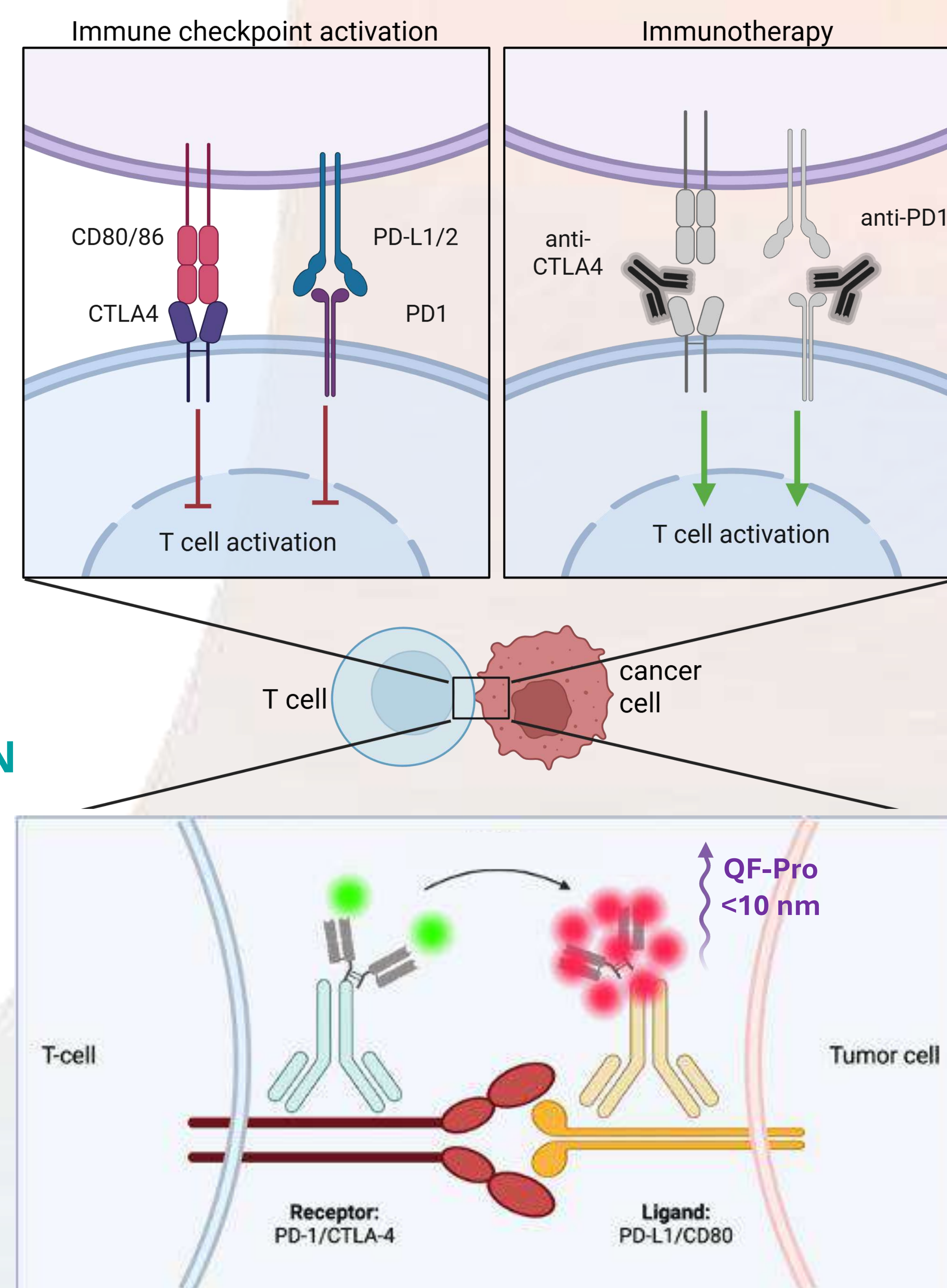


Immune Checkpoint Crosstalk: CTLA-4/CD80 Engagement as a Predictor of Anti-PD-1/PD-L1 Therapy Outcome in NSCLC

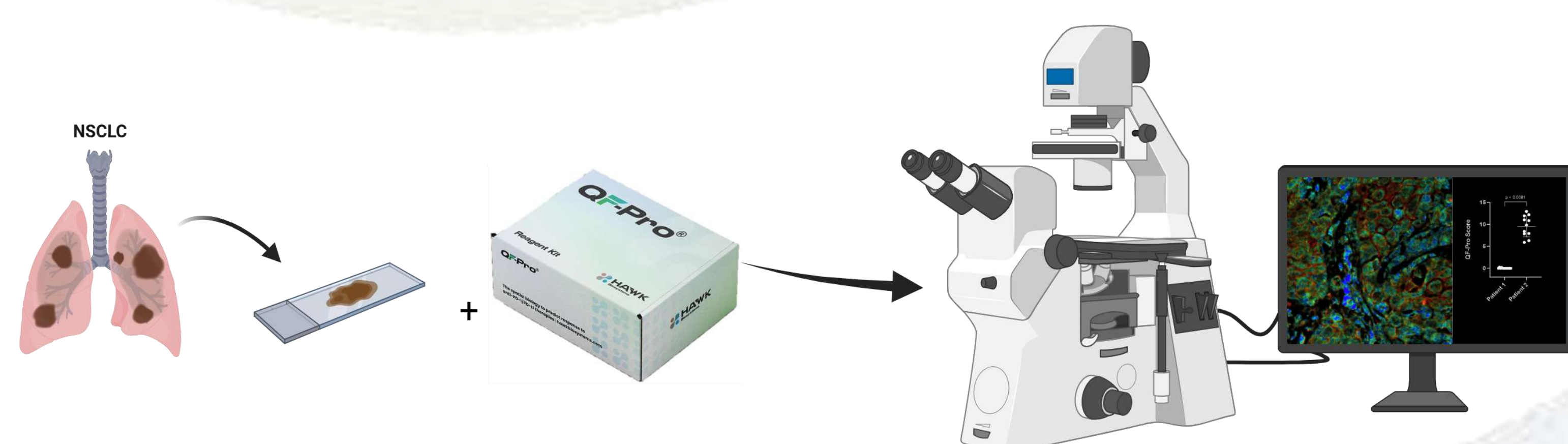
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- NSCLC, the lung cancer subtype with the **poorest outcomes**, exploits immune checkpoints such as **CTLA-4/CD80** and **PD-1/PD-L1** to **evade immunity**.
- ICIs targeting **PD-1/PD-L1** AND **CTLA-4/CD80** improve survival, but many patients do not respond, and current biomarkers -like PD-L1 TPS- are unreliable.
- In previous studies (*Sánchez-Magraner L. et al. 2023*) the predictive capacity of **PD-1/PD-L1** interaction vs **PD-L1 TPS** was assessed.
- This study explores whether the **additional information of immune crosstalk with CTLA-4/CD80** can better predict responses to ICI therapy.

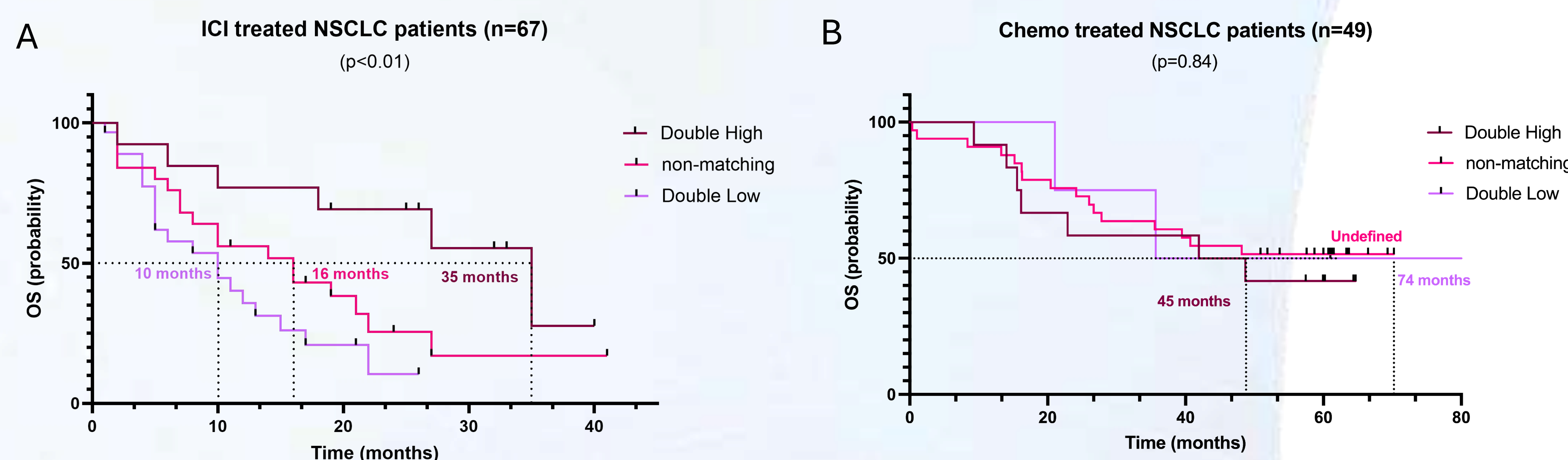


QF-Pro®: TECHNOLOGY FOR MEASSURING ICP ACTIVATION



- 67 NSCLC patients treated with anti-PD-1/PD-L1 therapies (pembrolizumab, nivolumab, durvalumab)
- 49 lung adenocarcinoma patients treated with chemotherapy, used as a control group.
- Quantification of ICP activation assessed through **QF-Pro® technology**.
- QF-Pro® is an enhanced and **simplified FRET-FLIM based bio-imaging technique**.
- Quantifies **receptor-ligand interactions directly in FFPE patient tissues at nanometer resolution**.
- Able to provide significant **cutoff values** (FRET efficiency).

DOUBLE ACTIVATION OF PD-1/PD-L1 AND CTLA-4/CD80 AXIS CORRELATES WITH BETTER RESPONSE TO IMMUNOTHERAPY



- High CTLA-4/CD80 interaction (FRET efficiency $\geq 6.41\%$) increased OS by 17 months and showed a favorable trend in PFS.
- High PD-1/PD-L1 interaction (FRET efficiency $\geq 2.13\%$) increased OS by 7.5 months and PFS by 10.5 months.
- Double high patients had the best outcomes, with OS up to 35 months and 24-month PFS of 53.8%.
- No predictive value was observed for **PD-L1 TPS score** or in control group.

CONCLUSIONS QF-Pro® has proven that:

- Dual **CTLA-4/CD80** and **PD-1/PD-L1** interactions predict **response** to anti-PD-1/PD-L1 therapy in NSCLC.
- Combining both biomarkers is **more effective** than single checkpoints or PD-L1 expression.
- QF-Pro® allows **functional patient stratification** for personalized immunotherapy.
- Results support using **functional interactomics** to guide immune-based treatment decisions.

