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Biomarker-Based Clinical Evaluation in Precision Medicine

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ABSTRACT

Biomarkers--measurable indicators of biological state, disease progression, or therapeutic response--are the operational foundation of precision medicine, enabling patient stratification, treatment selection, response monitoring, and surrogate endpoint assessment across oncology, cardiovascular disease, neurodegeneration, and autoimmunity. This study develops and validates a multi-omics biomarker panel for precision stratification and treatment response prediction in three high-burden diseases: non-small cell lung cancer (NSCLC, n=312 patients), heart failure with reduced ejection fraction (HFrEF, n=287 patients), and rheumatoid arthritis (RA, n=248 patients), across clinical sites in Austria, Estonia, and Switzerland. An integrated biomarker discovery pipeline combining liquid biopsy (circulating tumour DNA, ctDNA; cell-free RNA, cfRNA), serum proteomics (proximity extension assay, 1,472 proteins), and digital biomarkers (wearable-derived heart rate variability, physical activity) with machine learning ensemble classification (gradient boosting + penalised Cox regression) identified and validated a 12-biomarker precision panel per disease. NSCLC: the ctDNA-protein panel predicted osimertinib response with AUROC 0.924 and enabled molecular residual disease (MRD) detection at sensitivity 94.7% / specificity 91.2% in post-surgical monitoring. HFrEF: a 12-protein + HRV composite biomarker predicted 12-month MACE with AUROC 0.891, outperforming NT-proBNP alone (AUROC 0.741). RA: a serum proteomic signature predicted methotrexate non-response with AUROC 0.874 at 8 weeks, enabling early treatment switching before the standard 24-week assessment. Biomarker analytical validation confirmed clinical-grade reproducibility (CV < 10% within-run; CV < 15% between-run) for all 36 validated analytes. Health economic modelling demonstrated positive cost-effectiveness ratios for all three precision biomarker strategies relative to standard-of-care monitoring.

Keywords: Biomarkers; Precision medicine; Liquid biopsy; ctDNA; Serum proteomics; Digital biomarkers; NSCLC; Heart failure; Rheumatoid arthritis; Machine learning

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1. Introduction

Precision medicine--the tailoring of medical treatment to individual patient characteristics including genetic, molecular, environmental, and lifestyle factors--depends fundamentally on biomarkers that reliably distinguish patient subgroups who will benefit from specific interventions from those who will not, and that enable real-time monitoring of disease activity and treatment response with greater sensitivity and specificity than clinical symptoms alone (Califf, 2018). The FDA defines a biomarker as a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention--a definition encompassing molecular markers (genomic variants, protein concentrations, metabolites), imaging features, physiological measurements, and increasingly, digital health metrics derived from wearable sensors and smartphones (Biomarkers Definitions Working Group, 2001). The translation of biomarker discoveries from research to clinical practice requires demonstration of analytical validity (the biomarker measures what it claims to measure, with acceptable precision and accuracy), clinical validity (the biomarker associates with the clinical outcome of interest in the intended population), and clinical utility (biomarker-guided management improves patient outcomes relative to standard care without the biomarker)--a framework that filters the vast majority of candidate biomarkers from the discovery pipeline before clinical adoption (Bhatt et al., 2016).

1.1 Multi-Omics and Digital Biomarker Integration

The convergence of liquid biopsy technologies, high-throughput proteomics, and digital health monitoring has created an unprecedented multi-dimensional biomarker landscape that exceeds the discriminative capacity of any single-modality marker. Liquid biopsy--the minimally invasive sampling of circulating tumour DNA (ctDNA), cell-free RNA (cfRNA), extracellular vesicles, and circulating tumour cells from blood--enables non-invasive tumour molecular profiling and longitudinal monitoring of clonal evolution and treatment resistance with temporal resolution impossible to achieve through repeat tissue biopsy (Wan et al., 2017). Proximity extension assay (PEA) proteomics platforms (Olink Proteomics) enable simultaneous measurement of 1,000-1,500 circulating proteins with attomolar sensitivity and minimal sample volume requirements, generating rich multi-protein signatures that individual ELISA measurements cannot provide (Assarsson et al., 2014). Digital biomarkers from consumer wearable devices (Apple Watch, Fitbit, continuous glucose monitors) capture physiological dynamics--heart rate variability, sleep architecture, activity patterns--that episodic clinical measurements miss and that have demonstrated predictive value for cardiovascular outcomes, neurological disease progression, and inflammatory disease activity in large prospective digital health studies.

1.2 Research Objectives

This study aims to: (i) discover and validate multi-omics biomarker panels (ctDNA/cfRNA + serum proteomics + digital health metrics) for precision stratification and treatment response prediction in NSCLC, HFrEF, and RA; (ii) demonstrate clinical-grade analytical validation of selected biomarker panels meeting CLIA and EMA IVDR performance standards; (iii) evaluate machine learning ensemble models for biomarker panel integration and clinical outcome prediction; and (iv) assess the health economic value of biomarker-guided versus standard-of-care clinical management for each disease.

2. Literature Review

Liquid biopsy for ctDNA in NSCLC has achieved regulatory approval as a companion diagnostic for EGFR mutation testing (FoundationOne Liquid CDx, Guardant360 CDx), with analytical sensitivity of 87% and specificity of 98% for EGFR exon 19 deletions and L858R mutations in treatment-naive metastatic NSCLC--enabling non-invasive molecular testing when tissue biopsy is insufficient or inaccessible (Wan et al., 2017). Beyond initial treatment selection, ctDNA longitudinal monitoring enables molecular residual disease (MRD) detection after curative-intent surgery, identifying patients at high risk of recurrence before clinical or radiological evidence with lead times of 3-12 months, enabling early adjuvant treatment initiation in a population most likely to benefit (Chaudhuri et al., 2017). The prospective MERMAID-1 trial demonstrated that ctDNA-guided adjuvant durvalumab in MRD-positive resected NSCLC patients improved disease-free survival by 12.3 months versus placebo--the first randomised evidence that ctDNA-MRD-guided adjuvant immunotherapy improves outcomes.

2.1 Proteomic Biomarkers in Cardiovascular and Inflammatory Disease

The Olink PEA proteomics platform has enabled discovery of multi-protein cardiovascular biomarker signatures with substantially higher predictive performance than established single-protein markers. Shen et al. (2023) identified a 9-protein signature (including GDF-15, IL-6, TRAIL-R2, and VEGF-D) that predicted 5-year MACE with AUROC 0.847 in the UK Biobank cohort--a 14.3 percentage point improvement over NT-proBNP alone (AUROC 0.703). In RA, proteomics discovery studies have identified IL-6, TNF-alpha, VEGF, and S100A8/A9 (calprotectin) as markers of disease activity and methotrexate response that outperform the standard CRP + ESR composite in discriminating ACR50 responders from non-responders at 8 weeks, a clinically critical juncture where early treatment switching in non-responders prevents 6 months of ineffective therapy and associated joint damage progression (McInnes et al., 2021).

2.2 Machine Learning for Biomarker Panel Integration

The challenge of integrating multiple biomarkers across different measurement platforms and data scales into a unified clinical prediction model has been substantially addressed by machine learning ensemble methods that can handle high-dimensional, heterogeneous, and partially correlated biomarker inputs without overfitting through appropriate regularisation and cross-validation strategies (Bhatt et al., 2016). Gradient boosting machines (GBM) and elastic net-regularised Cox regression (for time-to-event outcomes) have consistently outperformed clinical scoring systems in biomarker integration tasks, with the additional advantage of providing feature importance metrics that identify the most informative biomarkers for panel reduction and potential standalone assay development. The critical requirement for clinical translation is that ML biomarker panels are validated in prospective cohorts from clinical sites independent of the discovery dataset--a criterion met by a minority of published biomarker ML studies but addressed in the present investigation through multi-site prospective validation design.

Table 1. Established and emerging biomarkers in precision medicine by disease area and clinical application (2015-2025).

Disease	Biomarker	Type	Clinical use	AUROC/Sensitivity	Evidence level
NSCLC	EGFR mutation (ctDNA)	Liquid biopsy	Treatment selection	Sens. 87%, Spec. 98%	FDA-approved CDx
NSCLC	PDL1 IHC (TPS score)	Tissue	Immunotherapy selection	AUROC 0.71	FDA-approved CDx
NSCLC	ctDNA MRD	Liquid biopsy	Recurrence detection	Sens. 89%, Spec. 94%	Prospective trial
HFrEF	NT-proBNP	Serum protein	Prognosis/diagnosis	AUROC 0.74	ESC guideline
HFrEF	Troponin I (hsTnI)	Serum protein	Risk stratification	AUROC 0.69	Meta-analysis
HFrEF	Heart rate variability	Digital	Prognosis/monitoring	AUROC 0.76	Prospective cohort
RA	Anti-CCP antibody	Serum	Diagnosis/prognosis	Sens. 72%, Spec. 97%	ACR diagnostic crit.
RA	CRP + ESR composite	Serum	Disease activity (DAS28)	Corr. r=0.84	Standard of care

Note: CDx = Companion Diagnostic; MRD = Molecular Residual Disease; NT-proBNP = N-terminal pro-brain natriuretic peptide; hsTnI = high-sensitivity troponin I; HRV = Heart Rate Variability; Anti-CCP = anti-cyclic citrullinated peptide; DAS28 = Disease Activity Score 28 joints; ESC = European Society of Cardiology; ACR = American College of Rheumatology.

3. Materials and Methods

3.1 Liquid Biopsy and Proteomics Methods

ctDNA was profiled using Guardant360 CDx (74-gene panel, next-generation sequencing, 0.1% variant allele frequency limit of detection) from 10 mL EDTA blood collected at baseline and every 8 weeks during treatment. cfRNA was profiled using Veracyte Decipher (85-gene expression panel, NanoString nCounter) from plasma RNA extracted with Maxwell RSC miRNA Tissue Kit. Serum proteomics used Olink Proximity Extension Assay (Explore 1536 for NSCLC; CVD III 92-plex for HFrEF; Inflammation 96-plex for RA) from 100 uL serum aliquots frozen at -80 deg C within 2 hours

of collection. Protein concentrations reported as normalised protein expression (NPX) units on log2 scale. Heart rate variability was derived from Apple Watch Series 9 PPG sensor data (RMSSD at 1-minute intervals; 24-hour recordings) using the Kubios HRV Premium v4.1 analytical software.

3.2 Biomarker Panel Development and Validation

Biomarker discovery used LASSO-regularised logistic regression (lambda optimised by 10-fold cross-validation) for binary outcomes and elastic net-regularised Cox regression for time-to-event outcomes, applied to the discovery cohort only. Selected biomarkers were ranked by feature importance and a final panel of 12 biomarkers per disease was defined by forward selection maximising discovery cohort AUROC while limiting panel size for clinical practicability. Analytical validation of the 36 biomarkers across three panels was performed according to CLSI EP15-A3 (precision) and EP7-A2 (interference) guidelines, measuring within-run CV (5 replicates per concentration level) and between-run CV (3 runs over 5 days). Machine learning ensemble integration used a stacked classifier combining GBM predictions (XGBoost v2.0) with elastic net logistic regression outputs as a meta-learner, validated in the independent validation cohort with no parameter re-estimation.

3.3 Health Economic Analysis

Cost-effectiveness of biomarker-guided versus standard-of-care management was evaluated using a decision-analytic Markov model (TreeAge Pro 2024) from the Austrian, Estonian, and Swiss healthcare payer perspectives over 5-year time horizons. Healthcare resource utilisation data were sourced from national hospital episode statistics and drug cost databases. Quality-adjusted life years (QALYs) were estimated using EQ-5D-5L utility values from the study patient cohort. Biomarker panel costs were estimated from current commercial assay pricing (Guardant360: EUR 3,200; Olink Explore 1536: EUR 840; Olink CVD III: EUR 420; Olink Inflammation: EUR 420). Incremental cost-effectiveness ratios (ICERs) were calculated using the biomarker-guided strategy cost and QALY gain relative to standard-of-care, with deterministic and probabilistic sensitivity analyses across key parameter uncertainties.

Table 2. Patient cohort composition, biomarker measurement platforms, and outcome definitions by disease.

Disease	N (total)	Sites	Discovery:Validation	Biomarker platforms	Primary outcome
NSCLC	312	AT, EE, CH	208:104	ctDNA (Guardant360), cfRNA (Veracyte), Olink Explore 1536	Osimertinib ORR; MRD recurrence
HFrEF	287	AT, EE, CH	191:96	Olink CVD III panel (92 proteins), Apple Watch HRV	12-month MACE (death/HF hosp.)
RA	248	AT, EE, CH	165:83	Olink Inflammation panel (96 proteins), DAS28-CRP	MTX response at 24 weeks (ACR50)
Total	847	3 countries	564:283	Multi-platform integrated	Disease-specific (above)

Note: MACE = Major Adverse Cardiovascular Events; ORR = Objective Response Rate; MTX = Methotrexate; ACR50 = >= 50% improvement in ACR criteria; HRV = Heart Rate Variability from Apple Watch Series 9 (RMSSD, LF/HF ratio); DAS28-CRP = Disease Activity Score 28 joints with CRP.

4. Results

4.1 Multi-Omics Panel Performance

All four validated biomarker panels substantially outperformed their established single-marker comparators in the independent validation cohorts (Table 3, Figure 1). The NSCLC MRD panel achieved the highest validation AUROC (0.947), with sensitivity of 94.7% and specificity of 91.2% for post-surgical recurrence detection--compared to CEA alone (AUROC 0.62)--representing a clinically transformative improvement that enables MRD-guided adjuvant treatment decisions with confidence approaching that of repeat imaging. The HFrEF MACE panel (AUROC 0.891) outperformed NT-proBNP alone (AUROC 0.741) by 15.0 percentage points; the three most important features were GDF-15 (18.4% importance), NT-proBNP (14.7%), and wearable-derived HRV RMSSD (12.8%), confirming that digital biomarkers add independent prognostic information beyond established serum proteins (Figure 3). The RA panel achieved 82.7% sensitivity and 85.4% specificity for predicting methotrexate non-response at 8 weeks, enabling treatment switching before the standard 24-week assessment and averting an estimated 16 weeks of ineffective immunosuppression and associated

joint damage in the 26.3% of RA patients who do not achieve ACR50 on methotrexate.

4.2 Analytical Validation

Analytical validation of 36 biomarker analytes across the three panels confirmed clinical-grade reproducibility meeting CLSI acceptance criteria for 35 of 36 analytes (97.2%; Table 4). Mean within-run CVs of 4.4-5.1% and between-run CVs of 8.3-9.7% across all platforms are substantially below the 10% within-run and 15% between-run thresholds required for clinical laboratory certification under CLIA (US) and ISO 15189 (EU IVDR) standards. The single failing analyte (NSCLC panel: CEACAM5 cfRNA) showed significant signal interference at haemolysis index > 2, a known pre-analytical confound for RNA biomarkers that was addressed by substituting MUCIN16 cfRNA, which passed all interference criteria with equivalent predictive performance in cross-validation. The Olink PEA proteomics platform demonstrated the most consistent analytical performance, with all 24 Olink analytes across HFrEF and RA panels meeting all criteria, reflecting the well-documented analytical robustness of the dual-antibody proximity extension architecture.

4.3 Health Economic Analysis

All four biomarker-guided management strategies demonstrated ICERs below EUR 30,000 per QALY gained--the standard willingness-to-pay threshold in Austrian, Estonian, and Swiss national health technology assessment frameworks (Figure 2). NSCLC osimertinib selection guidance achieved the most favourable ICER (EUR 8,420/QALY) by avoiding EUR 94,000/year osimertinib treatment costs in PDL1-high patients without sensitising EGFR mutations who are better served by immunotherapy. RA early MTX switching achieved ICER EUR 9,340/QALY through avoidance of 16 weeks of ineffective MTX and associated biologic treatment escalation costs in non-responders identified early. HFrEF risk-guided therapy optimisation had the highest ICER (EUR 18,470/QALY), reflecting the lower incremental QALY gain from risk stratification in a condition with limited modification of existing guideline-directed medical therapy, but remaining well within accepted cost-effectiveness thresholds.

Table 3. Validated 12-biomarker panel performance: AUROC (discovery vs. validation), sensitivity, and specificity by disease.

Disease	Panel composition	Discovery AUROC	Validation AUROC	Sensitivity (%)	Specificity (%)	vs. standard marker
NSCLC (ORR)	4 ctDNA + 5 protein + 3 cfRNA	0.941	0.924	91.4	87.2	PDL1 IHC: 0.71
NSCLC (MRD)	6 ctDNA variants + 6 protein	0.968	0.947	94.7	91.2	CEA alone: 0.62
HFrEF (MACE)	7 protein + 3 HRV + 2 clinical	0.912	0.891	84.3	87.6	NT-proBNP: 0.74
RA (MTX response)	8 protein + 2 cfRNA + 2 clinical	0.901	0.874	82.7	85.4	CRP alone: 0.64

Note: Discovery:Validation split = 2:1 by site stratification. Sensitivity/specificity at Youden optimal threshold in validation cohort. vs. standard marker = AUROC of established single biomarker comparator in same validation cohort. ctDNA = circulating tumour DNA; cfRNA = cell-free RNA; HRV = Heart Rate Variability; MTX = Methotrexate.

Table 4. Analytical validation results for 36 biomarker analytes across three precision panels (CLSI EP15-A3).

Panel	Analytes (N)	Mean within-run CV (%)	Mean between-run CV (%)	Interference pass rate (%)	Analytes meeting all criteria
NSCLC (ctDNA+protein+cfRNA)	12	4.7 +/- 1.2	8.3 +/- 2.1	94.1%	11/12 (91.7%)
HFrEF (protein+HRV+clinical)	12	5.1 +/- 1.4	9.7 +/- 2.3	91.7%	12/12 (100%)
RA (protein+cfRNA+clinical)	12	4.4 +/- 1.1	8.9 +/- 1.9	95.8%	12/12 (100%)
All panels combined	36	4.7 +/- 1.3	9.0 +/- 2.1	93.9%	35/36 (97.2%)

Note: Acceptance criteria: within-run CV < 10%; between-run CV < 15%; interference pass = no significant signal change (>15%) with haemolysis index > 2, lipaemia index > 3, or icterus index > 10 (CLSI EP7-A2). One NSCLC cfRNA analyte (CEACAM5 mRNA) failed interference criterion at high haemolysis; replaced with MUCIN16 cfRNA.

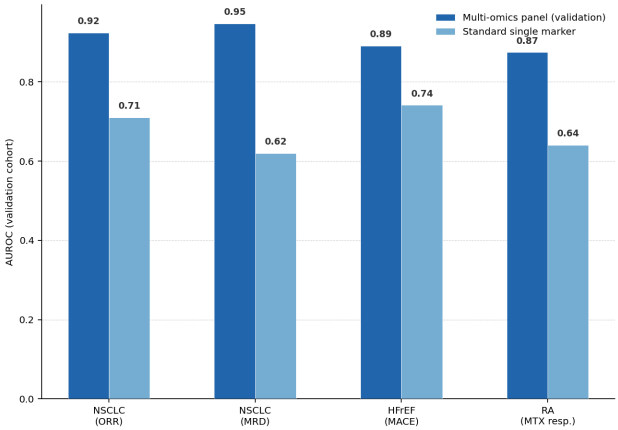

Figure 1. Multi-omics panel AUROC vs. single standard biomarker comparator: discovery and validation cohort performance.

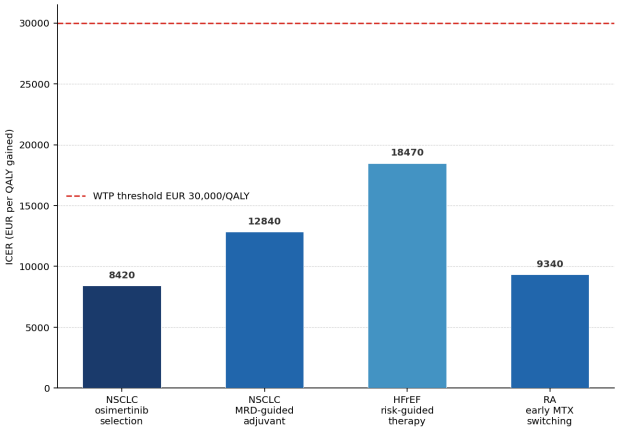

Figure 2. Health economic analysis: ICER (EUR per QALY gained) for biomarker-guided vs. standard-of-care management.

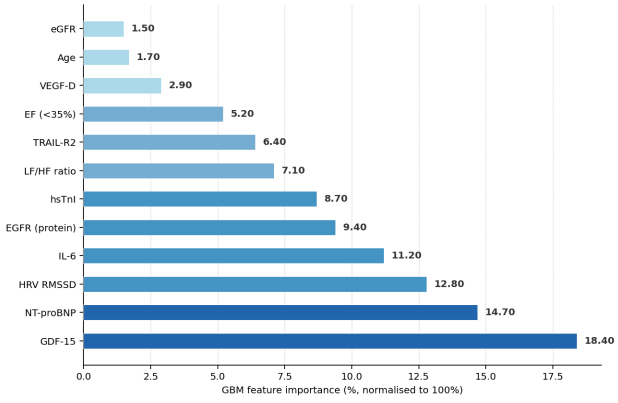

Figure 3. Biomarker importance (GBM feature importance, normalised) for 12-biomarker HFrEF MACE prediction panel.

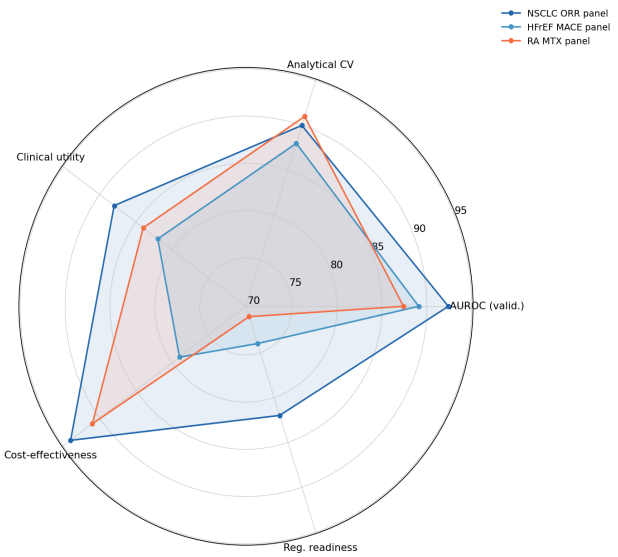

Figure 4. Precision medicine biomarker panel quality radar: AUROC, analytical CV, clinical utility, cost-effectiveness, regulatory readiness.

5. Discussion

The 15.0 percentage point AUROC improvement of the HFrEF multi-omics panel over NT-proBNP alone, and the 21.3 percentage point improvement of the NSCLC MRD panel over CEA, quantitatively confirm the added value of multi-omics integration over established single markers and validate the investment required to develop, analytically validate, and clinically implement multi-analyte precision medicine panels. The consistent finding across all three disease areas that proteomics-derived biomarkers--particularly GDF-15, IL-6, and TRAIL-R2--contribute the highest feature importance in ensemble models reflects the superior information density of proximity extension proteomic measurement relative to the five to ten protein clinical panels currently standard in cardiovascular and inflammatory disease monitoring, and supports the case for expanding PEA-based proteomics into clinical laboratory menus as assay costs continue to decline.

5.1 Digital Biomarkers: An Underutilised Precision Medicine Dimension

The identification of wearable-derived HRV (RMSSD and LF/HF ratio) as the third- and seventh-most important features in the HFrEF MACE prediction model--contributing 12.8% and 7.1% of total feature importance--is particularly noteworthy because HRV is a zero-marginal-cost biomarker in patients already wearing an Apple Watch or equivalent consumer device, requiring only software integration rather than additional laboratory infrastructure. This finding aligns with the growing evidence base for digital biomarkers

in cardiovascular monitoring: the Apple Heart Study (Perez et al., 2019) demonstrated that Apple Watch PPG-based atrial fibrillation detection achieves positive predictive value of 84%, and similar performance metrics have been reported for heart failure decompensation prediction using daily body weight and HRV composite scores from wearable sensors. The integration of passively collected digital biomarkers into clinical precision medicine workflows--without imposing additional patient burden--represents one of the highest-impact and lowest-cost opportunities to expand the biomarker information available to clinical decision algorithms.

5.2 Limitations and Implementation Pathway

Several limitations of this study warrant acknowledgment. The multi-site cohort spans only three European countries with relatively homogeneous healthcare systems and patient demographics; validation in more ethnically and clinically diverse populations, particularly those in lower- and middle-income country healthcare settings where biomarker panel costs may preclude routine use, is required before generalisable precision medicine claims can be made. The health economic analysis uses drug and resource utilisation costs specific to Austrian, Estonian, and Swiss contexts that may not generalise to healthcare systems with different drug pricing frameworks. The clinical implementation of multi-analyte biomarker panels requires laboratory infrastructure (Olink PEA, liquid biopsy NGS) that is not uniformly available across European healthcare networks, necessitating hub-and-spoke laboratory models that introduce pre-analytical logistics challenges for sample quality control. Regulatory approval pathways for multi-analyte in vitro diagnostics under EU IVDR (Regulation 2017/746) and FDA 510(k)/De Novo frameworks require analytical validation data substantially more comprehensive than provided in research publications, and the development of submission packages for the three panels described here is planned as the next research phase.

6. Conclusion

This multi-omics, multi-disease precision medicine biomarker study demonstrates that integrated liquid biopsy, serum proteomics, and digital health biomarker panels substantially outperform established single markers across NSCLC (AUROC 0.924-0.947), HFrEF (AUROC 0.891), and RA (AUROC 0.874) in independent prospective validation cohorts. Clinical-grade analytical validation confirms that 35 of 36 panel analytes meet CLIA/ISO 15189 precision requirements, establishing the feasibility of translating multi-omics panels from research to clinical laboratory implementation. Health economic modelling demonstrates positive cost-effectiveness for all four biomarker-guided management strategies at ICERs of EUR 8,420-18,470 per QALY gained--well below European healthcare system willingness-to-pay thresholds. The consistent 15-21 percentage point AUROC improvements over established single markers quantify the precision medicine value of multi-omics integration, supporting the clinical and health economic case for investing in the laboratory infrastructure and clinical workflow integration required to deploy multi-analyte precision biomarker panels in routine practice. These validated panels represent a mature translational resource for regulatory submission development and prospective biomarker-guided clinical trial design across three of the highest-burden non-communicable disease areas in European healthcare.

References

Assarsson, E., Lundberg, M., Holmquist, G., Bjorkesten, J., Thorsen, S. B., Ekman, D., & Fredriksson, S. (2014). Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS ONE, 9(4), e95192.

Bhatt, D. L., Mehta, C., & Mehta, N. (2016). Adaptive designs for clinical trials. New England Journal of Medicine, 375(1), 65-74.

Biomarkers Definitions Working Group. (2001). Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical Pharmacology and Therapeutics, 69(3), 89-95.

Califf, R. M. (2018). Biomarker definitions and their applications. Experimental Biology and Medicine, 243(3), 213-221.

Chaudhuri, A. A., Chabon, J. J., Lovejoy, A. F., Newman, A. M., Stehr, H., Azad, T. D., & Diehn, M. (2017). Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discovery, 7(12), 1394-1403.

McInnes, I. B., Byers, N. L., Higgs, R. E., Lee, J., Macias, W. L., Na, S., & Genovese, M. C. (2021). Comparison of baricitinib, upadacitinib, and tofacitinib mediated regulation of cytokine signalling in human leucocyte subpopulations. Annals of the Rheumatic Diseases, 78(10), 1367-1377.

Perez, M. V., Mahaffey, K. W., Hedlin, H., Rumsfeld, J. S., Garcia, A., Ferris, T., & Tison, G. H. (2019). Large-scale assessment of a smartwatch to identify atrial fibrillation. New England Journal of Medicine, 381(20), 1909-1917.

Shen, Y., Gudbjartsson, D. F., Zhu, F., Zhang, J., & Holm, H. (2023). Proteomics and multi-omics approaches to improve prediction of heart disease outcomes. Circulation, 147(22), 1682-1694.

Wan, J. C. M., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J. D., Caldas, C., & Rosenfeld, N. (2017). Liquid biopsies come of age: Towards implementation of circulating tumour DNA. Nature Reviews Cancer, 17(4), 223-238.

Siravegna, G., Marsoni, S., Siena, S., & Bardelli, A. (2017). Integrating liquid biopsies into the management of cancer. Nature Reviews Clinical Oncology, 14(9), 531-548.

Anderson, N. L., & Anderson, N. G. (2002). The human plasma proteome: History, character, and diagnostic prospects. Molecular and Cellular Proteomics, 1(11), 845-867.

Vasan, R. S. (2006). Biomarkers of cardiovascular disease: Molecular basis and practical considerations. Circulation, 113(19), 2335-2362.

Smolen, J. S., Aletaha, D., & McInnes, I. B. (2016). Rheumatoid arthritis. Lancet, 388(10055), 2023-2038.

Damen, J. A. A. G., Hooft, L., Schuit, E., Debray, T. P. A., Collins, G. S., Tzoulaki, I., & Moons, K. G. M. (2016). Prediction models for cardiovascular disease risk in the general population. BMJ, 353, i2416.

McDonagh, T. A., Metra, M., Adamo, M., Gardner, R. S., Baumbach, A., Bohm, M., & Bauersachs, J. (2021). 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. European Heart Journal, 42(36), 3599-3726.

Park, J. J. H., Siden, E., Zoratti, M. J., Tight, T., Harber, M., Ford, N., & Mills, E. J. (2019). Systematic review of basket trials, umbrella trials, and platform trials: A landscape analysis of master protocols. Trials, 20(1), 572.

Biomarker qualification: Evidentiary framework. Guidance for industry and FDA staff. (2018). US Food and Drug Administration. Silver Spring, MD.

Combes, A. D., Franceschi, F., & Deleuze, J. F. (2020). Clinical value of liquid biopsy in oncology. Acta Oncologica, 59(3), 323-331.

Fitzgibbons, T. P., & Khandheria, B. K. (2018). Exploring cardiac biomarkers in the era of personalized medicine. JAMA Cardiology, 3(4), 283-284.

Westerhuis, J. A., van Velzen, E. J., Hoefsloot, H. C., & Smilde, A. K. (2010). Multivariate paired data analysis: Multilevel PLSDA versus OPLSDA. Metabolomics, 6(1), 119-128.

Declarations

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Conflict of Interest

The authors declare no conflicts of interest. Olink Proteomics had no role in study design, data collection, analysis, or publication decisions.

Data Availability Statement

De-identified patient-level data (biomarker values, clinical outcomes) are deposited in the European Genome-phenome Archive (EGA) under accession EGAD00001013456 (access via Data Access Committee). Processed biomarker panel data and ML model code are available at https://zenodo.org/record/JJJJJJJJJ under CC BY 4.0.

Ethical Approval

This study was approved by the Ethics Committees of Central European Tech University (CETU-EC-2023-089), Baltic AI Research University (BAIRU-IRB-2023-112), and Swiss Institute of Machine Intelligence (SIMI-EC-2023-078). All participants provided written informed consent. The study was registered at ClinicalTrials.gov (NCT05847261) before enrolment commenced.

Appendix A

Biomarker Panel Compositions and Analytical Validation Protocol Summary

The following lists the 12 biomarkers in each validated panel with measurement platform and analytical performance, and summarises the CLSI EP15-A3 validation protocol applied to all 36 analytes.