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

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Abstract

Introduction: The tumor microenvironment (TME) often contains high levels of suppressive myeloid cells that may contribute to invade and acquired checkpoint inhibitor (CPI) resistance. Pionyr’s Myeloid Tumour™ approach involves altering the compartment and/or the 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 (NCT04961575). 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 multiplex TREM2 IHC assay was optimized and validated at Mosaic Laboratories, a CLIA-licensed and CAP-accredited laboratory, using an anti-TREM2 antibody on the Leica Bond Rx platform. Optimization included control selection, pretreatment selection and antigen detection experiments. The assay was validated for sensitivity, specificity, and inter-day precision. Tissues were evaluated by standard manual pathology review and pathologist guided image analysis using HALO (Indica labs). In a multiplex immunofluorescence (IF) assay, Mosaic’s antibody library was optimized and validated at Pionyr to include the anti-TREM2 antibody in a 5-plex panel (DAPI, TREM2, CD8, PD-L1, and pan-C6). Tissues were scanned on the Vectra 3 microscope (Akoya) and images were further processed digitally and analyzed using Immifore software.

Results: The multiplex TREM2 IHC assay was successfully optimized and validated at Mosaic Laboratories and achieved excellent sensitivity, specificity, and precision following pathologist guided image analysis. The optimal anti-TREM2 staining concentration was 1 μg/mL, with a high-µg/mL antigen release. The inter-day precision assay demonstrated that guided image analysis was more robust and reproducible than a standard manual pathology review for determining the percentage TREM2+ cells in the total tumor area. TREM2 expression was observed in direct correlation with the presence of PD-L1 in the tumor and stroma. The anti-TREM2 antibody was successfully integrated and validated into the Akoya’s 5-plex multiplex panel.

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 multiplex 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 compartment will be more favorable for patient response to P314 therapy. This assay may also be used to follow changes in the TME associated with PY314 treatment in preclinical models.

Optimization of the Monoplex TREM2 IHC Assay

The TREM2 IHC assay was optimized using the anti-TREM2 human antibody, FFPE HEK-293 cell line was used as the positive control, and normal colon tissue was used as negative control. Tissue sections for both TREM2 expressing and negative (GFP expressing control) were stained with antibodies against TREM2 and GFP, respectively. Tissue experiments demonstrated the necessity of antigen retrieval at a concentration of 0.5 µg/mL, stained on the Leica Bond Rx to be optimal using high pH antigen retrieval.

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

The TREM2 inter-day precision analysis was performed in 2 ovarian and 2 breast cancer tissues stained on 5 separate days, which were evaluated by using the anti-TREM2 positive cell and scored manually and digitally by pathologist guided image analysis. The 2D analysis showed robust and similar staining across different days with an average ±SD (coefficient of variation) of 34.50% for manual scoring and 22.33% for digital scoring. The concordance of the 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 IHC staining.

Optimization of a Multiplex IHC Assay (5-plex)

A multiplex immunofluorescence (F) assay using Akoya’s Opal™ reagents was optimized and validated at Pionyr to include the anti-TREM2 antibody in a 5-plex panel. Tissues were scanned on the Vectra 3 microscope (Akoya) and images were further processed digitally and analyzed using Immifore software.

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 (A) where we compared the distribution of TREM2+ cells across multiple tumor locations. The data demonstrated that the median distance to T-cell and CD8+ cell in the tumor compartment was significantly greater than the distance to CD8+ cell in the stroma 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 multiplex 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 compartment will be more favorable for patient response to P314 therapy.

We want to thank the P314 R&D and clinical teams at Pionyr Immunotherapeutics and our collaborators at Mosaic CAP-CIA (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.

References

1. To optimize and validate TREM2 DAB IHC assay that could aid in the selection of patients that may benefit of P314 therapy.
2. To optimize and validate a multiplex IHC assay to better understand the TREM2 expression, localization within the TME, and spatial interaction with other immune cells.

Abstract: TREM2 is an immunoreceptor expressed on TAMs. PY314 is a humanized mAb that specifically binds TREM2 and ‘re-tunes’ the TME via targeted depletion of TREM2+ TAMs via antibody-dependent cell-mediated cytotoxicity and/or cellular phagocytosis. P314 is currently in Phase 1a clinical trial in advanced solid tumors relapsed or refractory to standard of care (NCT04961575).