SignalStar™ multiplex immunohistochemistry is a novel technology capable of amplifying the signal of multiple biomarkers in the tumor microenvironment.

**Background**

There is a growing need to characterize multiple targets simultaneously in the tissue microenvironment (TME) in order to identify novel biomarkers that can be used to drive precision medicine. Multiplex immunohistochemistry (mIHC) enables the imaging and quantification of multiple biomarkers at a single cell level while retaining spatial context. SignalStar™mIHC by Cell Signaling Technology® is a fluorescent multiplexing approach that amplifies the signal of up to 8 protein biomarkers in FFPE tissues.

**Methods**

SignalStar oligo-conjugated antibodies were applied simultaneously to label markers of T cell phenotype and function (CD8, PD-1, TIM-3, LAG3, TACO/Tox, TCF1/TCF7, and Granzyme B) within the spatial context of the Pan-Keratin+ tumor. The signal of the first four targets was amplified and visualized, followed by removal of fluorescent signal, and the signal of 4 additional antibodies were amplified individually. In parallel, the CD8 and LAG3 were substituted into the 8-plex mIHC panel without additional protocol optimization required. The SignalStar assay underwent rigorous validation on tissue microarrays and whole tissues with diverse target expression levels to ensure assay reproducibility and dynamic range. SignalStar staining was compared to the chromogenic gold standard, tyramide signal amplification (TSA), indirect fluorescent, and direct fluorescent labeling, and quantified with respect to signal intensity and frequency.

**Results**

Our data demonstrates consistent staining with SignalStar mIHC compared to the chromogenic DAB in serial sections across replicates. SignalStar amplification enabled detection of targets in tissues with high, medium, and/or low levels of target expression, and produced signal intensity comparable to TSA while substantially greater than direct or indirect immunofluorescence.

**Conclusions**

Multiplex IHC is ideal for maximizing the data acquired from limited FFPE tissue, and can provide a detailed map of the spatial distribution and expression patterns of multiple targets. Current spatial imaging techniques limit forward progress due to the time-consuming methods needed for assay design and antibody panel development, optimization, validation and analysis.

Our data demonstrates SignalStar mIHC can eliminate these hurdles by providing customizable and highly validated antibody panels that work right out of the box. Here, the ability to characterize biomarkers of T cell exhaustion with ease and flexibility is demonstrated, enabling powerful phenotypic characterization with respect to signal intensity and expression patterns of multiple biomarkers. As new targets are identified and research needs evolve, researchers can switch out targets and quickly redesign panels without the need for additional panel or protocol optimization, as SignalStar antibodies and fluorophores are interchangeable.

Importantly, SignalStar provides amplification of signal for detection of targets with low expression. Our data indicates SignalStar can enable detection of low abundance proteins that may be beyond the detection limit for direct and indirect immunofluorescence.