be quantified, and IHC assays are amenable to future in vitro diagnostic development; however, there is limited capacity for multiplexing within IHC assays
- Gene expression profiling (GEP) provides an alternative approach for assessment of inflammation in the TME
- Associations between inflammatory gene signature scores and response to I-O therapy have been demonstrated in multiple tumor types⁸⁻¹²
- An inflammation gene panel comprising 95 genes for GEP, in combination with Al-based image analysis, was used to analyze patterns of T-cell infiltration in tumor parenchymal and stromal compartments and to evaluate potential biomarker assays

Objectives

- Quantify regional CD8+ T-cell localization using AI-based image analysis
- Identify gene signatures that define CD8+ T-cell infiltration and localization to parenchymal and stromal compartments in the TME

Methods

- IHC for CD8 expression (CD8 IHC) and GEP were performed on commercially procured melanoma (n = 158) and squamous cell carcinoma of the head and neck (SCCHN; n = 250) tumor samples
- CD8 IHC was performed by Mosaic Laboratories using a monoclonal CD8 (clone C8/144B) antibody (Dako, an Agilent Technologies Co, Santa Clara, CA)
- A convolutional neural network (PathAl Inc, Boston, MA) and Al-based image analysis algorithms were used to quantify the abundance of CD8+ T cells in tumor parenchymal and stromal regions (Figure 1)
- GEP was performed by next-generation sequencing (NGS) using an inflammation panel¹³
- The inflammation panel comprises 95 genes, including genes related to tumor inflammation and other I-O processes, housekeeping genes, and control genes. This inflammation panel measures mRNA expression levels of all 95 genes on the panel¹³
- Generalized constrained regression models were used to predict CD8+ T-cell abundance in the tumor parenchyma and stroma using specific gene signatures (Figure 2)
- Repeated cross-validation (100 repeats of 5-fold cross-validation) was performed to estimate generalization of the models
- Adjusted coefficient of determination (R²) was used to analyze the relationship between CD8+ T-cell abundance and the tumor parenchymal and stromal CD8 signatures

Results

 Immune phenotypes of melanoma and SCCHN samples were defined and characterized using Al-based quantification of CD8+ T cells (Figures 1 and 3)

Figure 1. Example images used for Al-based quantification of CD8+ T cells in tumor parenchymal and stromal regions

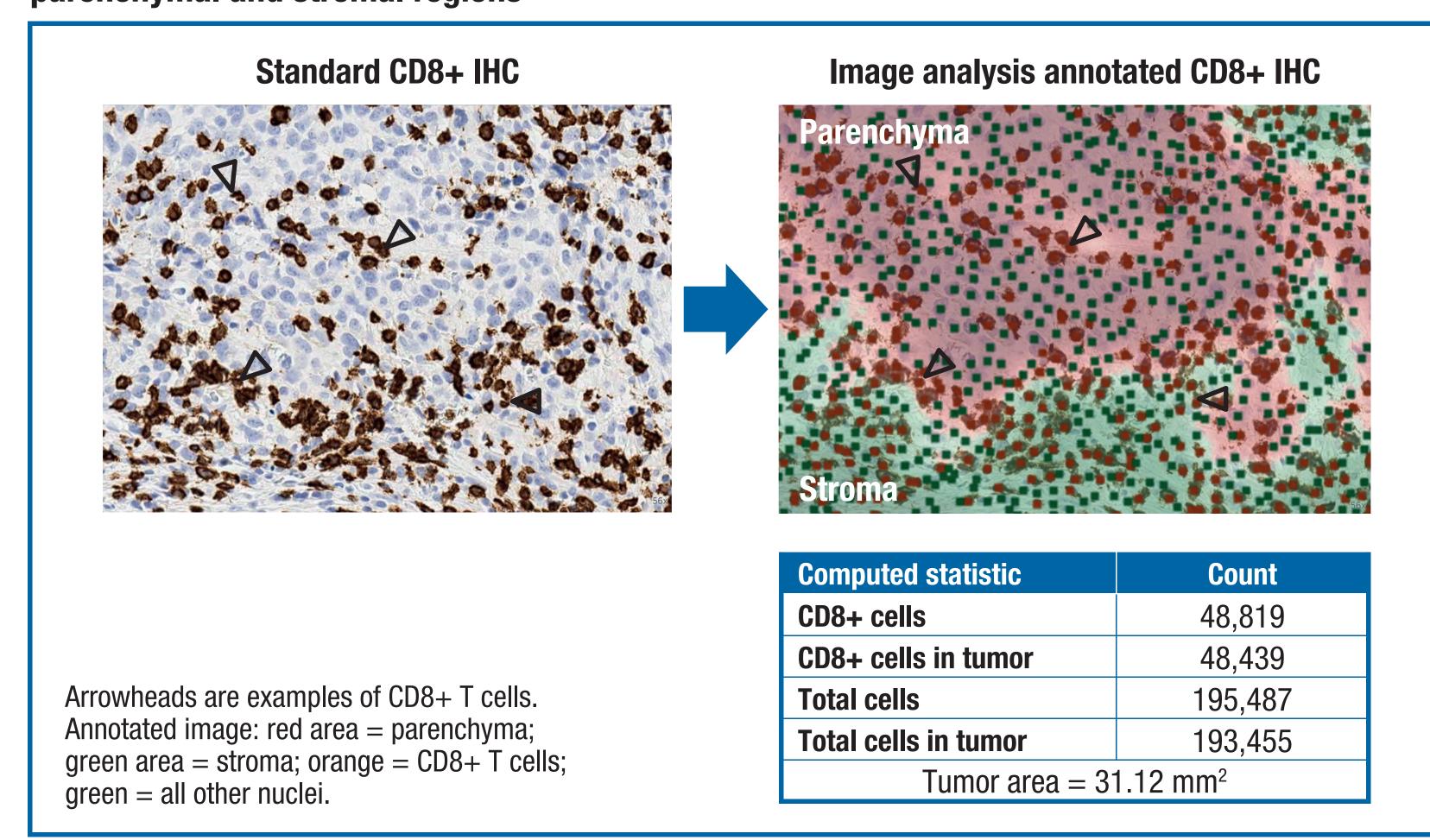
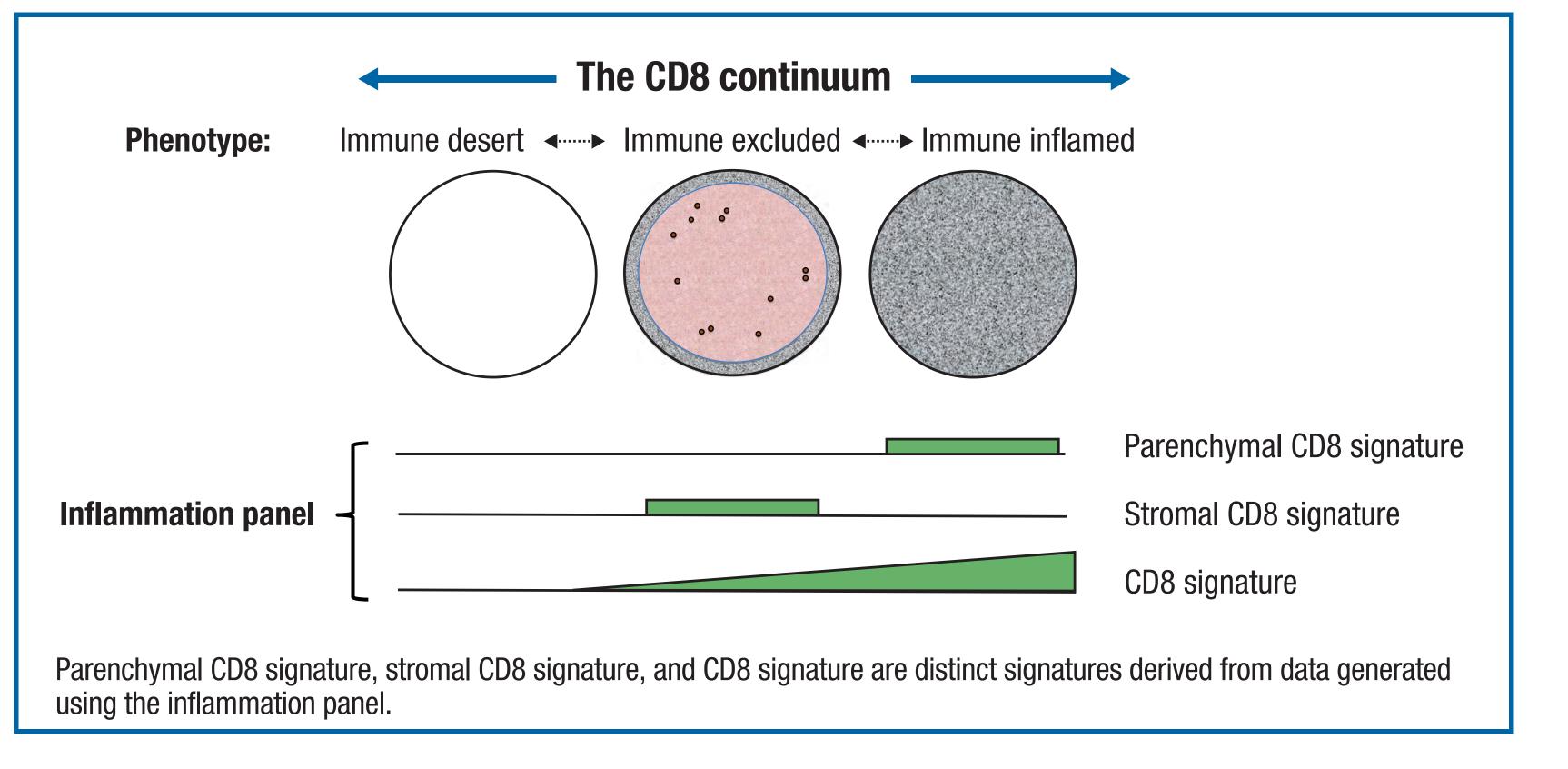
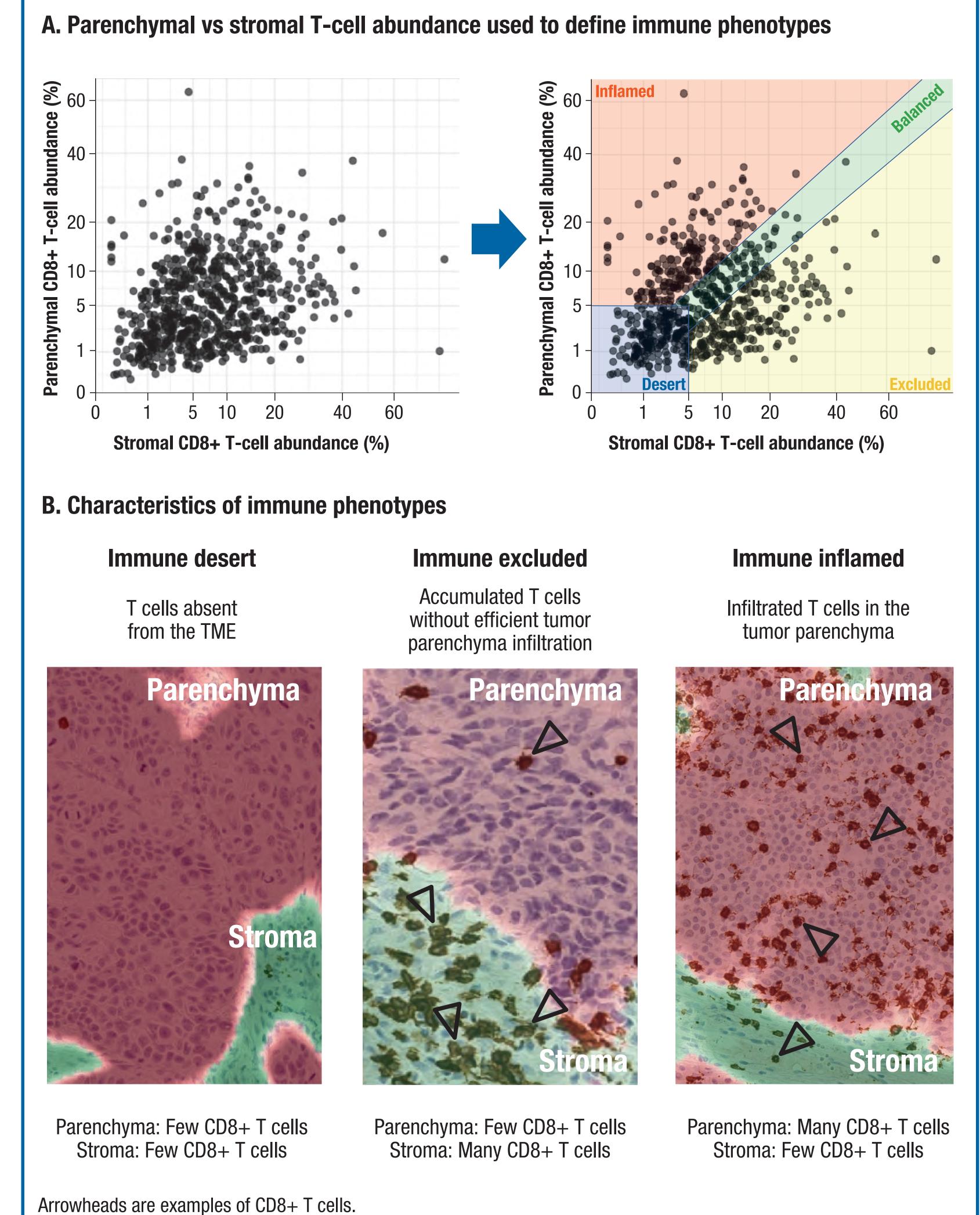


Figure 2. Association of tumor immune phenotypes and region-derived gene signatures



- Using the inflammation panel to analyze melanoma and SCCHN samples, gene signatures that correlated with parenchymal and stromal CD8+ T-cell abundance were derived, demonstrating that different gene signatures can be developed using the same assay (Figures 2 and 4)
- A parenchymal CD8 gene signature correlated with tumor parenchymal CD8+ T-cell abundance (Figure 4A)
- Of the 23 genes in the signature, 10 were upregulated and 13 were downregulated; top predictors of parenchymal CD8+ T-cell abundance included:
- Upregulation of STAT1 and IFN_Y
- Downregulation of NECTIN2 and CSF1R
- A stromal CD8 gene signature correlated with tumor stromal CD8+ T-cell abundance (Figure 4B)
- Of the 38 genes in the signature, 19 were upregulated and 19 were downregulated; top predictors of tumor stromal CD8+ T-cell abundance included:
- Upregulation of CSF1R and NECTIN2
- Downregulation of STAT1 and IFN_Y

Figure 3. Phenotypes characterizing immune activity in tumors by CD8 IHC



- Limited overlap was observed between the 2 sets of genes; some genes that correlated with stromal CD8+ T-cell localization showed inverse correlation with parenchymal CD8+ T-cell localization (Figure 4)
- Upregulation of STAT1 and IFN_Y correlated with parenchymal CD8+ T-cell abundance, whereas downregulation of these genes correlated with stromal CD8+ T-cell abundance
- Downregulation of CSF1R and NECTIN2 correlated with parenchymal CD8+ T-cell abundance, whereas upregulation of these genes correlated with stromal CD8+ T-cell abundance
- Gene signature scores were developed from the tumor parenchymal and stromal CD8 signatures
- For both tumor-specific and pooled analyses of melanoma and SCCHN samples, gene signature scores were highly concordant with Al-based quantification of CD8+ T cells, depending on regional CD8+ T-cell infiltration (Figure 5)
- Adjusted R² for parenchymal CD8 signature score (pooled analysis) = 0.67 (P < 0.01)
- Adjusted R^2 for stromal CD8 signature score (pooled analysis) = 0.65 (P < 0.01)

Figure 4. Gene expression signatures for tumor parenchymal and stromal CD8+ T-cell abundance

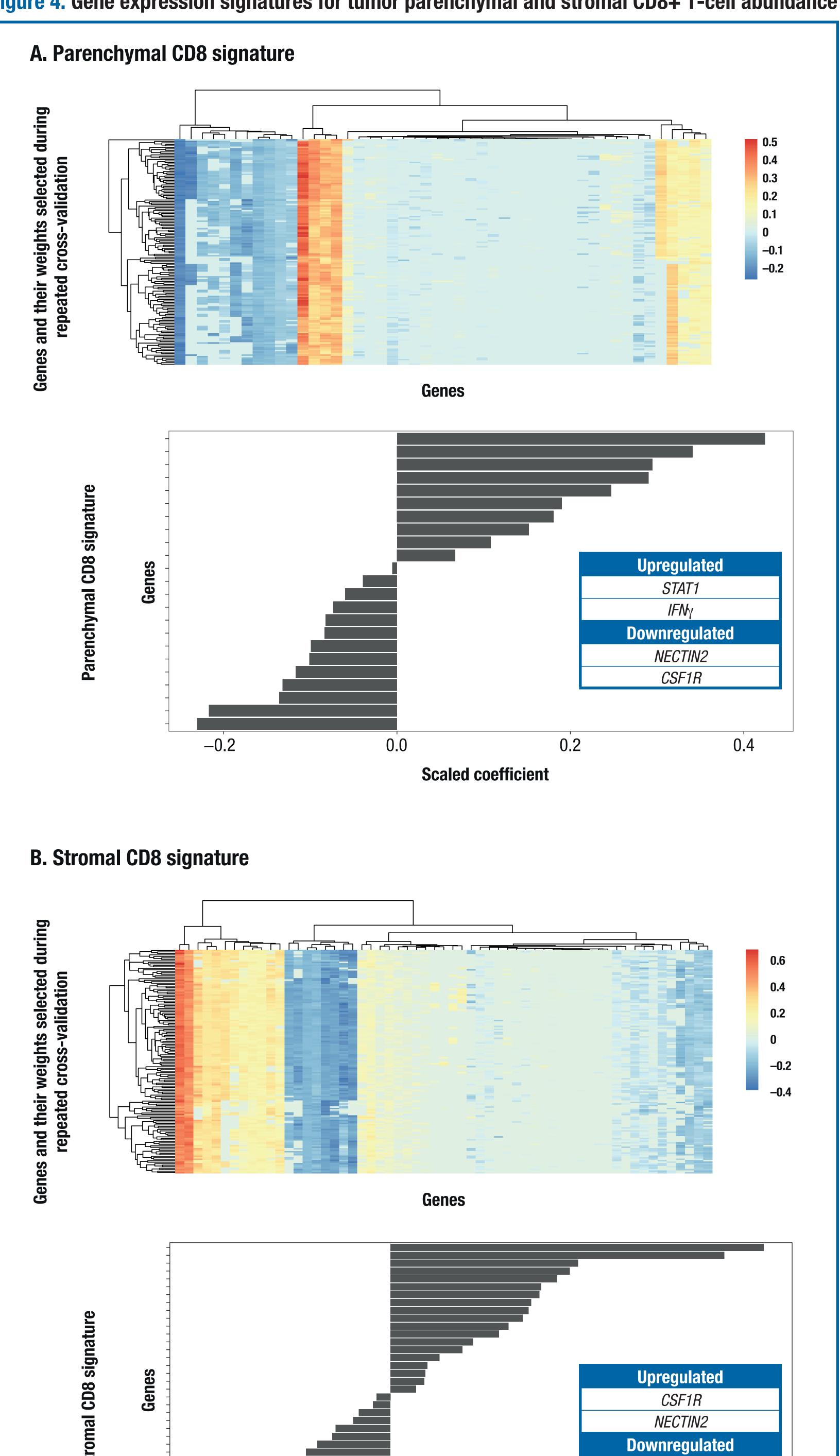
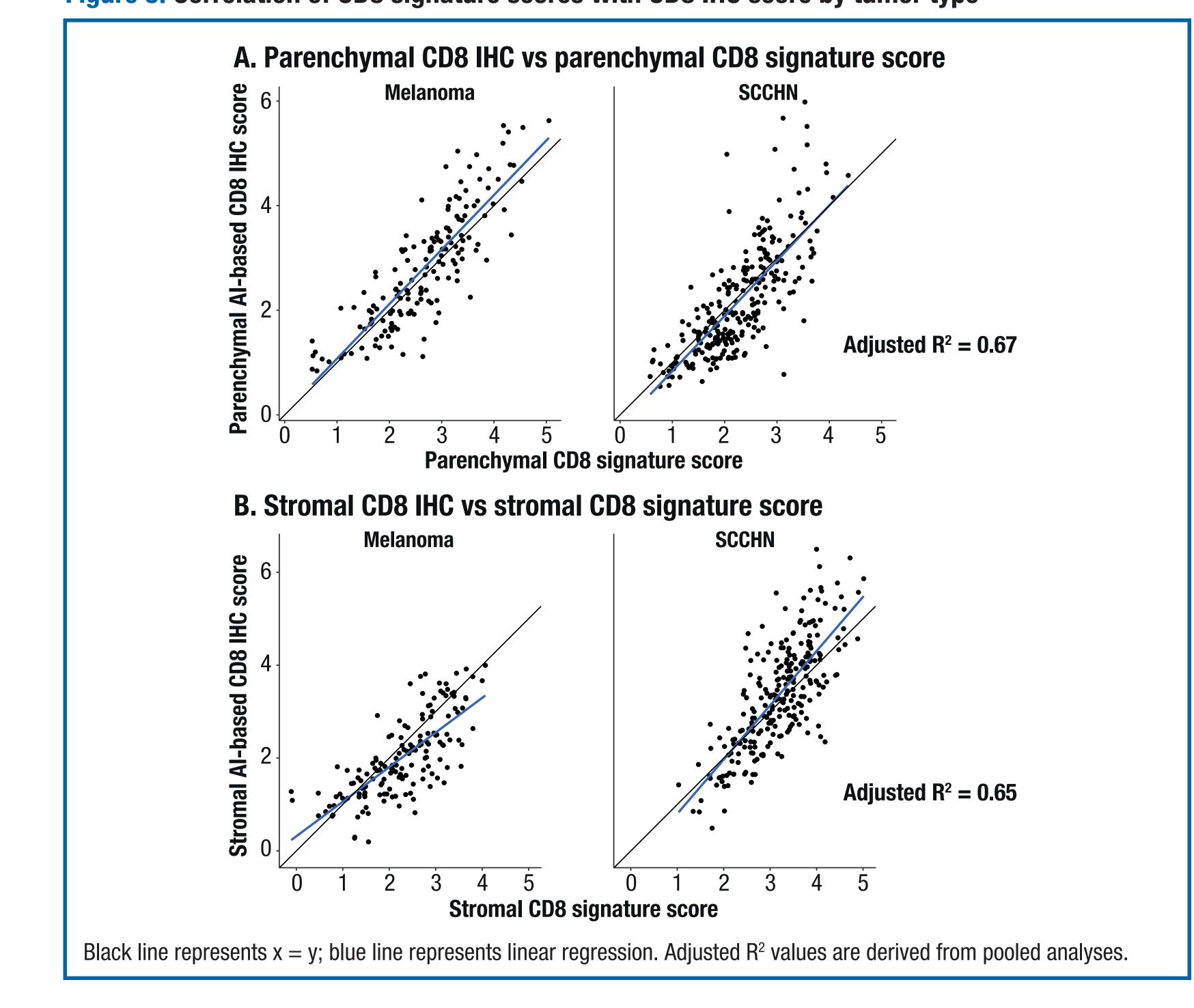


Figure 5. Correlation of CD8 signature scores with CD8 IHC score by tumor type



Conclusions

- We have developed a GEP-based, investigational-use-only (IUO) inflammation assay with the potential to be utilized prospectively in a clinical trial setting
- Using this panel, we have further derived gene signatures that predict CD8+ T-cell abundance in the tumor parenchyma and stroma
- Parenchymal and stromal CD8 signature scores were concordant with CD8+ T-cell abundance determined by IHC in melanoma and SCCHN samples (individually and pooled), indicating that these gene signatures may be utilized to assess T-cell abundance
- Combining GEP with Al-based image analysis could be developed as an analytical tool to characterize immune cell infiltration in the TME
- The clinical relevance of parenchymal and stromal CD8 signatures will be investigated in future studies to assess the value of these signatures as biomarkers for I-O therapy

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