Optimization and validation of immunohistochemical assays for the detection of TREM2 and evaluation by image analysis in FFPE human tissue


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Optimization of a Multiplex IHC Assay (5-plex)

Abstract

Introduction: The tumor microenvironment (TME) often contains high levels of suppressive myeloid cells that may contribute to inactive and acquired checkpoint inhibitor (CPI) resistance. Pionyr’s Myeloid Tumor™ approach involves altering the composition and/or function of myeloid cells in the TME. Pionyr has identified the transmembrane protein Triggering receptor expressed on myeloid cells-2 (TREM2) as a highly enriched target on tumor-associated macrophages (TAMs) and has developed an anti-TREM2 therapeutic monoclonal antibody, termed P314, which is currently being tested in a Phase 1 clinical trial (NCT04591379). To select patients that would most likely benefit from P314 therapy, Pionyr has developed a TREM2 immunohistochemical (IHC) assay for the detection of TREM2 in formalin-fixed, paraffin-embedded (FFPE) human tissue samples. Moreover, to better understand TREM2 expression localization within the tumor, and spatial interaction with other immune cells, a multiplex immunofluorescence IHC panel was developed.

Methods: The monoplex TREM2 IHC assay was optimized and validated at Mosaic Laboratories, a CLIA-licensed and CAP-CLIA-accredited laboratory, using an anti-TREM2 antibody on the Leica Bond Rx platform. Optimization included control selection, pretreatment selection and antibody titration experiments. The assay was validated for sensitivity, specificity, and inter-day precision. Tissues were evaluated by standard manual pathology testing. TREM2+ TAMs and their spatial relationship with other immune cells present in the TME to determine what immune composition will be more favorable for patient response to P314 therapy. This assay may also be used to follow changes in the TME associated with P314 treatment in pre- and post-tumor biopsies.

Optimization of the Monoplex TREM2 IHC Assay

The TREM2 IHC assay was optimized using the HEK293-TREM2 (human embryonic kidney-293 cell expressing human TREM2) human tumor xenograft, FFPE HCC-237 cell line was used as a positive control (TREM2 expressing) and negative (no TREM2 expressing) controls, and an ovarian cancer cell line was used as a negative control tissue. Titration experiments determined the optimal plasma concentration of Anti-TREM2 6F1 antibody at a concentration of 0.5 µg/mL started on the Leica Bond Rx to be optimal using high pH antigen retrieval.

Results: The monoplex TREM2 IHC assay was successfully optimized and validated at Mosaic Laboratories and showed robustness with sensitivity, specificity, and precision following pathologist guided image analysis. The optimal anti-TREM2 staining concentration was 0.5 µg/mL with a high-pH antigen retrieval. The inter-day precision assay was performed on a representative sample and was shown to be highly reproducible. The TREM2+ TAMs and their spatial relationship with other immune cells present in the TME determined what immune composition will be more favorable for patient response to P314 therapy. This assay may also be used to follow changes in the TME associated with P314 treatment in pre- and post-tumor biopsies.

Conclusions: Screening for TREM2 expression using the IHC assay demonstrated that TREM2+ TAMs were deeply enriched in the TME of the prioritized solid tumor indications currently being pursued in the P314 Phase 1a clinical trial. The monoplex TREM2 IHC assay is successfully being used on FFPE archival tumor tissues from enrolled patients to determine TREM2 expression. The multiplex IF assay is offering insights into the localization of TREM2+ TAMs and their spatial relationship with other immune cells present in the TME to determine what immune composition will be more favorable for patient response to P314 therapy. This assay may also be used to follow changes in the TME associated with P314 treatment in pre- and post-tumor biopsies.

Validation of the TREM2 IHC Assay : Inter-day precision, Sensitivity, and Specificity

Inter-day precision and manual versus guided image analysis scoring

The IHC inter-day precision analysis was performed in 2 ovarian and 2 breast cancer tissues stained on 5 separate slides, which were evaluated by manual and computer-aided and guided by pathologist guided image analysis. (A) The assay showed robust and similar staining across different slides with an average %CV (coefficient of variation) of 34.59% for manual scoring and 22.01% for digital scoring suggesting that pathologist guided image analysis is a robust and reproducible method for TREM2 scoring. (B) Digital scoring using the HALO image analysis tool shows accurate detection of TREM2 DAB staining.

Sensitivity analysis

The sensitivity of the TREM2 IHC assay was tested on 107 archival archival tumor tissues from Mosaic Laboratories and evaluated by pathologist guided image analysis. (A) Representative IHC images (20x) of high and low TREM2 expression on FFPE tissues from various tumor indications stained with the anti-TREM2 antibody. Note: Due to tissue size and heterogeneity of staining, images may not be truly representative of the analysis.

Specificity analysis

The specificity of the TREM2 IHC assay was tested on 3 colorectal tumor samples. (A) Schematic diagram showing the percentage of positive TREM2 expression for 3 colorectal tumor samples. (B) Representative IHC images (20x) of high and low TREM2 expression on FFPE tissues from various tumor indications stained with the anti-TREM2 antibody. Note: Due to tissue size and heterogeneity of staining, images may not be truly representative of the analysis.

Using Image Analysis to Understand Spatial Localization of TREM2 in Relation to Other Markers in the Tumor Microenvironment

Using our multiplex IHC assay we can study the composition of different cell phenotypes, including TREM2, in the tumor and stroma compartments. This is exemplified in Table 1 where we compared the TREM2 expression data with collagen IV and DAB (CD8+) expression, which are known to be markers for immune infiltration and stromal composition. The median distance is also much larger between a TREM2+ cell and CD8+ cell in the tumor compartment compared to the stromal compartment.

Summary & Acknowledgements

- TREM2 expression using the TREM2 IHC assay demonstrated that TREM2+ TAMs were highly enriched in the TME of the prioritized solid tumor indications currently being pursued in the P314 phase 1a clinical trial.
- The monoplex TREM2 IHC assay is successfully being used on FFPE archival tumor tissues from enrolled patients to determine TREM2 expression.
- The multiplex IF assay is offering insights into the localization of TREM2+ TAMs and their spatial relationship with other immune cells present in the TME to determine what immune composition will be more favorable for patient response to P314 therapy.
- We want to thank the PSY314 R&D and clinical teams at Pionyr Immunotherapeutics and our collaborators at Mosaic Laboratories (Forest Lake, CA) for their work on the optimization and validation of the anti-TREM2 IHC assay and for screening FFPE tumor tissues of patients enrolled in the PIONYR Phase 1 clinical trial.