

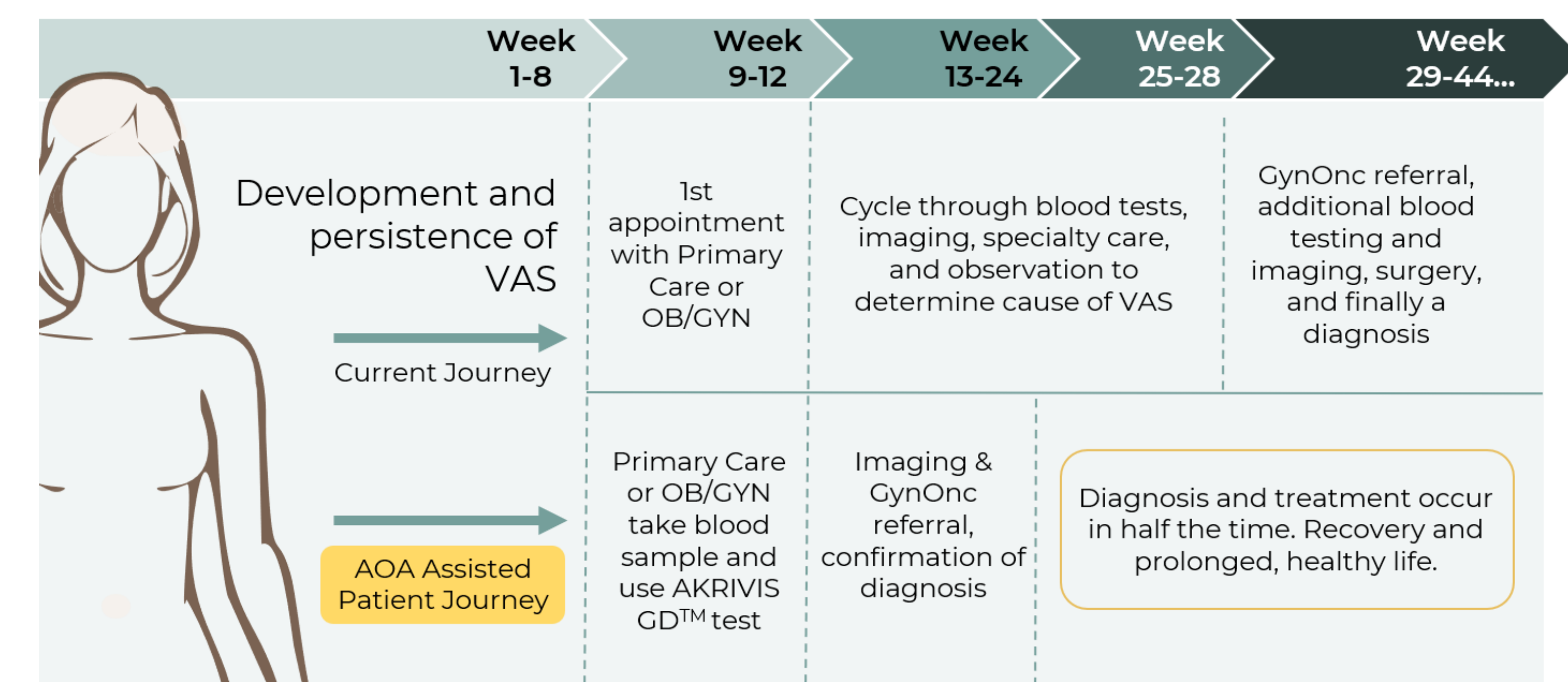
<sup>1</sup>Nichols CM, <sup>1</sup>Culp-Hill R, <sup>1</sup>Han E, <sup>1</sup>Giles B, <sup>1</sup>Zapata M, <sup>1</sup>Goldberg M, <sup>1</sup>Law RA, <sup>1</sup>Radnaa E, <sup>1</sup>Kilkenny S, <sup>1</sup>Wong M, <sup>1</sup>Hansen C, <sup>1</sup>Fa VS, <sup>1</sup>Bystrom C, <sup>2</sup>Zhao L, <sup>3</sup>E Kroos K, <sup>1</sup>McElhinny A  
<sup>1</sup>AOA Dx, Denver, CO | <sup>2</sup>CompleteOmics | <sup>3</sup>Lipidomics Consulting Ltd.

## Clinical Need

Most ovarian cancer (OC) patients are diagnosed at late stage when survival outcomes are poor.<sup>2</sup>

- 80% of women with OC present with vague abdominal symptoms (VAS) early<sup>3</sup>
- Symptoms overlap with benign conditions<sup>3</sup>
- Diagnostic journey averages 9 months in the U.S.<sup>4</sup>
- No blood-based rule-out test exists for symptomatic women<sup>5</sup>

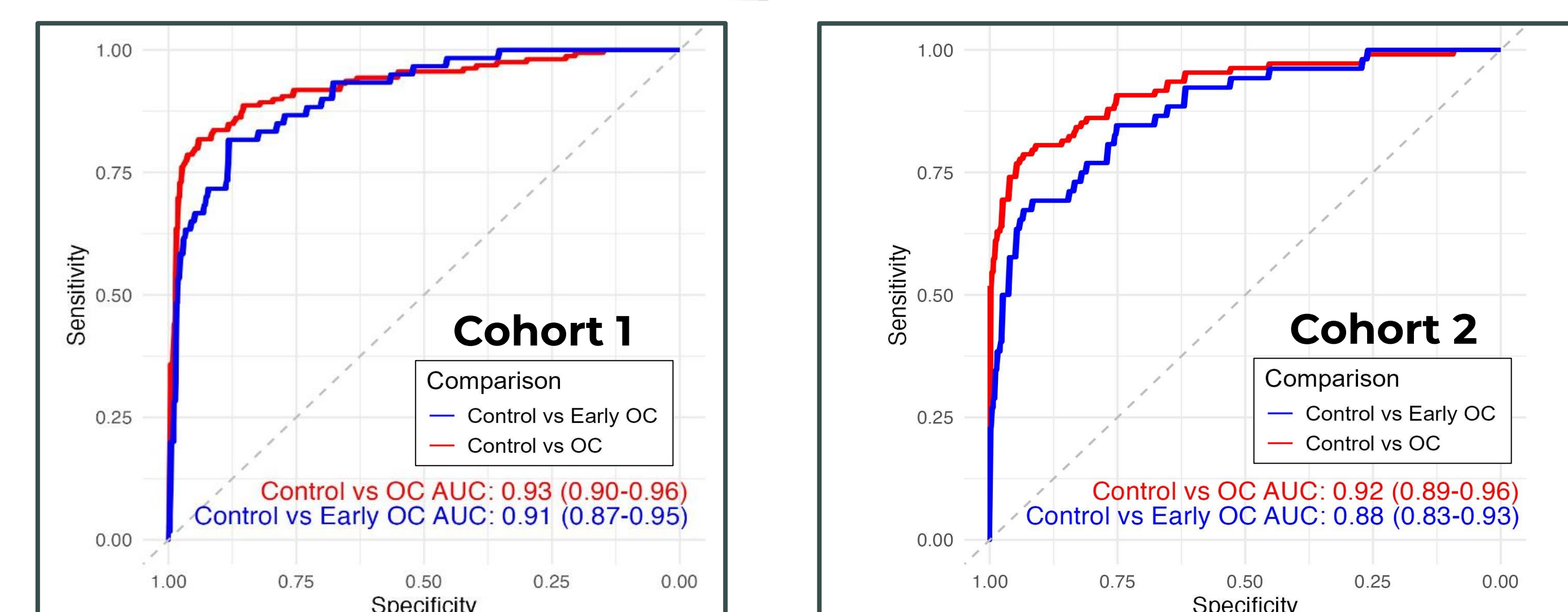
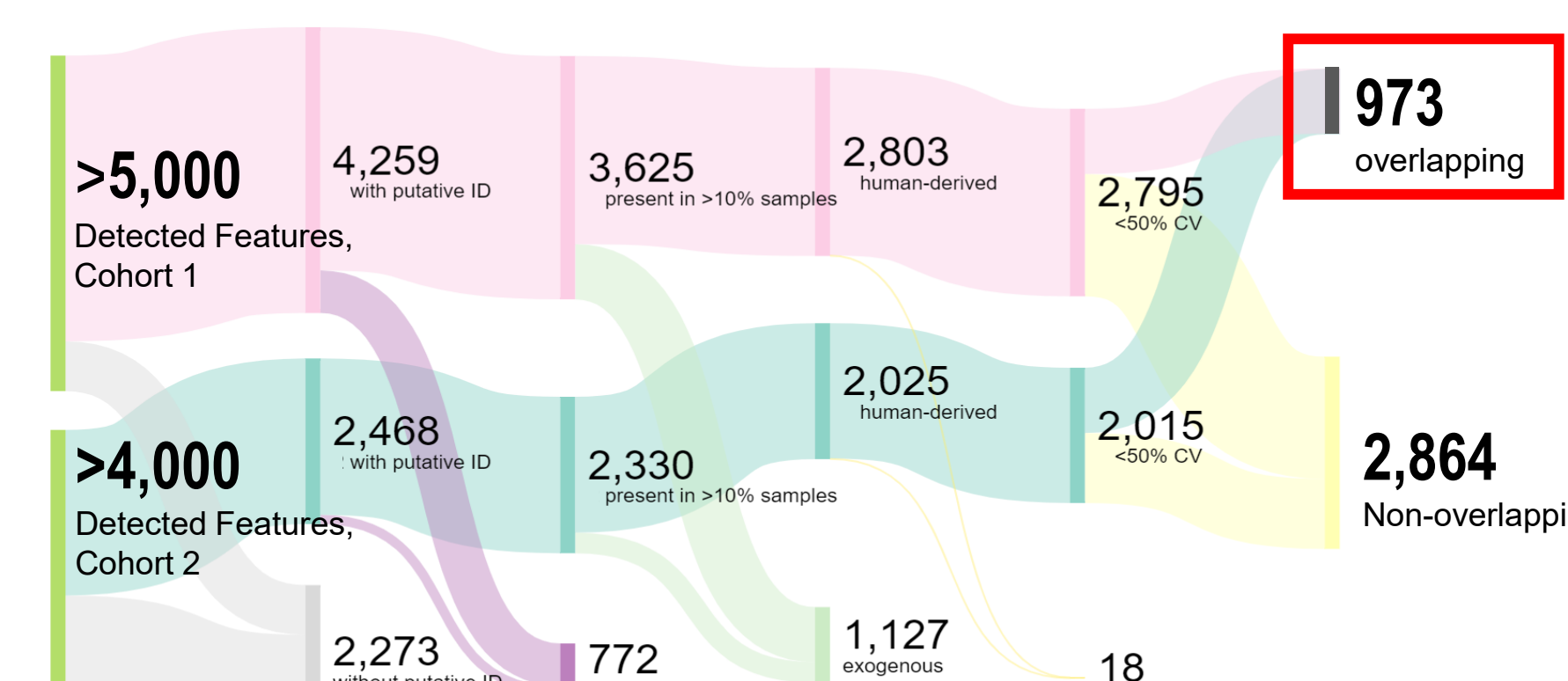
Our goal is to develop a non-invasive, **multi-omic blood test** to aid diagnosis at first presentation and shorten time to imaging and referral.



## Discovery Lipidomics + Modeling

Features detected across both cohorts were first down selected prior to inclusion in machine learning-based modeling.

Feature down-selection included:  
(1) without library ID,  
(2) present in <10% of samples,  
(3) of exogenous origin,  
(4) technical CV > 50%, and  
(5) not detected in both cohorts.



A proof-of-concept multi-omic model distinguished OC from symptomatic controls, maintaining early-stage detection in an independent cohort.

## Performance + Normalization

### Analytical Precision

- Targeted assay shows stable extraction replicates and technical controls
- Quantitative reproducibility improved relative to discovery HRMS workflows

### Functional Controls

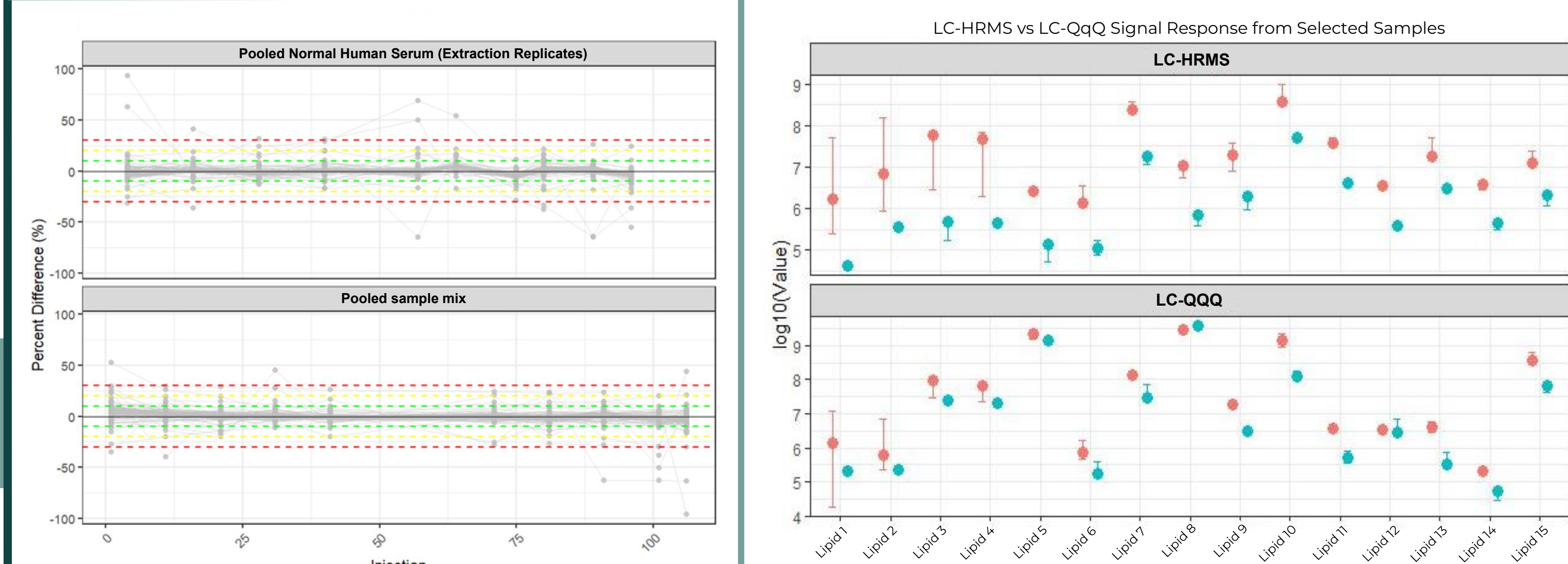
- Lipids retain fold-change directionality between discovery and targeted platforms
- Confirms preservation of biological signal during assay translation

### Statistical Discrimination

- Targeted lipid panel maintains separation between OC and controls
- Univariate analysis confirms multiple discriminatory lipid features

### Data-Driven Normalization

- Internal standards assigned using algorithmic lipid-standard pairing
- Optimization accounts for retention time, ion mode, and lipid class
- Reduces post-normalization variability and improves quantitative stability



## Scientific Rationale

Protein biomarkers such as CA125 and HE4 provide clinical value but have **limited discrimination in early-stage disease**.<sup>5</sup>

Lipids represent a complementary biological signal, and reprogrammed lipid metabolism is a known cancer hallmark<sup>8-10</sup> that affects:

- ↔ Membrane remodeling
- ▮ Cell proliferation
- ⚡ Tumor signaling pathways
- 🛡 Immune modulation

Combining **lipids with proteins** has been shown to improve OC detection within a clinically complex symptomatic population.<sup>11</sup>

## The Translational Challenge

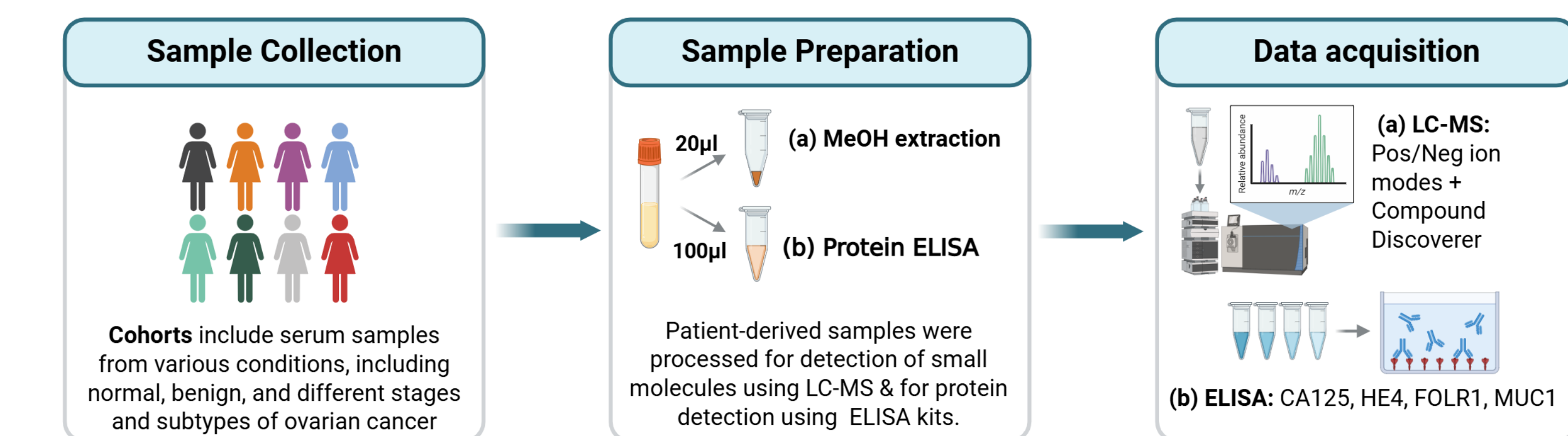
Discovery lipidomics generates powerful biological insights but presents challenges for clinical implementation.

	Discovery Lipidomics	Targeted Clinical Assay
Analytical Scope	Untargeted – thousands of features	Targeted quantification of defined panel
Dimensionality	High-dimensional data, complex processing	Focused measurement of selected transitions
Quantitation	Semi-quantitative, relative abundance	Precise quantitative measurement
Throughput	Research-scale workflows	High-throughput clinical testing
Clinical Readiness	Not easily standardized across labs	Transferable, reproducible clinical assay

**Goal:** Convert discovery lipid biomarkers into a **robust targeted MRM assay** capable of clinical deployment while preserving diagnostic signal.

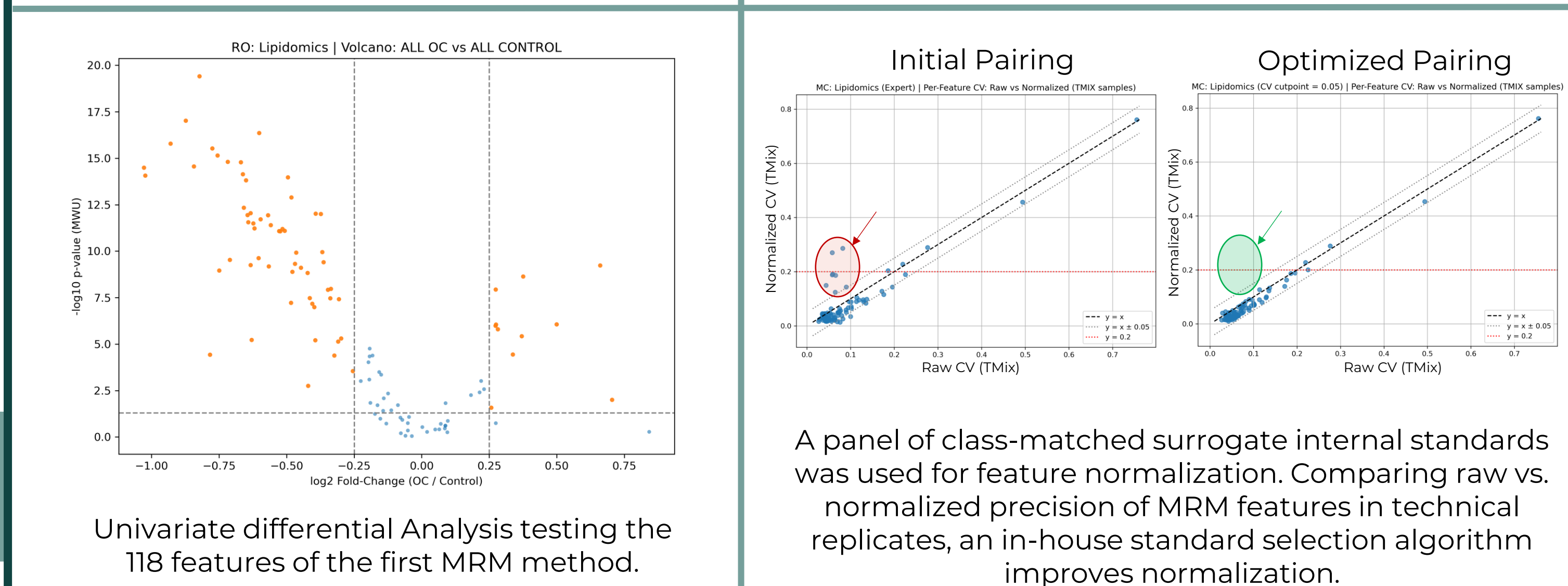
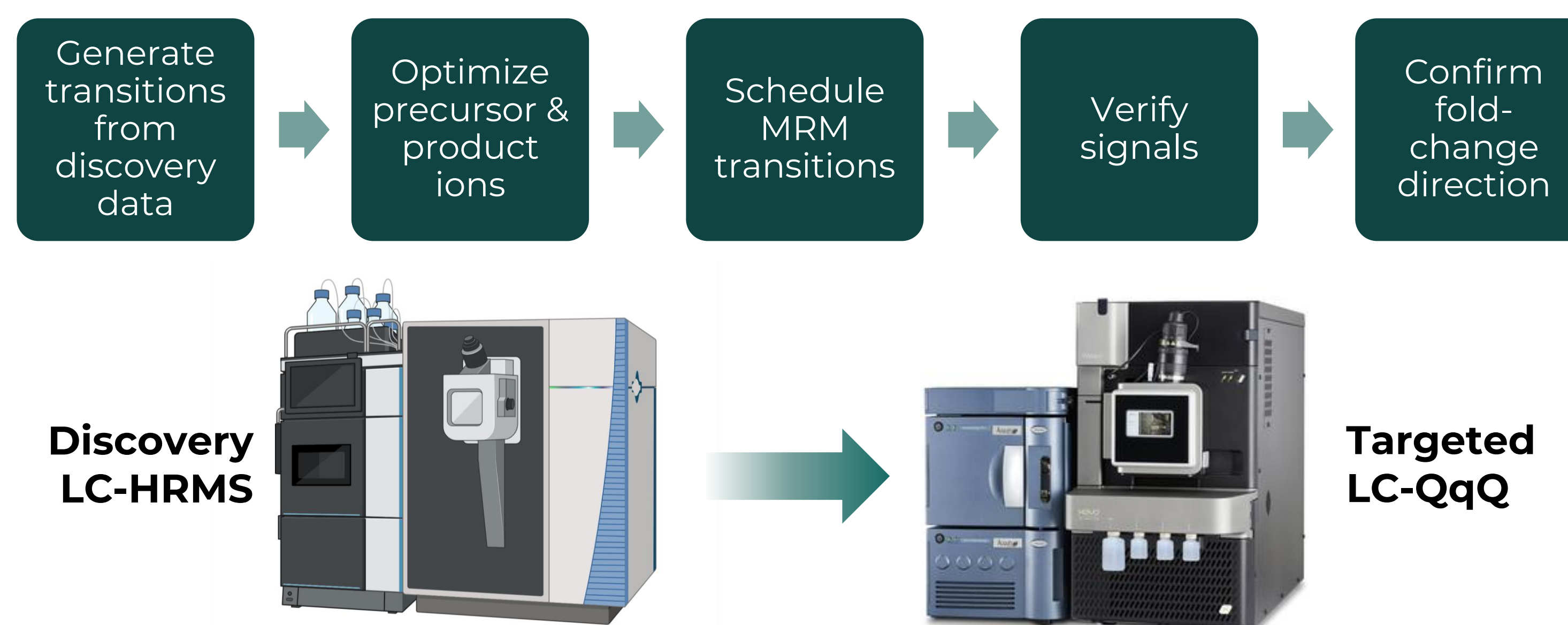
## Discovery Cohort Study Design + Workflow

Condition	Cohort 1	Cohort 2
Normal/Symptomatic Healthy	82	208
Gastrointestinal Disorder	50	0
Benign Gynecological Conditions and Masses	168	86
Early-Stage OC (I/II)	80	52
Late-Stage OC (III/IV)	139	57
<b>Grand Total</b>	<b>519</b>	<b>403</b>



## Targeted Assay Development

Candidate lipid biomarkers identified in discovery were transferred into a **targeted assay**, enabling **precise quantification of selected lipid biomarkers** in clinical serum samples.



## Conclusions

- Multi-omic modeling improves discrimination in symptomatic women.
- Discovery lipidomics biomarkers can be successfully translated into a targeted assay.
- Analytical precision improves while clinical performance is retained.
- This workflow provides a framework for translating omics discovery findings into clinically deployable diagnostics.

(1) Sundar, S., Agarwal, R. et al. Risk-prediction models in postmenopausal patients with symptoms of suspected OC in the UK (ROCKET): a multicentre, prospective diagnostic accuracy study. *Lancet Oncol.* 2024, 25 (10), 1371 (2) Siegel, R.L., Miller, T.B. et al. "Cancer Statistics" *CA Cancer J Clin.* 2025, 75(1), 10 (3) Carr, B.A., Matthews, B.L. et al. "Are there any symptoms of OC management?" *Cancer* 2020, 137(10), 4434 (4) Huseronberger, S.P., Sun, C.S. et al. "Factors impacting the time to ovarian cancer diagnosis based on classic symptom presentation in the United States" *Cancer* 2021, 127(22), 4181 (5) Lu, K.H. "Screening for OC in Asymptomatic Women" *JAMA* 2018, 319(6), 557 (6) Kwong, P.L.A., Krishnan, C. et al. "Symptom-triggered testing detects early stage and low volume resectable advanced stage OC" *Int. J. Gynecol. Cancer* 2020, 30(7), 1284-8 (7) Estrada, M.F., Aronoff, P. et al. "Substantiated: A functional precision model to personalize OC treatments: Results from a co-clinical study." *Cell Rep. Med.* 2020, 7(1), 102530 (8) Buas, M.F., Drescher, C.W. et al. "Quantitative Global Lipidomics Analysis of Patients with OC versus Benign Adrenal Mass." *Sci. Rep.* 11, 2021, 18156 (9) Sah, S., Bhatn, O.O. et al. "Serum Lipidome Profiling Reveals a Distinct Signature of OC in Korean Women." *Cancer Epidemiol Biomarkers Prev.* 33(5), 2024, 681 (10) Tzafetsis, V., Gika, H. et al. "The Contribution of Lipidomics in OC Management: A Systematic Review." *Int. J. Mol. Sci.* 24(28), 2023, 15961 (11) Giles, B.M., Culp-Hill, R. et al. "Utilizing Serum-Derived Lipidomics with Protein Biomarkers and Machine Learning for Early Detection of OC in the Symptomatic Population." *Cancer Res. Comm.*, 2025, 5(8), 1516