Leveraging Olink in Oncology Research: Summaries of Key Publications

Compiled by Marijana Rucevic and Gary Franklin
Update: 15 May 2024

Executive Summary: Over the past few years Olink technology has emerged as an essential tool to advance cancer research and its clinical translation. Here we focus on recent publications using Olink technology from early cancer detection to immunotherapy. Publications are broadly categorized and cover key application areas including early cancer detection, identifying causal targets in biomarker research, prediction of treatment responses and adverse events, mechanism of resistance, drug repurposing and improving success of clinical trials. Many publications have leveraged Olink's Proximity Extension Assay (PEA) technology with either the qPCR redout (Olink Target 96, Olink Target 48, Olink Focus or Olink Flex products) or the Olink Explore products with current capability to use PEA to measure over 5,400 proteins per sample in up to 344 samples at a time using a Next Generation Sequencing (NGS) redout (Olink Explore 384, 1536, 3072, HT). Many recent publications have combined proteomics with other omics to enable deeper biological findings. The Table of Contents outlines key applications where Olink publications illustrate value from proteomics to achieve goals in precision medicine. **This document is intended to highlight the impact of Olink's technologies. As such, these summaries are not intended to be comprehensive descriptions of the listed studies for all findings/results. Each paper should be read in its entirety for all background and conclusions.**

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Early Detection of Disease

Budnik B, Amirkhani H, Forouzanfar MH, et al. 2024. Novel proteomics-based plasma test for early detection of multiple cancers in the general population. BMJ Oncology, DOI: 10.1136/bmjonc-2023-000073
https://bmjoncology.bmj.com/content/3/1/e000073
Novelna Inc.

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- Comprehensive plasma proteomics analyses performed by Explore 3072 in treatment naïve patients diagnosed with solid tumors for 18 different types of cancer and healthy individuals' subset.
- Observed sex-specific differences in the cancer-associated proteins, ~80% of significant proteins in men (p = <0.05) showed no significant differences in women, and vice versa.
- Models for all cancer vs controls (i.e., a pan-cancer panel) performance increased quickly when adding more proteins but levelled out after just 10 proteins were included for both men and women (AUCs >0.98).
- Overall, these models identified the tissue of origin of most cancers in more than 80% of cases, overperforming sensitivity of ctDNA approach.

Álvez MB, Edfors F, von Feilitzen K, et al. 2023. Next generation pan-cancer blood proteome profiling using proximity extension assay. Nature Communications, DOI: 10.1038/s41467-023-39765-y
https://www.nature.com/articles/s41467-023-39765-y

Olink® Explore 1536

- The team at Science for Life, KTH Sweden used Olink Explore 1536 to characterize 1,463 plasma proteins collected from >1,400 patients representing 12 most common types of cancer collected at the time of diagnoses and before treatment.
- Developed a set of proteins associated with the 12 cancers; a panel comprising 83 proteins was defined that can identify each individual cancer type with high accuracy.
- This 83-proteins panel identified the right cancer with AUCs ranging between 0.93 and 1 for all cancer types. The models also discriminated cancer vs healthy controls with AUCs between 0.83 to 1.
- Preliminary analysis also indicated that the protein panel showed promising performance in staging some of the cancer types, and in detecting very early-stage cancer.

Novel Drug Target Identification

Mälarstig A, Grassmann F, Dahl L, et al. 2023. Evaluation of circulating plasma proteins in breast cancer using Mendelian randomization. Nature Communications, DOI: 10.1038/s41467-023-43485-8
https://www.nature.com/articles/s41467-023-43485-8

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- Measured 2929 unique proteins in plasma from 598 women selected from the KARMA prospective cohort (Karolinska Mammography Project for Risk Prediction of Breast Cancer) to

explore the association between protein levels, 7 clinical variables (age, births, smoking status, BMI, HRT, menopause, alcohol intake) and gene variants to identify proteins with a causal role in breast cancer.
- Identified 812 cis-Protein Quantitative Loci (pQTLs) for 732 proteins.
- Found strong evidence of causality in breast cancer risk for 7 proteins, five of which reached statistical significance (CD160, DNPH1, LAYN, LRRC37A2 and TLR1) and were replicated with data from previous case/control GWAS studies from UK Biobank and FinnGen.
- Concluded: By applying MR approach to a broad range of circulating proteins found that genetically elevated CD160, DNPH1, LAYN, LRRC37A2 and TLR1 were associated with breast cancer. Data suggest that these five proteins could play an etiological or causal role in breast cancer and provide basis for further functional evaluation of their potential as de-novo biomarkers and drug targets for breast cancer.

Novel IO Drug Target Identification

Loriot Y, Marabelle A, Guégan JP, et al. 2021. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune-checkpoint blockade resistance. Annals of Oncology,DOI:10.1016/j.annonc.2021.08.1748
https://www.sciencedirect.com/science/article/pii/S0923753421039788.

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- Identified Leukemia Inhibitory Factor (LIF) as predictor of response to Immune Checkpoint Inhibition in patients with solid tumors (NSCLC, bladder, others) using high throughput proteomics.
- Plasma LIF was identified as novel and robust baseline predictive biomarker associated with resistance to immunotherapy in cancer patients. High baseline levels of LIF were associated with poor clinical outcome versus low LIF levels associated with significantly better clinical outcome (PFS: LIF^{Low} vs LIF^{High} was 7.4 months vs 1.7 month and OS: LIF^{Low} vs LIF^{High} was 21.7 months vs 4.3 months).
- Levels of LIF in plasma inversely correlated with Tertiary Lymphoid Structure (TLS) in tumor microenvironment (TME) representing a potentially strong surrogate marker for TME.
    - LIF is also an important pleiotropic cytokine and may represent a new promising therapeutic target: clinical trials ongoing with anti-LIF specific antibodies. This publication demonstrates the ability of proteomics to identify novel drug targets, identify responders/non-responders, and act as surrogate markers for safety and efficacy.

Mechanistic Insight/Mechanism of Action

van Eijck CWF, Strijk G, Vietsch EE, et al. 2023. FOLFIRINOX chemotherapy modulates the peripheral immune landscape in pancreatic cancer: Implications for combination therapies and early response. European Journal of Cancer, DOI: /10.1016/j.ejca.2023.113440
https://www.sciencedirect.com/science/article/pii/S0959804923007426

Olink® Explore 384

- Investigated potential immune modulatory effects of the 4-component combination chemotherapy, FOLFIRINOX in patients with lethal pancreatic ductal adenocarcinoma (PDAC) with blood samples taken before and after the first of 4 cycles of FOLFIRINOX chemo.
- Proteomic data showed a cluster of 89 enriched proteins at baseline and 103 on-treatment. Pathway analysis at baseline vs on-treatment showed differential enrichment of pathways.
- This shift from tumor- to immune response-related pathways identified at the protein level was mirrored in a PCA obtained from the flow cytometry data at the cellular level.
- Concluded: data showed that a single cycle of FOLFIRINOX exerts immunological effects that bolster the immune cell-mediated anti-tumor response in PDAC patients.

Parra ER, Zhang J, Duose DY, et al. 2024. Multi-omics analysis reveals immune features associated with immunotherapy benefit in squamous cell lung cancer patients from Phase III Lung-MAP S1400I trial. Clinical Cancer Research, DOI: 10.1158/1078-0432.CCR-23-0251
https://aacrjournals.org/clincancerres/article/doi/10.1158/1078-0432.CCR-23-0251/733908/Multi-omics-analysis-reveals-immune-features

Olink® Target 96 Immuno-oncology Panel

- This immunotherapy study used proteomics in a multiomic analysis of tumor and serum samples from a partially failed phase III clinical trial to identify molecular and immunological characteristics associated with response and mechanism of immune checkpoint resistance.
- Trial compared ipilimumab combined with nivolumab vs. nivolumab monotherapy in patients with metastatic lung squamous cell carcinoma (SqNSCLC), but results could not show that the combination therapy improved clinical outcomes.
- In follow-up, progression-free survival (PFS) and overall survival (OS) curves separated, suggesting a subset of patients may benefit from combination treatment with ipilimumab and nivolumab.
- A multiomic approach combining multiplex immunofluorescence (mIF), targeted transcriptomics, whole-exome sequencing, and Olink provided better understanding of the immune and molecular mechanisms that affect therapeutic benefit and mechanism of drug resistance. This paper demonstrates the ability for proteomics to reveal mechanistic insight, to help stratify responders and non-responders and identify a signature suitable for clinical translation.

Reverse Translation/Repurposing in New Indications

Diorio C, Shraim R, Myers R, et al. 2022. Comprehensive Serum Proteome Profiling of Cytokine Release Syndrome and Immune Effector Cell-Associated Neurotoxicity Syndrome Patients with B-Cell ALL Receiving CAR T19. Clinical Cancer Research, DOI: DOI: 10.1158/1078-0432.CCR-22-0822 https://aacrjournals.org/clincancerres/article/28/17/3804/708069/Comprehensive-Serum-Proteome-Profiling-of-Cytokine.

Olink® Explore 1536

- Measured 1,463 proteins in the serum of pediatric and young adult B-cell acute lymphoblastic leukemia patients prior to and following chimeric antigen receptor T-cell (CAR T) infusion.
- Such serial and comprehensive proteomic analysis helped characterize the biology underlying severe cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS) and identified potential biomarkers of severity and targets for cytokine blockade.
- FLT3 and MILR1 were identified as pre-treatment predictive markers of CRS, with sensitivity, specificity, and accuracy superior to the current gold standard disease burden.
- Specifically, IL-18 was identified as a marker associated with the development of ICANS that could represent a target for future therapeutic development or provide drug retargeting opportunities. IL-18 blockade is currently being evaluated in other inflammatory conditions and could rapidly be translated into clinical trials in ICANS.

Al-Sawaf O, Weiss J, Skrzypski M, et al. 2023. Body composition and lung cancer-associated cachexia in TRACERx. Nature Medicine, DOI: 10.1038/s41591-023-02232-8 https://www.nature.com/articles/s41591-023-02232-8.

Olink® Explore 3072

- Explore 3072 was used to characterize biology behind cancer-associated cachexia (CAC) in NSCLC patients from the TRACERx clinical trial.
- CAC is a complex metabolic syndrome shown to be associated with cancer treatment toxicity and reduced overall survival in NSCLC patients.
- Proteomic data identified circulatory GDF15, at the time of relapse, to be significantly associated with the loss of body weight, skeletal muscle and adipose tissue, all of which are characteristics of CAC.
- Concluded: data expand the mechanistic understanding of how GDF-15 plays a key role in CAC and highlight the potential therapeutic relevance of targeting GDF15 in the management of cachexia. This publication demonstrates the ability for proteomics to facilitate repurposing opportunities and identify adverse events/toxicity.

Disease Prediction

Davies MPA, Sato T, Ashoor H, et al. 2023. Plasma protein biomarkers for early prediction of lung cancer. EBioMedicine, DOI: 10.1016/j.ebiom.2023.104686 https://www.sciencedirect.com/science/article/pii/S2352396423002517.

Olink® Explore 3072

- Liverpool Lung Project (LLP) study on biomarkers for early diagnosis and risk prediction for lung cancer with validation using the UKB-Pharma Proteomics Project data.
- The study included 131 cases taken 1–10 years prior to diagnosis, 237 controls, and 90 subjects at diagnosis, 1-3, 3-5, or 5-10 years prior to diagnosis.
- Four machine learning algorithms gave median AUCs of 0.76–0.90 and 0.73–0.83 for the 1–3 years pre-diagnosis and 1–5 years pre-diagnosis proteins respectively. External validation from UKBB data gave AUCs of 0.75 (1–3 year) and 0.69 (1–5 year), with AUC 0.7 up to 12 years prior to diagnosis. All models identified were independent of other confounding factors, including smoking.
- Concluded: plasma proteome provides biomarkers that can be used to identify those at greatest risk of lung cancer.

Lung Cancer Cohort Consortium (LC3). 2023. The blood proteome of imminent lung cancer diagnosis. Nature Communications, DOI: 10.1038/s41467-023-37979-8 https://www.nature.com/articles/s41467-023-37979-8.

Olink® Target 96

- This study identified predictive risk markers in the samples from the INTEGRAL (Integrative Analysis of Lung Cancer Etiology and Risk) project composed of 6 independent genetically diverse cohorts from the US, Europe, Singapore, and Australia.
- 36 proteins biomarkers were identified with independently reproducible associations with risk of imminent lung cancer diagnosis and included primarily novel potential diagnostic markers.
- Among identified biomarkers, 31 mapped to pathways expected based upon the hallmarks of cancer (activation of invasion and metastasis, proliferative signaling, tumor-promoting inflammation, and angiogenesis were most frequently implicated biological processes and pathways).
- Concluded: study provides a potential view of the blood proteome in the years leading up to diagnosis of smoking-related lung cancer and identified risk biomarkers may enhance early detection of smoking-related lung cancer.

Feng X, Wu WY, Onwuka JU, et al. 2023. Lung cancer risk discrimination of prediagnostic proteomics measurements compared with existing prediction tools. Journal of the National Cancer Institute, DOI: 10.1093/jnci/djad071 https://academic.oup.com/jnci/advance-article-abstract/doi/10.1093/jnci/djad071/7186270

Olink Target 96

- This work was published simultaneously with LC paper, as a part of the same INTEGRAL project.
- Evaluated proteins identified as potential predictive risk markers for lung cancer in the main LC study for the specific comparison of predictive performance against the commercialized

autoantibody-based Early Cancer Detection Test (EarlyCDT-Lung) and PLCOm201 risk factor-based selection criteria.

- 22 proteins were confirmed to be associated with lung cancer risk, and final risk model included 4-protein signature. The protein-based risk model outperformed other models.
    - Concluded: circulating proteins showed promise in predicting incident lung cancer and outperformed a standard risk prediction model and the commercialized EarlyCDT-Lung. This study demonstrates proteomics application in both disease risk prediction and clinical translation.

Responders/Non-Responders

Parra ER, Zhang J, Duose DY, et al. 2024. Multi-omics analysis reveals immune features associated with immunotherapy benefit in squamous cell lung cancer patients from Phase III Lung-MAP S1400I trial. Clinical Cancer Research, DOI: 10.1158/1078-0432.CCR-23-0251
https://aacrjournals.org/clincancerres/article/doi/10.1158/1078-0432.CCR-23-0251/733908/Multi-omics-analysis-reveals-immune-features

Olink® Target 96 Immuno-oncology Panel

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Stratification/Patient Enrichment/Identification of Disease Endotypes

Tang Z, Gu Y, Shi Z, et al. 2023. Multiplex immune profiling reveals the role of serum immune proteomics in predicting response to preoperative chemotherapy of gastric cancer. Cell Reports Medicine, DOI: 10.1016/j.xcrm.2023.10093 https://www.cell.com/cell-reports-medicine/fulltext/S2666-3791(23)00023-X

Olink® Target 96 Inflammation Panel

- This study examined serum proteomic markers before, during and after preoperative chemotherapy in patients with gastric adenocarcinoma to investigate the relationship between systemic immunity and chemotherapy response.
- Both local and systemic immune features identified were associated with treatment response. More dynamic systemic immune changes were observed in responders compared to non-responders.
- Established a 4-protein "pretreatment serum response predictive score (PSRscore)" that could predict response prior to starting treatment with an AUC of 0.907.Concluded: these findings highlight the fundamental but largely underestimated role of systemic immunity in the treatment of gastric cancer, suggesting a patient stratification strategy based on pretreatment serum immune proteomics. This publication demonstrates the ability for proteomics to identify likely responders/non-responders as well as to stratify patients and classify disease endotypes.

Thiis-Evensen E, Kjellman M, Knigge U, et al. 2022. Plasma protein biomarkers for the detection of pancreatic neuroendocrine tumours and differentiation from small intestinal neuroendocrine tumours. Journal of Neuroendocrinology, DOI: 10.1111/jne.13176 https://onlinelibrary.wiley.com/doi/abs/10.1111/jne.13176

Olink® Target 96 Oncology II Panel

- Leveraged proteomics and supervised machine learning to identify a high-performance protein profile to diagnose and differentiate pancreatic neuroendocrine tumors (PanNET).
- Several plasma proteins (CPE, MAD homolog 5, GPNMB and LYN) were identified important for both, detection of PanNET and discrimination versus small intestinal NET (SI-NET).
- The best-performing protein signature for PanNET vs controls comprised 9 top performing biomarkers with an AUC=0.99 while the best model to discriminate PenNET from Si-NET had an AUC=0.98. This PanNET diagnostic signature outperformed currently used biomarker CgA and performed better when CgA was excluded from the model.

- Concluded: this approach can provide a new tool in the work up of a patient with known, or suspected, neuroendocrine tumors and has the potential to be used in the follow-up of patients after radical resection of neuroendocrine tumors.

Li M, Chen J, Yu H, et al. 2023. Cerebrospinal fluid immunological cytokines predict intracranial tumor response to immunotherapy in non-small cell lung cancer patients with brain metastases. OncoImmunology, DOI: 10.1080/2162402X.2023.2290790
https://www.tandfonline.com/doi/full/10.1080/2162402X.2023.2290790

Olink® Target 96 Immuno-oncology Panel

- Included both plasma and Cerebrospinal Fluidclinical trial samples to look for predictive markers of intracranial tumor response to immunotherapy in non-small cell lung cancer (NSCLC) patients with brain metastases.
- Among 28 patients prospectively enrolled in this study, most baseline levels of immunological cytokines were significantly lower in CSF than in plasma.
- In patients who showed a cranial response to therapy, baseline CSF level of LAMP3 was significantly higher and CXCL10, IL-12, CXCL11, IL-18, TIE2, HGF, and PDCD1 levels were significantly lower. These levels were also associated with PFS and these 7-protein signature could predict responses with high accuracy and ROC = 0.91 compared to PD-L1 expression (AUC of 0.72).
- Concluded: Immunological cytokines in CSF could predict intracranial tumor response to immunotherapy in NSCLC patients with brain metastases. These findings could have clinical implication and warrant validation in a larger prospective cohort study.

Surrogate Markers for Safety and Efficacy

Loriot Y, Marabelle A, Guégan JP, et al. 2021. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune-checkpoint blockade resistance. Annals of Oncology, DOI: 10.1016/j.annonc.2021.08.1748
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Clinical Translation

Enroth S, Berggrund M, Lycke M, Broberg J, et al., 2019. High throughput proteomics identifies a high-accuracy 11 plasma protein biomarker signature for ovarian cancer. Communications Biology, DOI: 10.1038/s42003-019-0464-9. https://www.nature.com/articles/s42003-019-0464-9

Olink® Target 96 panels

- Demonstrates the ability to go from broad-scale discovery to protein signature validation through to low-plex custom panel for clinical utility to improve early detection and diagnoses of ovarian cancer.
- Measured 593 plasma proteins to identify novel biomarkers across three cohorts of patients with ovarian cancer and benign tumors.
- A final model consisting of 11 biomarkers was developed into a multiplex Proximity Extension Assay (PEA) test reporting in absolute concentrations. The final model was evaluated in a fourth independent cohort and has an AUC = 0.94, PPV = 0.92, sensitivity = 0.85 and specificity = 0.93 for detection of ovarian cancer stages I–IV.
- Concluded: the novel plasma protein signature could be used to improve the diagnosis of women with adnexal ovarian mass or in screening to identify women that should be referred to specialized examination.

Feng X, Wu WY, Onwuka JU, et al. 2023. Lung cancer risk discrimination of prediagnostic proteomics measurements compared with existing prediction tools. Journal of the National Cancer Institute, DOI: 10.1093/jnci/djad071 https://academic.oup.com/jnci/advance-article-abstract/doi/10.1093/jnci/djad071/7186270

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Adverse Events irAEs/Toxicity

Flora C, Olesnavich M, Zuo Y, et al. 2024. Longitudinal plasma proteomics in CAR-T cell therapy patients implicates neutrophils and NETosis in the genesis of CRS. Blood Advances. DOI: 10.1182/bloodadvances.2023010728
https://ashpublications.org/bloodadvances/article/doi/10.1182/bloodadvances.2023010728/514722

Olink® Target 96

- This study examined longitudinal plasma samples from 26 patients receiving CD19 CAR-T cell therapies, 21 of whom developed cytokine release syndrome (CRS). Results confirmed many previously reported CRS related proteins along with a novel protein pathway.
- At baseline, 11 proteins were found differentially expressed between CRS and non-CRS patients with SERPINE1, TNFSF11, and PON3 having specifically strong association with CRS. Longitudinal profiling beyond baseline identified 21 proteins with increasing or decreasing temporal dynamics leading to CRS development compared to non-CRS patient. Of highest significance was MPO which is released upon neutrophils activation and is a marker of NETosis (a specialized form of neutrophil-dependent cell death) among other significantly altered proteins DEFA1, RETN and DPEP linked to neutrophil function.
- Concluded: Neutrophil activation, and in particular the process of NETosis might be associated with development of CRS. These findings can have strong implications for the understanding and prediction of severe CRS and may represent potential new therapeutic targets. The NETosis connection is of particular interest because FDA-approved drugs that can inhibit neutrophil activation or NETosis are already available.

Al-Sawaf O, Weiss J, Skrzypski M, et al. 2023. Body composition and lung cancer-associated cachexia in TRACERx. Nature Medicine, DOI: 10.1038/s41591-023-02232-8 https://www.nature.com/articles/s41591-023-02232-8.

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- Concluded: data expand the mechanistic understanding of how GDF-15 plays a key role in CAC and highlight the potential therapeutic relevance of targeting GDF15 in the management of cachexia. This publication demonstrates the ability for proteomics to facilitate repurposing opportunities and identify adverse events/toxicity.

Nuñez NG, Berner F, Friebel E, et al. 2023. Immune signatures predict development of autoimmune toxicity in patients with cancer treated with immune checkpoint inhibitors. Med, 10.1016/j.medj.2022.12.007https://www.sciencedirect.com/science/article/pii/S2666634022005232

Olink® Target 96

- This study combined mass cytometry (Cy-ToF) and Olink Target 96 Inflammation panel to look for markers associated with serious immune-related adverse events (irAEs) in melanoma and non-small cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitors (ICIs).
- Proteomics data showed an early increase in CXCL9, CXCL10, CXCL11 and IFN-γ as well as a decrease in IL-10 in the period 1 to 2 weeks after the start of therapy indicate a heightened risk of developing irAEs.
- Cy-ToF also showed that early expansion of Ki-67+ regulatory T cells and Ki-67+ CD8+ T cells is also likely to be associated with increased risk of irAEs.
- Conclusion: we propose that the analysis of serum proteins combined with the detection of proliferating T cell subsets 1 to 2 weeks after the start of therapy can help identify those patients at higher risk of later ICIs toxicity.

Assay and Technology Validation

Ghorbani A, Avery LM, Sohaei D, et al. 2023. Discovery of novel glioma serum biomarkers by proximity extension assay. Clinical Proteomics, DOI: 10.1186/s12014-023-09400-5 https://clinicalproteomicsjournal.biomedcentral.com/articles/10.1186/s12014-023-09400-5

Olink® Explore 3072

- This study looked for early diagnostic markers for gliomas by comparing plasma from samples collected retrospectively at first diagnosis of glioma (n=20) with meningioma patients as controls (n=20).

- Data revealed 12 proteins differentially expressed in gliomas v meningiomas: The strongest individual protein for glioma diagnosis was GFAP, with an AUC=0.86 while the combination of GFAP and FABP4 had AUC=0.98.
- Most proteins identified have known links to brain tumor biology lending confidence to the results.
- Characterized well the differentiation Olink offers as compared to mass spec and ELISAs citing PEA properties qualify superior to LC/MS/MS and single ELISAs for liquid biopsy-based biomarker discovery applications.

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