Anti-PD-1/PD-L1 are now established agents in the clinical management of advanced NSCLC patients. Although PD-L1 role in the immune response to cancer, could also be predictive of the response to Immune Checkpoint Inhibitors (ICI). In addition to their co-presence, the proximity between PD-L1+ and CD8+ cells in the tumor microenvironment is correlated to the response to ICI treatment in melanoma (Tumeh, P. C. et al, Nature, 2015). Determination of this relative proximity, as evidence of a physical interaction between PD-L1+ and CD8+ cells, might provide complementary information to stratify NSCLC patients and adapt therapeutic strategy.

**Method**

PD-L1 clinical approach: A total of 46 NSCLC tumors were analyzed with three PD-L1 IHC commercial assays: dual staining with Halioseek™ PD-L1/CD8 (HalioDx), single staining with 22C3 (Dako) and SP263 (Ventana). An expert trained in interpreting PD-L1 staining according to recommendations of each manufacturer estimated the percentages of PD-L1+ tumor cells (TC).

**Digital pathology analysis:** To evaluate the spatial relationship between PD-L1+ and CD8+ cells, Halioseek™ PD-L1/CD8 double-stained virtual slides were analyzed with a proprietary software for automated recognition and localization of tissue, antracence, PD-L1 and CD8 staining and quantification of PD-L1+ cells.

Three outputs are generated for each sample: CD8+ cell density, Proximity index between CD8+ cells and PD-L1+ staining, CD8+ cells clustering index.

**Correlation between Halioseek™ PD-L1/CD8 and commercial PD-L1 tests**

- Virtual slides (x20) of 5 NSCLC samples stained with Dako 22C3 (top), Halioseek™ PD-L1/CD8 dual stain (middle) or Ventana SP263 (bottom).
- Agreement between methods assessed at 1% and 50% cut-offs.
- Proximity index between CD8-positive cells and PD-L1 staining.
- References

**Conclusions**

- Halioseek™ PD-L1/CD8 is highly correlated to existing commercial PD-L1 assays across a wide range of PD-L1 expression.
- High overall agreement, sensitivity and specificity with both SP263 and 22C3 commercial assays suggest equivalent clinical (diagnostic) sensitivity of 95% and 90% cut-off values.
- The detection and quantification of CD8-positive cell density on the same slide may improve the stratification of NSCLC patients.
- CD8/PD-L1 proximity assessment may be useful to further refine that stratification.

**In summary:**

- The accurate PD-L1 expression biomarker.
- The quantification of PD-L1+ TC with Halioseek™ PD-L1/CD8 compared with 22C3 (Dako) and SP263 (Ventana) commercial tests.
- Discordant samples analysis: Three negative samples (<1%) with Halioseek™ PD-L1/CD8 test combines on a single slide: Halioseek™ digital pathology software establishes a proximity index based on the measure of the distance between CD8+ cells and PD-L1 signal.
- A proximity index between PD-L1+ and CD8+ cells.