Advancing biomarker discovery in ovarian cancer with Olink® Explore HT

A case study performed by Olink in collaboration with Professor Ulf Gyllensten, Department of Immunology, Genetics and Pathology at University of Uppsala, Sweden

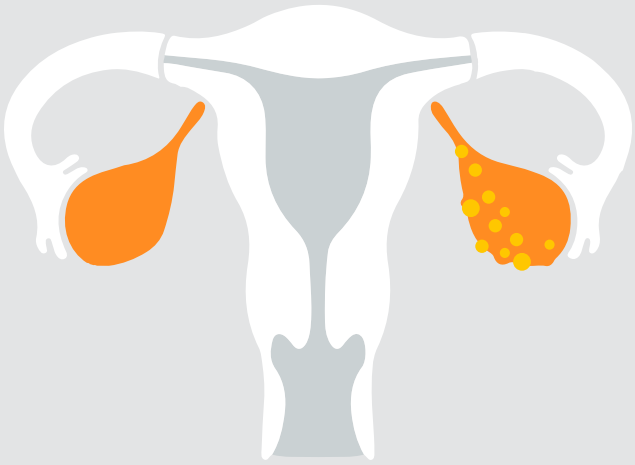
Background

Olink® protein panels are widely employed for broad biomarker discovery studies, allowing the accurate measurement of many proteins simultaneously in large numbers of samples. Increasingly, such studies aid in identifying protein signatures for cancer diagnosis, prognosis, and risk prediction (1). The widespread adoption of Olink technology is evident from nearly 200 oncology publications referencing its use (2).

Ovarian cancer (OvCa) claims about 140,000 lives each year worldwide, and the lack of accurate biomarkers for early detection significantly hinders effective treatment.

Professor Ulf Gyllensten's group at Uppsala University previously utilized Olink to screen for circulating protein biomarkers of OvCa, identifying high performance diagnostic protein signatures (3).

In this case study, they extended their investigations via early access to Olink Explore HT, the new solution for high-throughput proteomics.



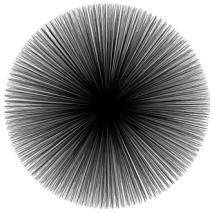
Study aims

Measure and compare 5400+ proteins in plasma samples from women with either malignant or benign ovarian tumors using Olink Explore HT, using separate discovery and validation cohorts.

Identify differentially expressed proteins and derive multi-protein diagnostic signatures using multivariate modeling.

Use pathway analysis to gain insights into ovarian cancer pathophysiology.

Evaluate the performance of Olink Explore HT compared to its predecessor, Olink® Explore 3072.



Proteins

5,400+

with proven specificity

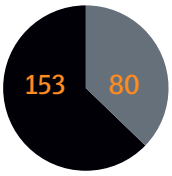
Results

Olink Explore HT identifies proteins associated with OvCa.

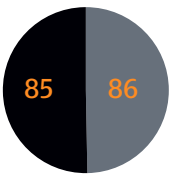
Over 5400 proteins were measured in a discovery cohort of patients with malignant (n=153) or benign (n=80) ovarian tumors. Validation was performed in a separate cohort of 85 cases and 86 controls.

485 proteins were significantly associated with OvCa vs benign controls in the discovery cohort, 128 of the significant protein associations were replicated in the validation set.

Discovery cohort samples



Validation cohort samples



● Malignant ● Benign

> 26 novel proteins associated with OvCa in the validation dataset were unique to the expanded protein library of Olink Explore HT

A diagnostic protein model for OvCa

In a first preliminary analysis of the data, a multivariate model was trained with a set of 19 proteins to discriminate between OvCa and benign ovarian tumors, and the resulting model was tested in the independent validation cohort. The performance of this 19-protein model was compared to that of the well-established OvCa biomarker, MUC16 (also known as CA-125).

This model could discriminate OvCa from benign samples better than MUC16, with area under the curves (AUCs) of 0.93 and 0.867, respectively, based on receiver operating characteristic (ROC) curve analyses (Fig. 1).

> 3 of the 19 proteins in the high-performance model were unique to Olink Explore HT, providing potential new biomarkers for the study and treatment of OvCa

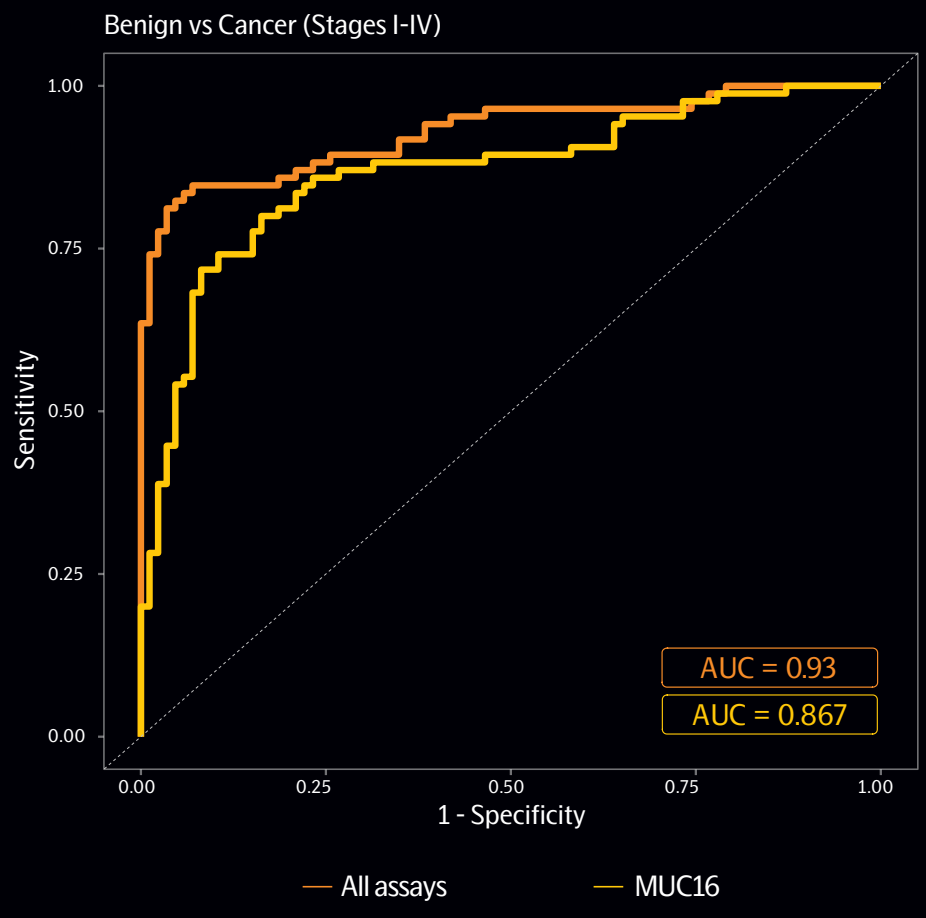


Figure 1. ROC curve comparing the 19-protein model for OvCa with one based on MUC16 alone

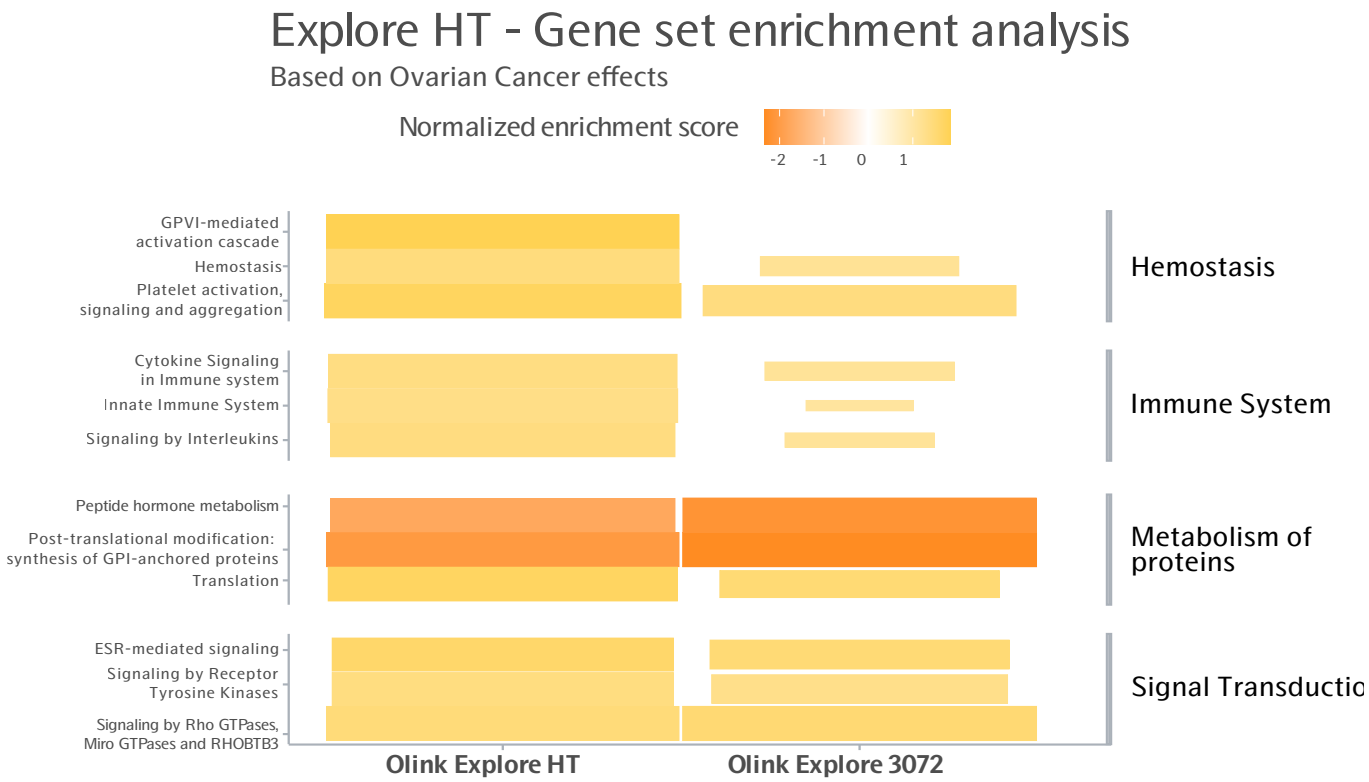


Figure 2. In this snippet from the gene set enrichment analysis, color indicates the direction of the overall alterations in the biological pathway while color intensity indicates the strength of the alteration. The size of each tile indicates statistical significance.

A broader protein library enables more confident pathway analysis

The biological pathways associated with disease biomarkers provide invaluable insights into the underlying pathophysiology and may point towards novel therapeutic approaches. The data obtained here were used to perform a gene set enrichment analysis based on the Reactome database. The enrichment analysis obtained using Olink Explore HT was also compared to the smaller protein library available in Olink Explore 3072.

Several pathways achieved better statistical significance and higher normalized enrichment score using the new assays unique to Olink Explore HT, while others that were significant based on the overlapping assays were confirmed by the new set of assays (Fig. 2).

> Several pathways showed increased statistical significance when utilizing the full set of assays from Olink Explore HT

Expanded proteome coverage with retained high data quality

Olink Explore HT (new features summarized in Fig. 3) enabled Prof. Gyllensten's team to evaluate 80% more proteins compared to its predecessor, Olink Explore 3072 (5416 unique targets vs 2925). More than 95% of the biomarker assays measured with Olink Explore 3072 are also included in Olink Explore HT, enabling direct comparison between the two platforms.

Figure 3. Key features of Olink Explore HT

Comparative analysis of 86 samples from the present ovarian cancer study showed that the data from shared assays between Olink Explore HT and Olink Explore 3072 were highly correlated, with a median Pearson correlation coefficient of 0.91 and a mean of 0.83 (Fig. 4).

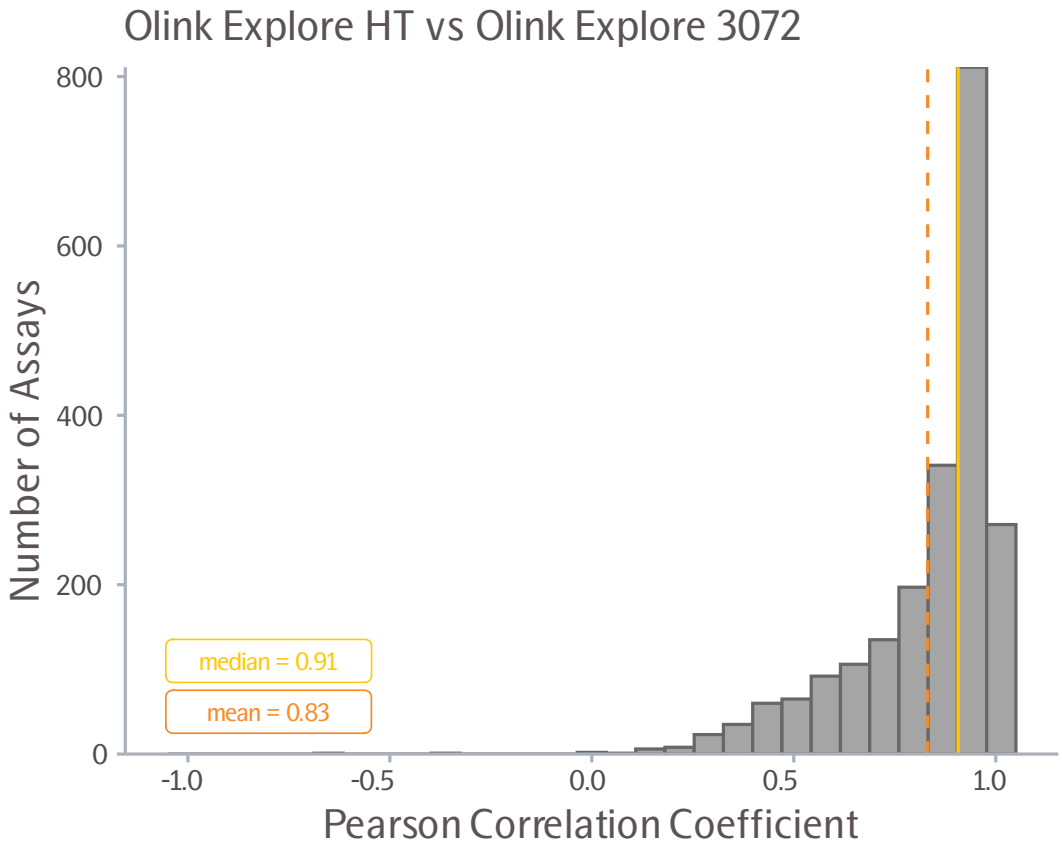


Figure 4. Pearson correlation coefficients of overlapping protein assays measured with Olink Explore HT and Olink Explore 3072.

The correlation of measured values for the differentially expressed OvCa markers detected by the two platforms was excellent. To minimize the influence of sample size differences on the data comparisons, the replication of significant protein findings between the two datasets was further assessed by comparing their effect sizes. The correlation between the effect sizes was 0.9, indicating a very high degree of reproducibility of the findings. (Fig. 5).

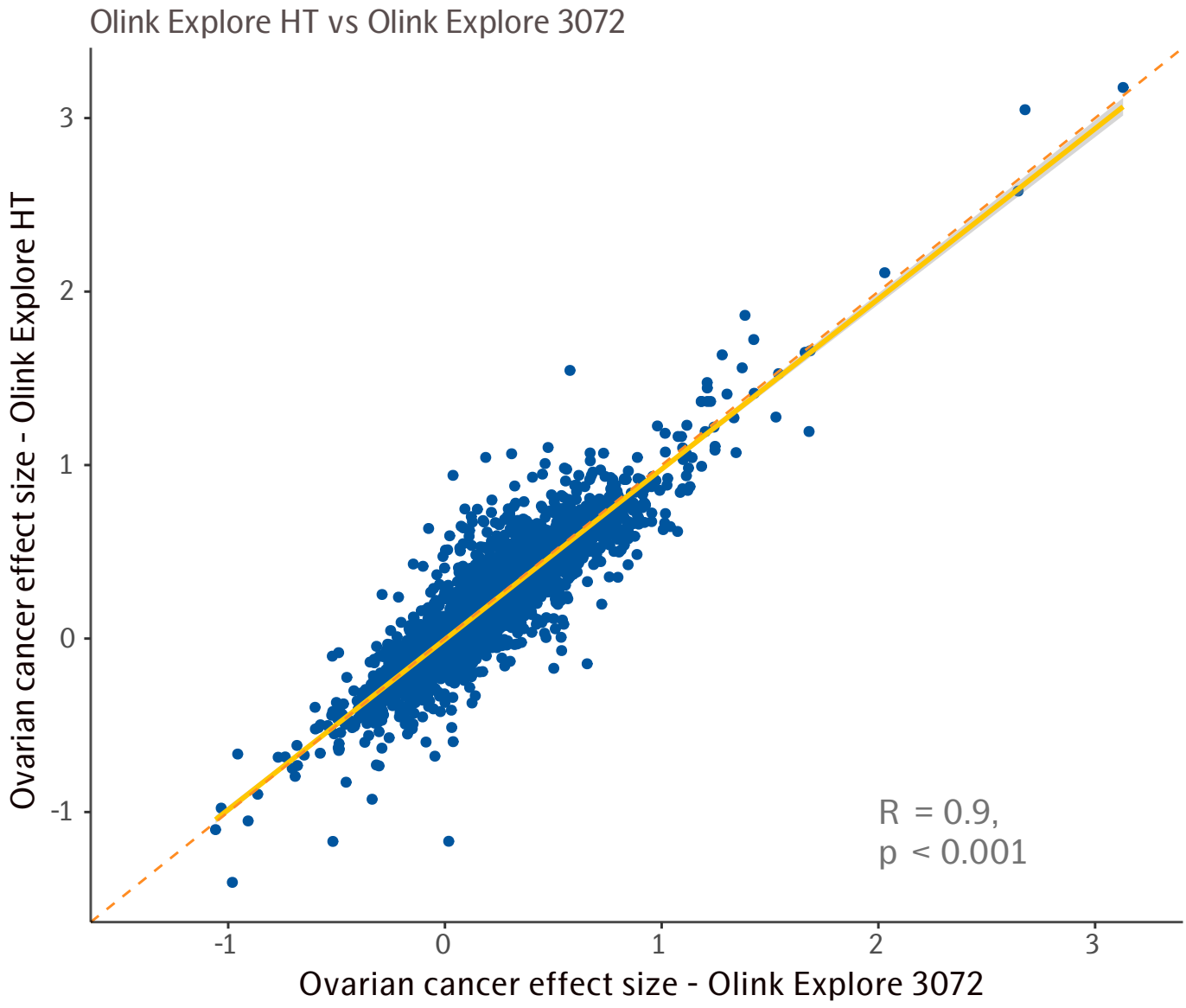


Figure 5. Pearson correlation of estimated effect sizes for Olink Explore HT and Olink Explore 3072 data.

Conclusions

This preliminary analysis of the pilot study shows the technical quality of Olink Explore HT, a recently launched, next-generation solution for high-throughput proteomics, while also demonstrating its great potential to generate new, actionable insights into important biological and clinical questions. It is anticipated that more extensive biostatistical analysis of this data will reveal more powerful insights into OvCa.

→ **The significantly expanded protein library enabled the identification of many additional protein biomarkers that are differentially expressed in OvCa compared to benign ovarian tumors. These additional findings:**

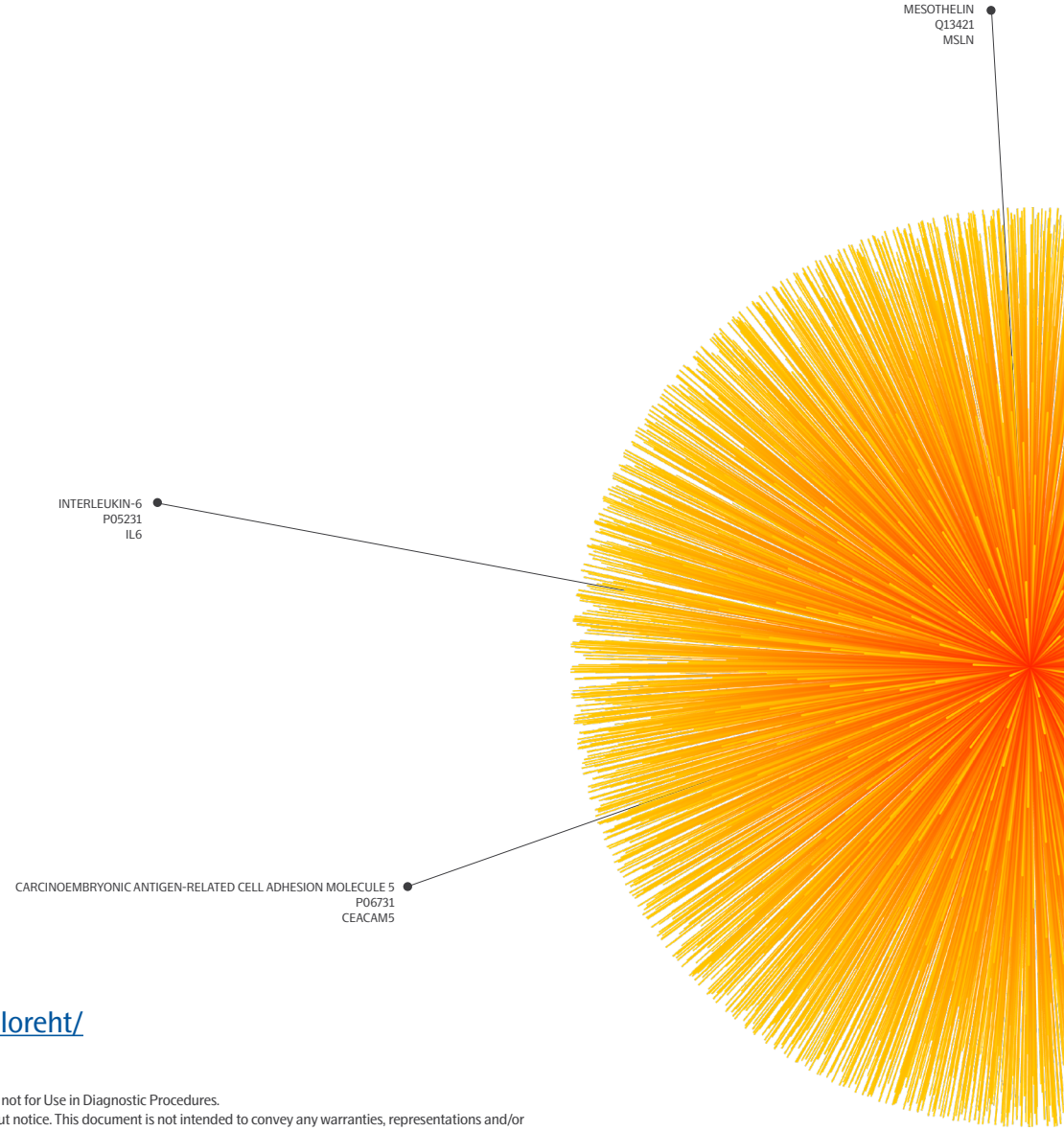
- Enhance the strength and statistical significance of pathway enrichment analysis, providing deeper insights into disease biology, hinting at possible future therapeutic avenues
- Facilitate identification of high-performance protein-based diagnostic models to accurately identify patients with OvCa, potentially improving clinical management
- Expand the list of cancer-related biomarkers for future studies and flags potential drug targets if causality can be demonstrated

→ **Olink Explore HT features significant technical advances including:**

- 80% more protein assays & 4x faster sample throughput
- A streamlined workflow and new software suite optimized for high-throughput data acquisition

References

1. Álvez et al. (2023) Nature Communications, DOI: 10.1038/s41467 023-39765-y
2. https://olink.com/resources-support/publications/disease-area/#onc
3. Gyllensten et al. (2022) Cancers, DOI: 10.3390/cancers14071757



Learn more at: olink.com/exploreht/

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