Utilizing Tissue Microarrays for Medium-Throughput Validation of Antibodies Against Immune Markers

Sujay Marisetty[1], Joseph R. Daniele, PhD[2], Annette Machado, PhD[2], Jing Han, PhD[2], Rocio Isabel Zorrilla-Veloz[3], Daniel B. Zamler*[1,3], Joe Marszalek*, PhD[2], Jian Hu*, PhD[3], and Giulio Draetta*, MD, PhD[1,2]

[1]Department of Genomic Medicine, The University of Texas MD Anderson Cancer, Houston, TX; [2]The Translational Research to AdvanCe Therapeutics and Innovation in ONcology (TRACTION), Houston, TX; [3]Department of Cancer Biology, The University of Texas MD Anderson Cancer, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Background:

- Novel immune biomarkers are constantly discovered, sometimes replacing old markers for identification.
- Antibodies – including those targeting immune markers – should be rigorously validated via methods such as immunohistochemistry (IHC) to ensure specificity.
- Single-tissue IHC staining and analysis is resource and time-consuming.
- Tissue Microarrays (TMAs) are an efficient platform to implement validation using multiple tissues, which allows technical replicates and on-slide controls.[1,2]

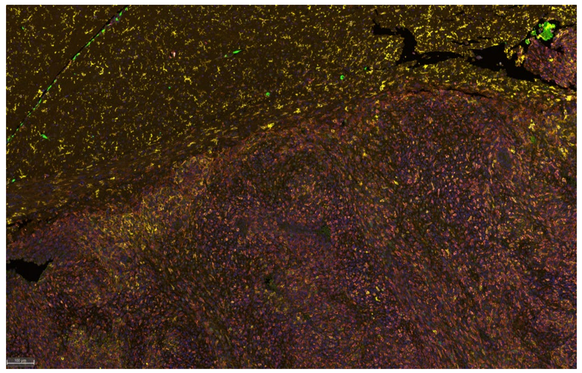
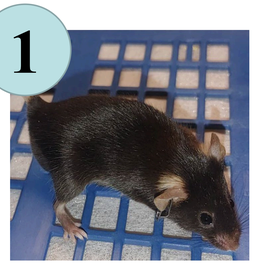
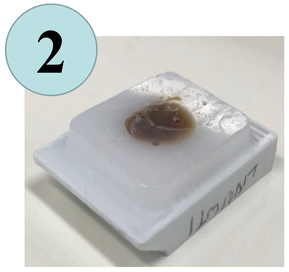


Fig 1. Sample Multiplex Stain of brain tumor border. Rigorous antibody validation has significant implications for many procedures, such as mIHC.

Methods:





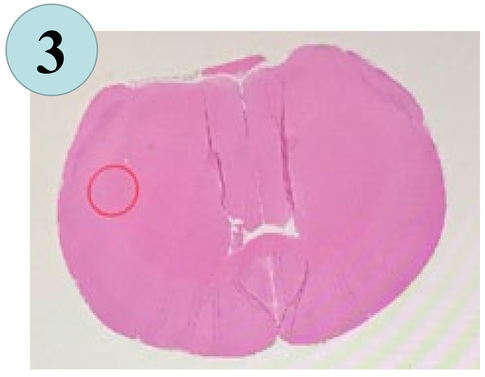


Fig 2. (Step 1-3). **(1) Image of C57BL/6J (wild-type) mouse.** Organs were harvested from 3 wild-type (WT) and 3 NSG mice. **(2) Paraffin-embedded WT mouse liver.** Paraffin-embedded single-organ blocks were sectioned. **(3) Sample QuPath annotation of WT mouse brain.** H&E-stained 5µm slices are scanned and resulting scans are annotated with QuPath.

Methods-cont.:

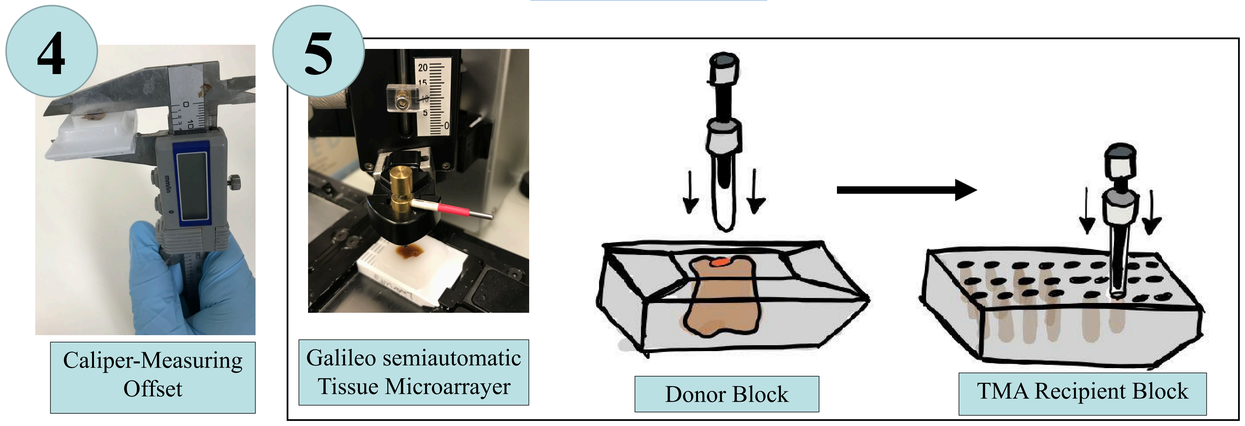


Fig 3. (Steps 4 -5). (**4) Caliper used to measure donor block offset.** Offsets of the donor blocks relative to the TMA recipient block were measured prior to tissue core extraction. **(5) TMA generation using the Galileo semiautomatic tissue microarrayer.** Cylindrical tissue cores were extracted from single-organ blocks (donor blocks) and inserted into the recipient TMA block.

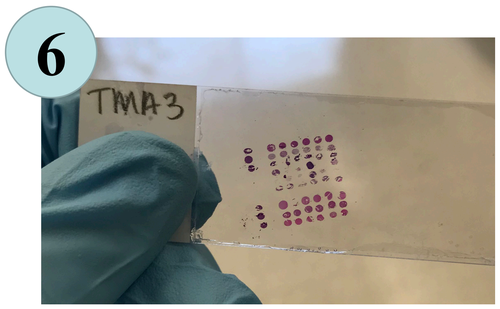


Fig 5. (Step 6). **Image of H&E stained TMA section.** 5 µm sections underwent IHC staining with respective antibodies.

Fig 6. (Step 7). **Image of Vectra Polaris.** Slides are scanned on H&E, Brightfield preset.

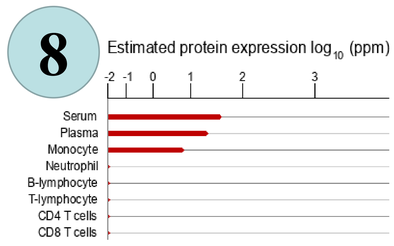


Fig 7. (Step 8). **Sample portion of GeneCards protein expression profile of CSF1R.** IHC-stained sections are analyzed using existing literature, as well as protein and gene expression databases. Adapted from GeneCards. (https://www.genecards.org/cgi-bin/carddisp.pl?gene=CSF1R).

Fig 6. Map of TMA design with 60-1mm cores. Tissues from WT mice and NSG mice are labeled blue and white, respectively. Spln – spleen. Liv – liver. Lng – lung. Panc – pancreas. Thy - thymus. LN – lmph node. Ret – retina. Brn – brain. Kid – kidney. Hrt – heart.

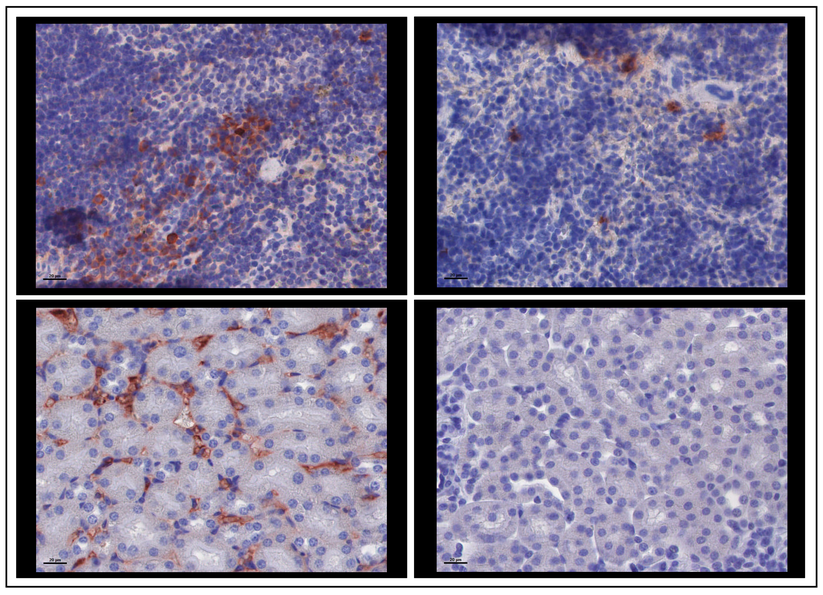


Fig 7. Panel of Anti-CD20 stained tissues (moderately specific). WT Spleen scanned at 20x (**Top left**). NSG spleen scanned at 20x (**Top right**). WT kidney scanned at 20x (**Bottom left**). WT Brain scanned at 20x (**Bottom right**).

Methods-cont.:

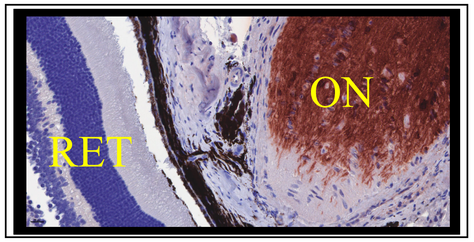


Fig 6. Image of Anti-MBP stained WT eye tissue (specific). RET – retina. ON – optic nerve.

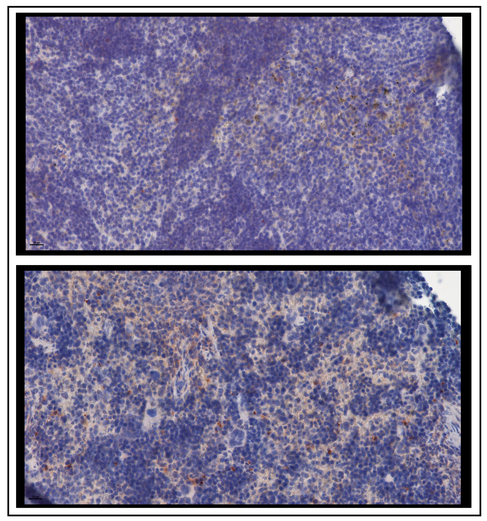


Fig 7. Panel of Anti-LAG3 stained tissues (nonspecific). WT Spleen at 20x – specific (**Top**). NSG spleen at 20x - nonspecific (**Bottom**).

Results:

We classified anti-CD20 as moderately specific because while the NSG spleen showed positive signal, the NSG kidney showed no signal as expected despite the positive signal in the WT kidney. We classified anti-CSF1R as moderately specific because the WT spleen showed positive signal, as expected, but the NSG spleen showed considerable background staining. We classified anti-LAG3 as nonspecific because the WT spleen shows positive signal, but the NSG spleen showed more positive signal in comparison to the WT spleen. Similarly, we classified anti-NCR1 as nonspecific because the WT spleen shows positive signal, but the NSG spleen showed more positive signal in comparison to the WT spleen. Lastly, we classified anti-FOXP3 as nonspecific because both the WT and NSG spleen show similar staining intensity.

Conclusions:

- TMAs mediate rapid validation of antibodies against immune markers, eliminating the need for single-tissue IHC staining and analysis.
- mIHC paired with TMA utilization could further increase the efficiency of validating antibodies.

References:

- 1. Zhang X, Su D, Cheng Q. Advantages and applications of tissue microarray technology on cancer research. Chin J Cancer Res. 2003;15(1):74-78. doi:10.1007/s11670-003-0017-z
- 2. Jawhar NM. Tissue Microarray: A rapidly evolving diagnostic and research tool. Ann Saudi Med. 2009;29(2):123-127. doi:10.4103/0256-4947.51806

Acknowledgements:

- The authors would like to thank Brittney Edwards, Kara Lewis, Khandan Keyomarsi, and Stephanie Watowich for facilitating this research through the CPRIT-CURE program and the NIH R25 Training Grant.