Halioseek®, a dual CD8 and PD-L1 IVD assay to improve NSCLC patients stratification

Florence Monville[1], Emmanuel Prestat[1], Laurent Vanhille[1], Julien Adam[2], Sergiu Coslet[3], Caroline Laugé[1], Caroline Davin[1], Merle Sophie Losch[1], Anne Legrand[1], Nicolas Brandone[4], Luciana Batista[1], Jacques Fieschi[1]

1 - HalioDx, Marseille, France I **2** - Gustave Roussy Cancer Campus, Villejuif, France I **3** – MI-mAbs, Marseille, France I **4** - Service de pathologie, Hôpital Nord, Marseille, France.

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Background

Immune checkpoint inhibitors (ICI) can dramatically improve the survival of non-small cells lung cancer (NSCLC) patients. ICI administration is decided according to the expression level of PD-L1 on tumor cells assessed by pathologists despite limited predictive value[1]. Recent studies show that the density of tumor-infiltrating lymphocytes (TILs), especially CD8-positive lymphocytes, may also be a predictive marker in several cancers, including NSCLC[2–9]. Moreover, the proximity between PD-L1+ and CD8+ cells in the tumor microenvironment is correlated to the response to ICI treatment in melanoma[10,11].

We have developed Halioseek® PD-L1/CD8, a standardized dual-staining IVD assay which, in addition to PD-L1 detection, provides critical information on the immune infiltrate through the detection of CD8+ cells on the same tissue section. Halioseek® PD-L1/CD8 includes a Digital Pathology (DP) analysis module to determine PD-L1+ and CD8+ cell densities as well as the proximity between these cell types. Here we show the main analytical performance of Halioseek® and concordance with two PD-L1 IVD assays.

1. Yu H *et al.* J. Thorac. Oncol. 2016
2. Takahashi K *et al.* Anticancer Res. 2017
3. Conde E *et al.* Histopathology. 2017
4. Velcheti V *et al.* Lab. Investig. 2014
5. Schalper KA *et al.* J. Natl. Cancer Inst. 2015
6. Zhuang X *et al.* Mol. Morphol. 2010
7. Parra ER *et al.* Clin. Cancer Res. 2016
8. De Meulenaere A *et al.* Oncotarget. 2017
9. Hiraoka K *et al.* Br. J. Cancer. 2006
10. Tumeh PC *et al.* Nature. 2014
11. Teng MWL *et al.* 2015

Halioseek® PD-L1/CD8 precision for PD-L1+ TC quantification

The precision of Halioseek® PD-L1/CD8 for PD-L1+ tumor cells quantification was assessed according to LA28-A2 standard on 11 NSCLC tumor resections representative of distinct PD-L1 expression level. 30 consecutive slides per sample were stained across 14 IHC runs on 2 Benchmark XT instruments with 3 batches of antibodies and of revelation kit. 318 NSCLC slides were dual-stained and randomized prior to PD-L1+ TC % assessment by a pathologist. For each class, Fisher's exact tests were performed.

Figure 1: Distribution of the PD-L1+ TC % across the 11 samples covering the assay range.

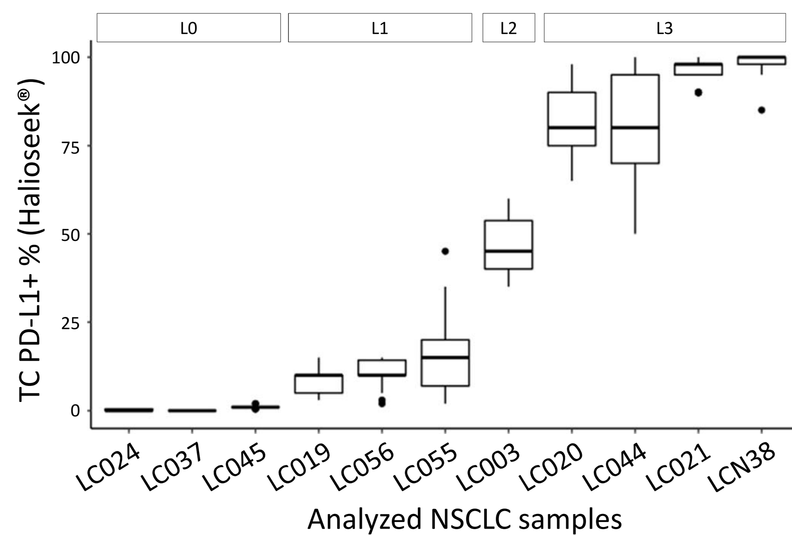
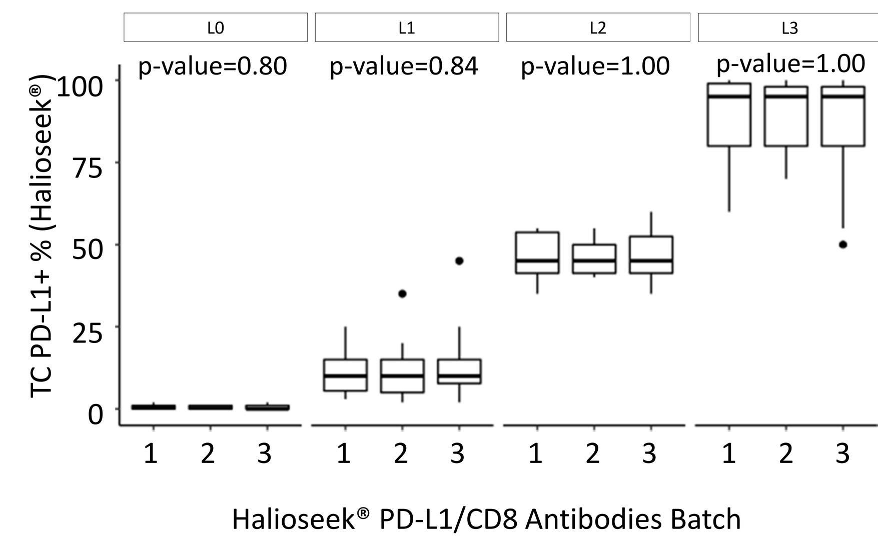


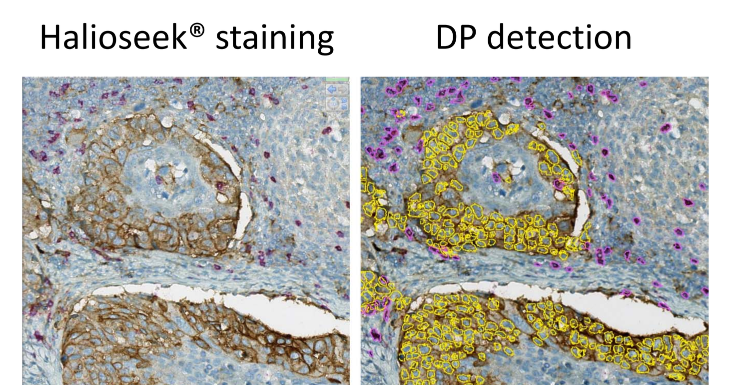
Figure 2: Distribution of PD-L1+ TC % for each Halioseek® batch at 4 levels of PD-L1 expression.



None of the tested variables had an impact on PD-L1 result according to Fisher's exact test.

Halioseek® PD-L1/CD8 Digital Pathology tool performance

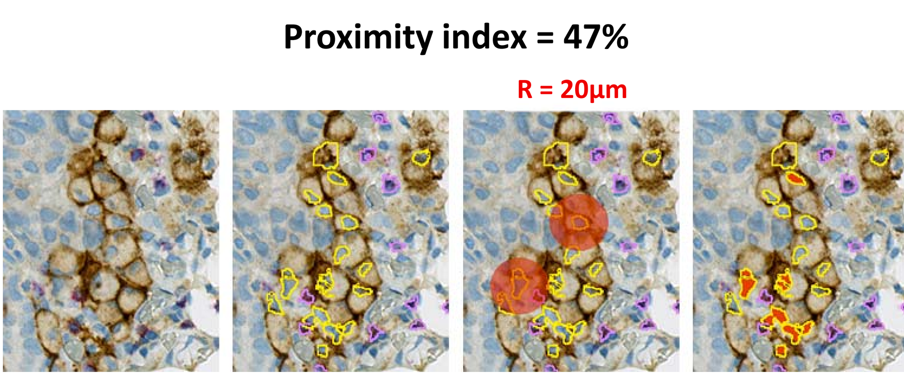
Figure 3: CD8+ and PD-L1+ cell detection



PD-L1+ cell nucleus detected by Halioseek® software

CD8+ cell detected by Halioseek® software

Figure 4: Proximity index calculation between CD8+ and PD-L1+ cells.



20µm radius circles surrounding PD-L1+ cells

PD-L1+ cell with at least 1 CD8+ cell at less than 20µm

Figure 5: Detection Accuracy. 34 zones from 25 NSCLC samples (CD8) and 22 zones from 11 NSCLC samples (PD-L1) were analyzed: pathologist absolute cell count of positive cells is highly correlated to DP cell count for both CD8 and PD-L1.

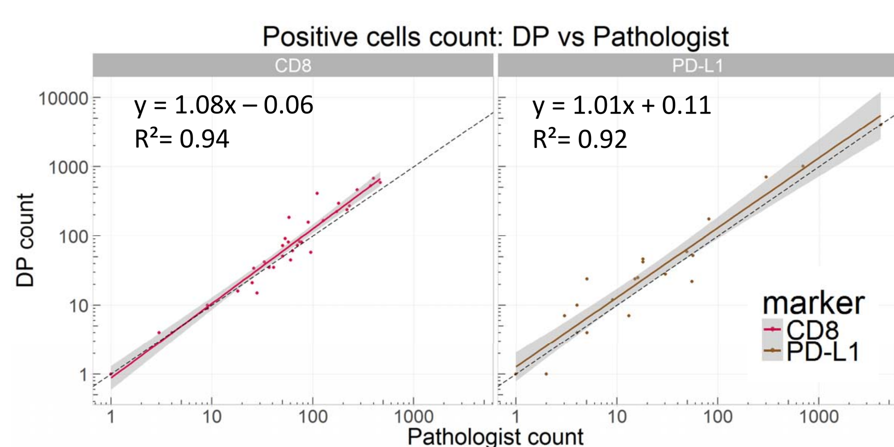
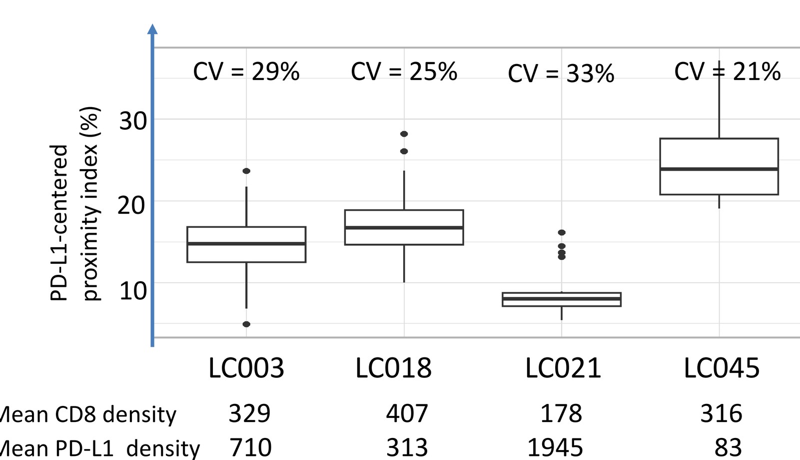


Figure 6: Precision of the proximity index. 21 to 28 consecutive slides from 4 NSCLC samples were stained across 4 IHC runs with 3 antibody batches, 3 revelation kit batches on 2 Benchmark XT instruments.



Comparison of Halioseek® PD-L1/CD8 to SP263 and 22C3 assays on 216 NSCLC samples

216 NSCLC tumors were analyzed with three PD-L1 IHC assays: Halioseek® PD-L1/CD8, DAKO 22C3 and VENTANA SP263. Two pathologists participated to the study. For each sample, the same pathologist interpreted the three slides, according to manufacturers' instructions. PD-L1+ TC % obtained with each test were confronted to each other.

Figure 7: Digital slides (x20) of a NSCLC sample stained with Dako 22C3, Halioseek® PD-L1/CD8 and Ventana SP263 assays.

Figure 8: Concordance between Halioseek® PD-L1/CD8 and SP263 (A) or 22C3 (B) at 1% and 50% cut-off.
The 11 false positive samples at the 1% cut off are ≤5%. Of 12 false negative samples, one is common to SP263 and 22C3. Only two are >5% with 22C3 only. The analysis based on the 1% and 50% clinical cut-off shows high concordance between Halioseek® PD-L1/CD8 and the two other CE-IVD tests.

A: Halioseek® PD-L1/CD8 vs SP263

1 % cut-off

		< 1%	≥ 1%
Halioseek®	< 1%	101	2
	≥1%	3	110

Overall agreement [95%CI]: **97.7 [94.7 - 99.0]**

50 % cut-off

		< 50%	≥ 50%
Halioseek®	< 50%	162	2
	≥ 50%	0	52

Overall agreement [95%CI]: **99.1 [96.7 - 99.7]**

B: Halioseek® PD-L1/CD8 vs 22C3

		< 1%	≥ 1%
Halioseek®	< 1%	83	11
	≥1%	8	114

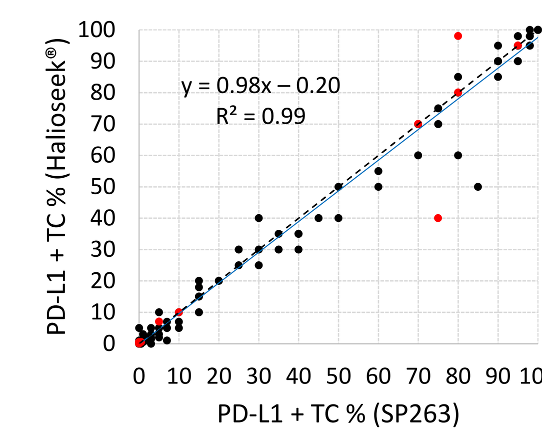
Overall agreement [95%CI]: **91.2 [86.7 - 94.3]**

		< 50%	≥ 50%
Halioseek®	< 50%	163	7
	≥ 50%	2	44

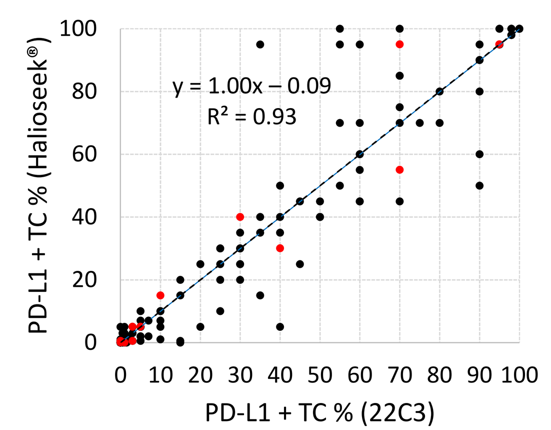
Overall agreement [95%CI]: **95.8 [92.3 - 97.8]**

Figure 9: PD-L1+ TC % determined with Halioseek® PD-L1/CD8 vs. SP263 (A) or 22C3 (B) on tumor Resections (black) and Biopsies (red).
The blue line depicts Deming regression curve and the black dashed line corresponds to y=x.
PD-L1+ TC % assessed with Halioseek® PD-L1/CD8 is highly correlated with both 22C3 and SP263 IVD assay results.

A: Halioseek® PD-L1/CD8 vs SP263



B: Halioseek® PD-L1/CD8 vs 22C3



108 NSCLC samples stratification with Digital Pathology outputs

Figure 10: **The distribution of PD-L1+ TC % and CD8+ cell densities across 108 NSCLC samples.** PD-L1+ TC % was assessed by pathologists. CD8+ cell density within the tumor area was determined by DP. Different T cell infiltration levels are observed for NSCLC samples of same PD-L1 status.

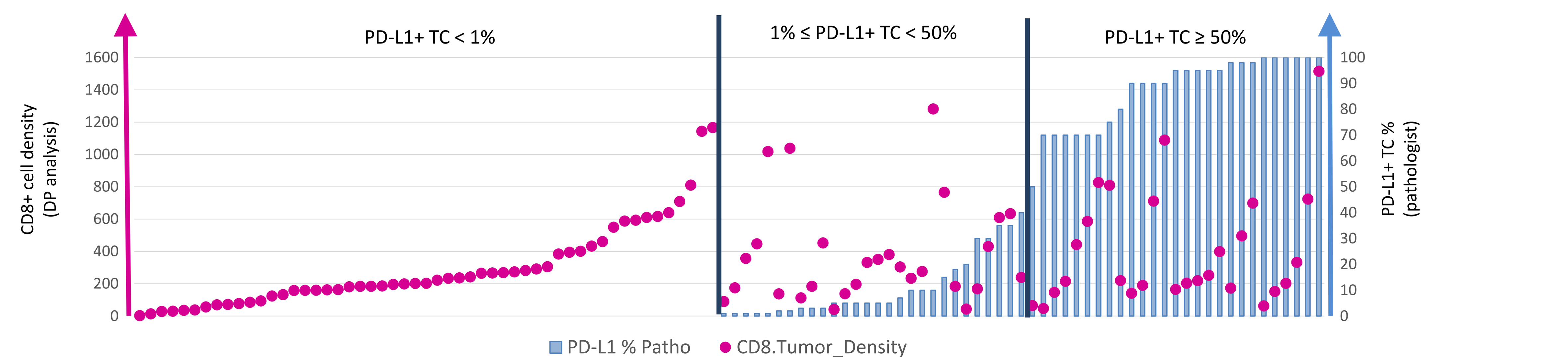
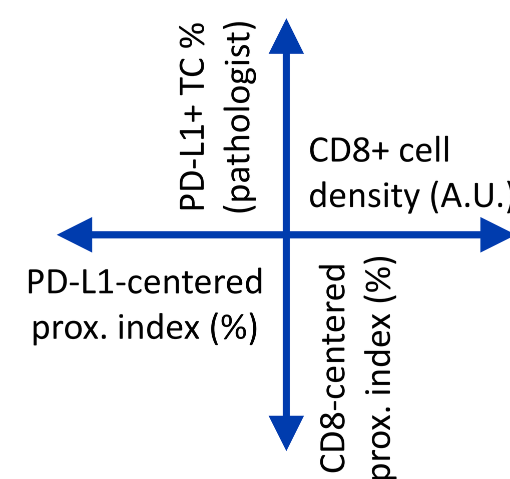
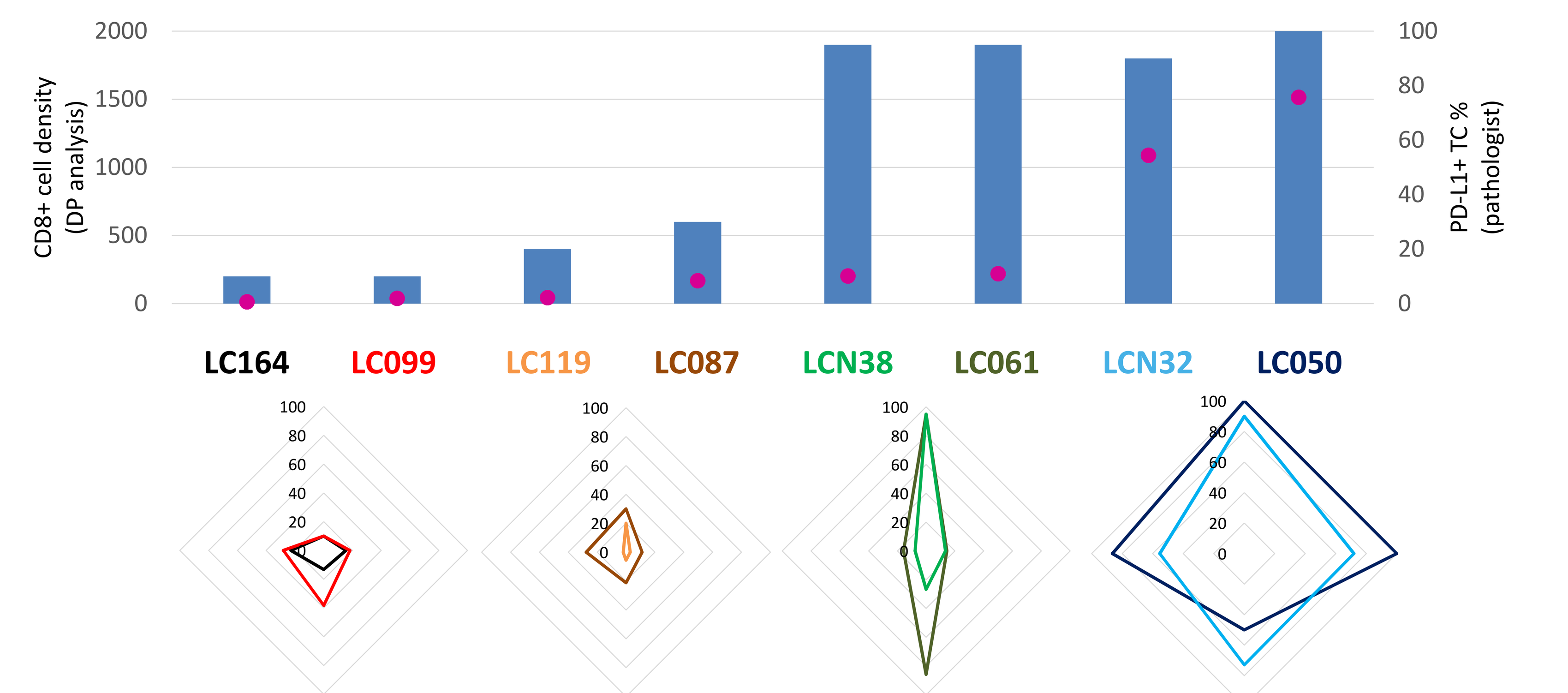


Figure 11: Examples of distinct proximity indexes for samples with similar PD-L1 and CD8 levels, showing that proximity indexes could allow further stratification of patients.





Conclusion

Halioseek® PD-L1/CD8 assay:

- is **equivalent to other validated IVD assays** SP263 (Ventana) and 22C3 (Dako). Therefore it can be used by clinicians for therapeutic indications in NSCLC.
- allows to **quantify CD8** T-cells infiltration on the same slide.
- provides **additional digital pathology tools** which could further improve patients selection for treatment with ICI.

Halioseek® PD-L1/CD8 is a new robust IVD assay leveraging the advantages of DP to combine TILs and PD-L1 quantification within the tumor microenvironment.
Halioseek® PD-L1/CD8 could have a higher predictive performance than existing IVD tests and could fill a major gap in the management of ICI administration. In a next step we intend to investigate the predictive value of the assay on samples from ICI treated patients.