Computational Analysis of Multiplexed Immunohistochemistry for Understanding Immune Profiles in Clinical Biopsy Samples

Brooke Hirsch, Joseph Krueger, Emily Mason, Karen Ryall, Kile McFadden

Flagship Biosciences, Inc, Westminster, CO

Flagship BIOSCIENCES

POSTER# 1694

Abstract

Using immunohistochemistry (IHC) to examine immune infiltrates in tissue biopsy samples to support exploratory investigations for immuno-oncology drug development is critical for understanding the abundance and spatial relationships of different immune cell types and how they may change with drug treatment. Multiplex IHC approaches are utilized to examine these types of pharmacodynamic responses because of the limited tissue available from repeat needle core biopsies and the need to visualize multiple biomarkers in the same tissue section. While several different fluorescent multiplexing approaches exist, the complexity of these fluorescent assays limits the ability to develop and validate bespoke assays to meet the needs of hypothesis-driven research, which aims to elucidate predictors of clinical response. Furthermore, these methodologies present known challenges for design control processes and regulatory approval as companion diagnostics, preventing wide use beyond exploratory research settings.

In contrast, the development and analytical validation of chromogenic IHC assays enables an agile and bespoke approach to IHC assay development that can support all phases of drug development, including direct translations of these methods into companion diagnostic building approaches. Investigating multiple biomarkers using chromogenic assays presents its own challenges, however, as there is a more limited repertoire of chromogens than fluorophores, which have more significant spectral overlap in wavelength absorbance and optical density. As such, a multiplex chromogenic IHC assay requires specialized performance specifications as well as sophisticated interpretation methods to ensure accurate interpretations.

While these limitations often create too great a challenge for pathologist interpretation, computational analysis of tissue using Flagship's cTA® platform resolves these challenges and enables drug developers to rely on chromogenic IHC approaches to meet the needs of drug and diagnostic development.

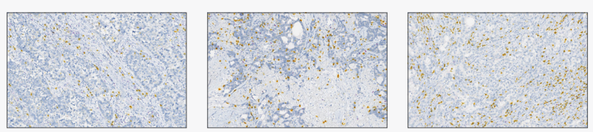
Immune Profiling Using Multiplex IHC Assays

CD8/CD68/FoxP3 triplex: General immune profile

CD8/Ki67 duplex: T-cell activation

CD8/CD3 duplex: T-cell ratio

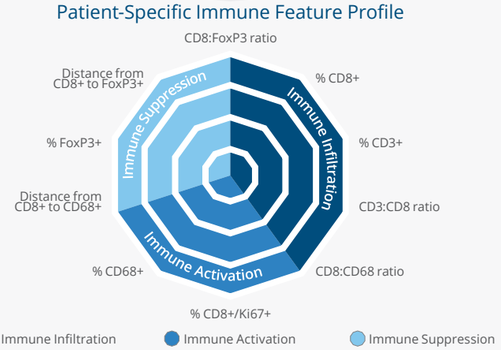
CD8/FoxP3 duplex: T-cell suppression



Patient A Low CD8 Expression | **Patient B** Moderate CD8 Expression | **Patient C** High CD8 Expression

Flagship's cTA Platform → Biofeatures™ Data

Patient-Specific Immune Feature Profile



©2018 Flagship Biosciences, Inc. • www.flagshipbio.com • info@flagshipbio.com

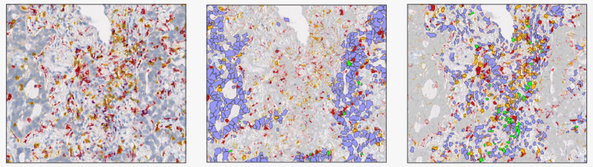
cTA Profiling of Multiplexed IHC in Tumor Biopsies

cTA distinguishes the tumor from tumor microenvironment (TME) without requiring tumor-specific stains.

Any immune marker is reliably distinguished and quantified in the tumor and TME compartments independently across a whole tissue section without requiring additional stains like pan-cytokeratin and without altering a typical IHC workflow for simplicity and scalability.

CD8/CD68/FoxP3 Triplex

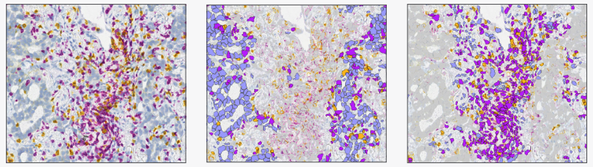
CD3+ Cell | CD68+ Cell | CD8+ Cell | FoxP3+ Cell | Ki67+ Cell | Negative Cell | Nonselected Cell



Digital Image | cTA Tumor Markup | cTA TME Markup

CD8/CD3 Duplex

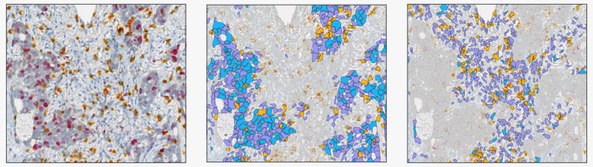
CD3+ Cell | CD68+ Cell | CD8+ Cell | FoxP3+ Cell | Ki67+ Cell | Negative Cell | Nonselected Cell



Digital Image | cTA Tumor Markup | cTA TME Markup

CD8/Ki67 Duplex

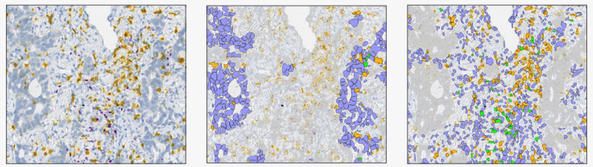
CD3+ Cell | CD68+ Cell | CD8+ Cell | FoxP3+ Cell | Ki67+ Cell | Negative Cell | Nonselected Cell



Digital Image | cTA Tumor Markup | cTA TME Markup

CD8/FoxP3 Duplex

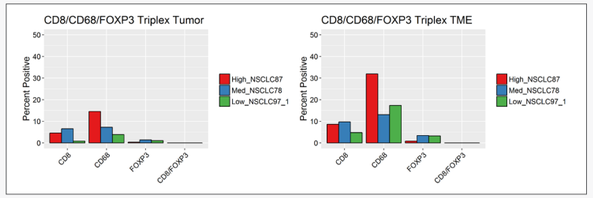
CD3+ Cell | CD68+ Cell | CD8+ Cell | FoxP3+ Cell | Ki67+ Cell | Negative Cell | Nonselected Cell



Digital Image | cTA Tumor Markup | cTA TME Markup

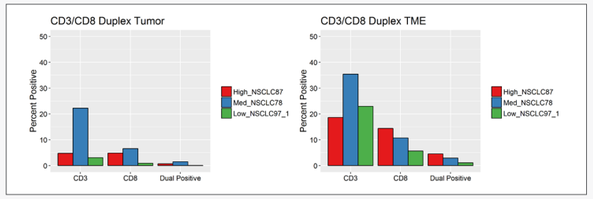
cTA-Reported Immune Content and Context Information

CD8/CD68/FoxP3 Triplex: General Immune Profile



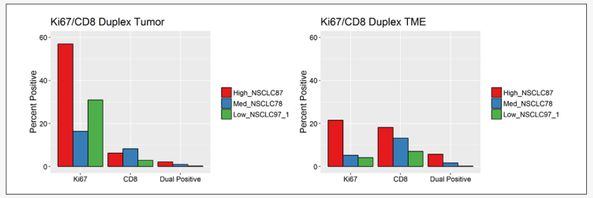
- Samples show differential CD8+, CD68+, and FoxP3+ infiltrates in the tumor syncytium and TME.
- Little information is captured in dual-positive cells (eg, CD8/FoxP3).

CD8/CD3 Duplex: T-Cell Ratio



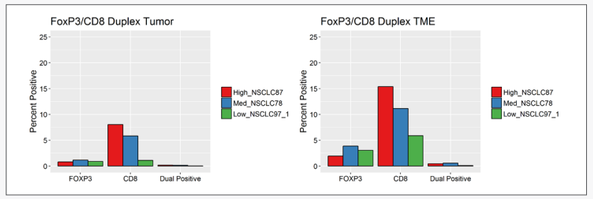
- Samples show differential CD3+, CD8+, and dual-positive infiltrates in the tumor syncytium and TME.
- Differential ratios of CD3 versus CD8 and double-positive infiltrates indicate an immune response.

CD8/Ki67 Duplex: T-Cell Activation



- Tumor Ki67 reflects cancer proliferation, whereas dual CD8+/Ki67+ infiltrates reflect an immune response.
- Key differences in dual CD8+/Ki67+ infiltrates in the tumor and TME may distinguish immune competency.

CD8/FoxP3 Duplex: T-Cell Suppression



- Samples show differential CD8+ but similar FoxP3+ infiltrates in the tumor syncytium and TME.
- Spatial relationships between FoxP3+ and CD8+ cells may capture the suppression phenotype better.

Developing Immune Response Profiles Using Biofeatures

Translating IHC Quantification Into Biofeatures Profiles

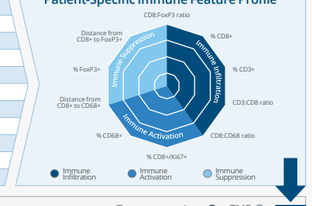
cTA Analysis of Multiplexed IHC in Tumor Biopsies

Wet Assays	**TYPE** Lineage, Activation state Phenotype	CD3, CD8, PD-L1, ETC.
Scoring	**ABUNDANCE** Number, Percentage Density	%POS Cells/mm² Ratios
Computation	**LOCATION** Adjacent/distal (tumor microenvironment/stroma), Compartment (nucleus, cytoplasm, membrane), Defined areas (intraepithelial, vasculature)	Flagship cTA
Biofeatures	**CONTEXT** Distance from other cell types, Clusters/Foci, Ratios	Flagship cTA

Biofeatures Profile

1. % CD8+
2. % CD68+
3. % CD3+
4. CD8:CD68 ratio
5. CD3:CD8 ratio
6. Distance from CD8 to CD68
7. % CD8+/Ki67+
8. % FoxP3+
9. CD8:FoxP3 ratio
10. Distance from CD8 to FoxP3

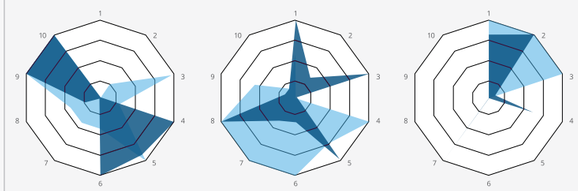
Patient-Specific Immune Feature Profile



Multiparametric Immune Response Patient Profile

Tumor syncytium ● TME ●

- Immune Infiltration
- Immune Activation
- Immune Suppression

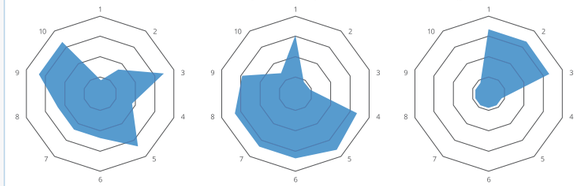


Patient A T-cell infiltration in tumor with indication of suppression | **Patient B** Active immune response in TME with little infiltration in tumor | **Patient C** Competent infiltration in tumor and TME

Immune Response Profile

Tumor vs TME Response Profile — Similarity between tumor and TME ●

- Plots show degree of similarity between tumor and TME values
- Key differentials show immune phenotype profiles by unique shape
- Plots distinguish resistant from responding immune profiles



Immune Tolerance Patient A: Suppressed Infiltrate | **Adaptive Resistance** Patient B: Excluded Infiltrate | **Immune Induction** Patient C: Competent Infiltrate

Conclusions

Multiparametric cTA profiles can capture key immune response phenotypes by:

- Utilizing multiplexed IHC images to profile pharmacodynamic changes of immune content and context in tumor biopsy samples.
- Combining multiple measurements to identify specific immune phenotypes.
- Incorporating spatial relationships of different cell types to represent biological behaviors of a patient's tumor that are key for predicting a response.