Validation of Brightplex, a multiplex IHC solution for immune cell phenotyping of the tumor microenvironment

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Background

- Multiplex immunohistochemistry is developing rapidly to respond to the demand of pharma industry for analyzing the complexity of the tumor microenvironment in situ.
- Brightplex, a chromogenic multiplex approach was recently developed by HalioDx to assess immune cells phenotyping on FFPE tumor tissue sections combining the power of multiplex IHC and the ease of chromogenic HIC.
- Among others, a panel called IMMUNOSCORE® was developed to quantify the exhaustion of tumor infiltrating lymphocytes.
- In the present work, data on the analytical validation of Brightplex, a multiplex IHC solution using an automated sequential chromogenic IHC approach (Brightplex) is reported to highlight the performance and robustness of Brightplex.

Methods

- Brightplex: A panel of 5 markers CD3, CD8, PD-1, TIM-3 and LAG-3 (IMMUNOSCORE® TCE) on NSCLC tissue was evaluated.
- Analytical validation:
  1. Evaluation of intra-run, inter-run, inter-operator (IHC) and inter-analyst (Digital Pathology, DP) variability.
  2. Comparison of the accuracy of automated identification of cell phenotype to a trained reader (reference method).
  3. Evaluation of the potential loss of staining intensity to determine the best staining sequence.
  4. Verification of the efficiency of the destaining/stripping step.
  5. The Brightplex chromogen was compared to the DAB chromogen.

Previous related work

1. T cell exhaustion assessment using an automated sequential chromogenic multiplex assay (Brightplex).
2. Myeloid-derived suppressor cells (MDSC) assessment using an automated sequential chromogenic multiplex assay (Brightplex).

Poster presentation at ASCO18
Poster presentation at SITC2018

Precision and accuracy

- Accuracy: 50 areas from 3 NSCLC tumors (3 slides/sample) were assessed by digital pathology (DP). The accuracy of the automated cell detection was compared to a trained pathologist (reference method).
- Concordance of both detection was assessed for each phenotype of interest.
- Acceptance criteria: PPV >75%.
- The following metrics were computed:
  - Sensitivity:
    - True Positive Cells (TP) / All Positive Cells (TP+FP)
    - All DP Positive Cells (TP+FP) / All TP+FP
  - PPV:
    - True Positive Cells (TP) / All Positive Cells (TP+FN)
    - All DP Positive Cells (TP+FP) / All TP+FP
  - PPV Sensitivity mean (PPV x Sensivity)

Comparison of Brightplex with DAB

Brightplex represents a powerful and reliable tool to quantify cell populations of complex phenotypes within the TME:
- Low improvement even at very low cell density
- Excellent accuracy of the digital pathology tool compared to reference
- Fast customization of complex panels
- Simple instrumentation requirements
- Brightfield analysis allowing control by a pathologist
- Cell densities with Brightplex comparable to DAB chromogen
- Accelerated method development compared to other multiplex technologies