

Ovarian cancer (OC) remains highly lethal primarily due to late-stage diagnosis¹: most OC is detected **at stages III/IV**, when **5-year survival is <30%**. Existing tools lack sensitivity and specificity needed for early detection².

~85% of patients present with symptoms³, but vague, non-specific presentation leads to a 9-month diagnostic delay⁴. HGSOC **doubles in volume every 2-4 months**, leaving a narrow window for intervention; early diagnosis is associated with improved survival^{5,6}.

There is a clear **unmet need for improved diagnostic tools** to accurately triage symptomatic women and enable earlier detection. Our discovery work shows this is achievable, with **replicated performance of >90% sensitivity** for early-stage OC detection in the target symptomatic population.

Lipidomics remains challenging due to the structural complexity of lipids. Discovery datasets produce thousands of features^{7,8}, but they must be identified and validated. Characterization by tandem mass spectrometry (MS2) is critical, yet full structural verification can remain elusive without additional advanced techniques.

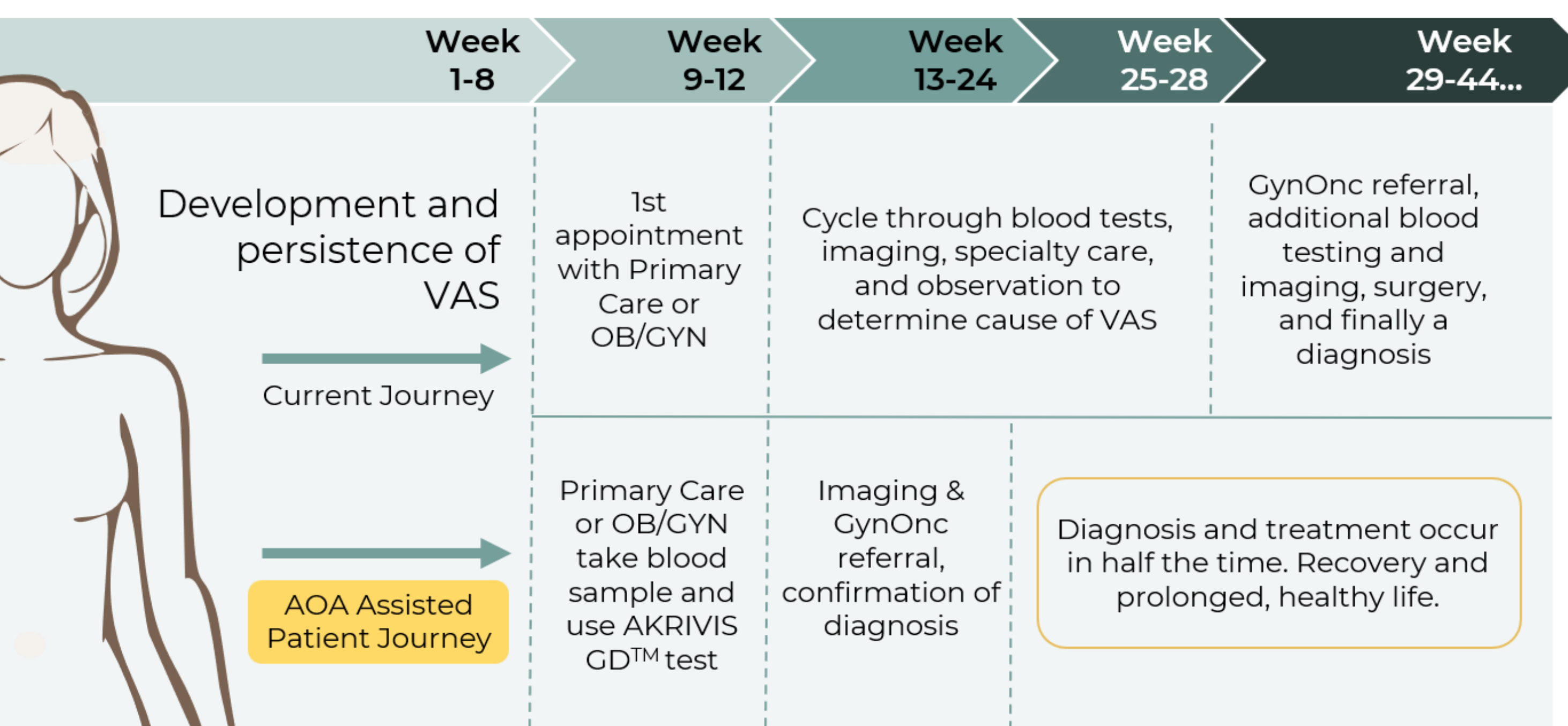


Figure 1. Comparing current standard-of-care path to OC diagnosis and the AKRIVIS GD™ supported patient care pathway.

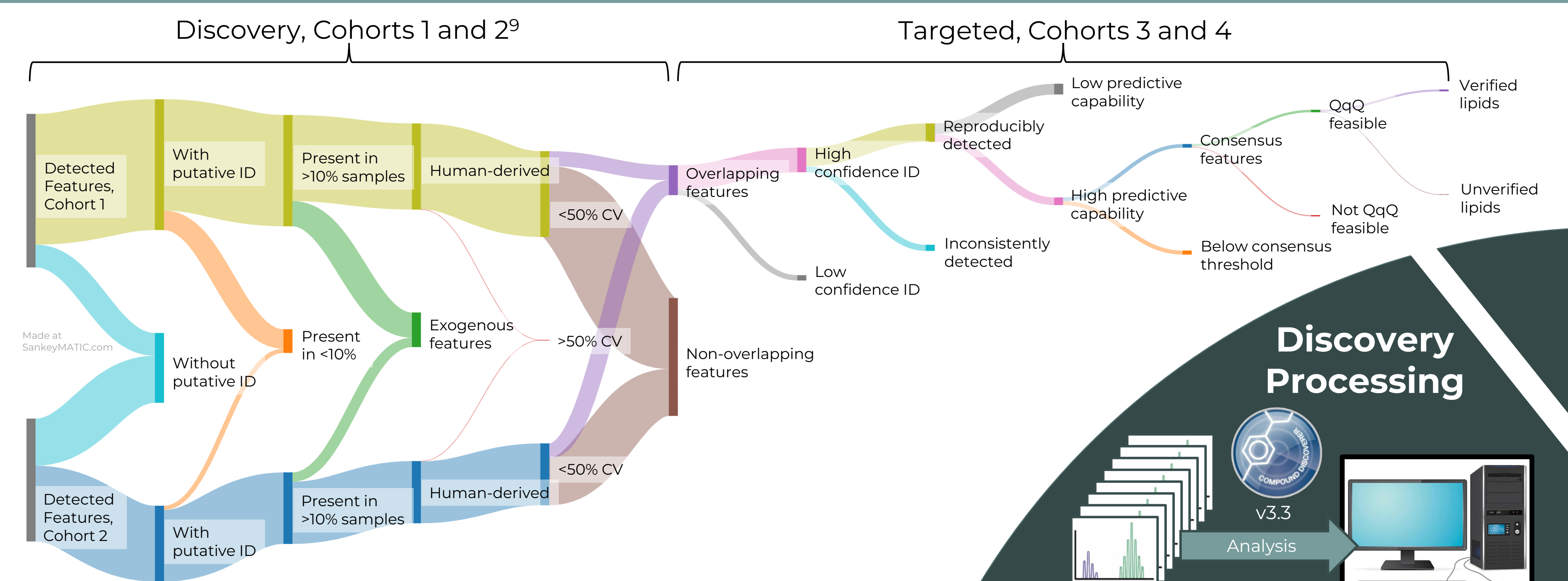


Figure 3. Untargeted-to-targeted lipidomics feature filtering pipeline. Sankey diagram tracking lipid features from two independent discovery cohorts through sequential QC filters: putative library ID, prevalence ($\geq 10\%$ samples), of human origin, and analytical reproducibility (CV $< 50\%$). Features detected in both cohorts ($n=973$ overlapping) were carried forward as the Discovery feature pool. Features were further filtered in two additional independent cohorts by ID confidence, detection reproducibility, and predictive performance, yielding 118 consensus features that advanced to targeted QqQ-MRM method development; iterative verification and performance evaluation reduced the panel to ~65 lipids confirmed to MS2 identification level 3-4.

Condition	Cohort 1	Cohort 2	Cohort 3	Cohort 4
Role	Discovery	Discovery	Validation	Validation
Early-Stage OC (I/II)	80	52	50	20
Late-Stage OC (III/IV)	139	57	66	20
Benign Gynecological Conditions + Masses	168	86	115	40
Symptomatic Normal	—	208	115	40
Healthy	82	—	35	40
Grand Total	469	403	381	160

Table 1. Cohort description for four independent serum cohorts spanning discovery and validation phases. All cohorts include women diagnosed with OC across subtypes and stages, and non-cancerous controls designed to mimic symptomatic populations. Samples sourced from biobanks and prospective collections, supplemented with commercial vendors

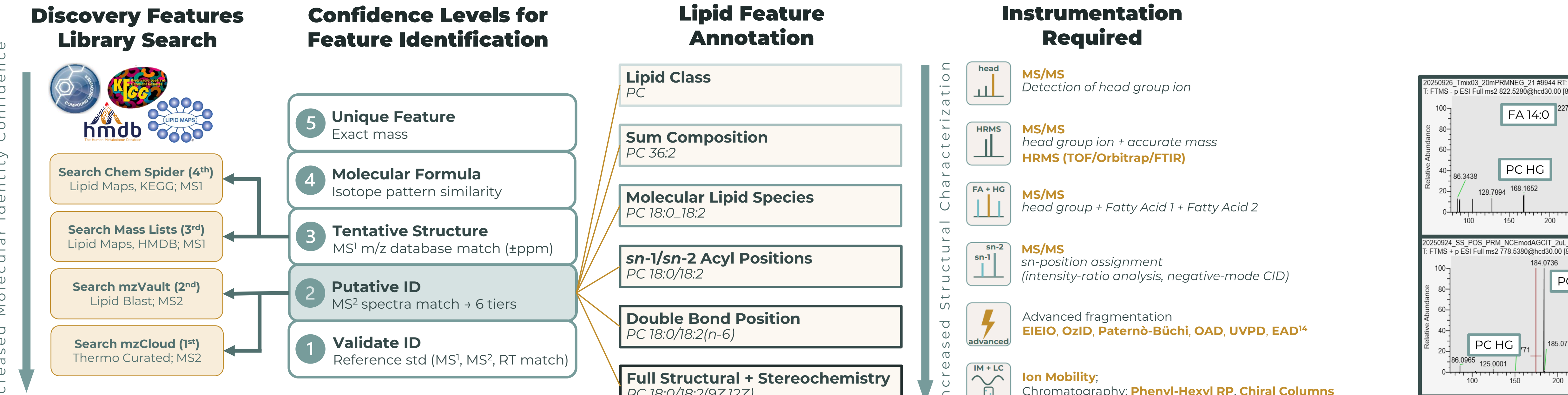
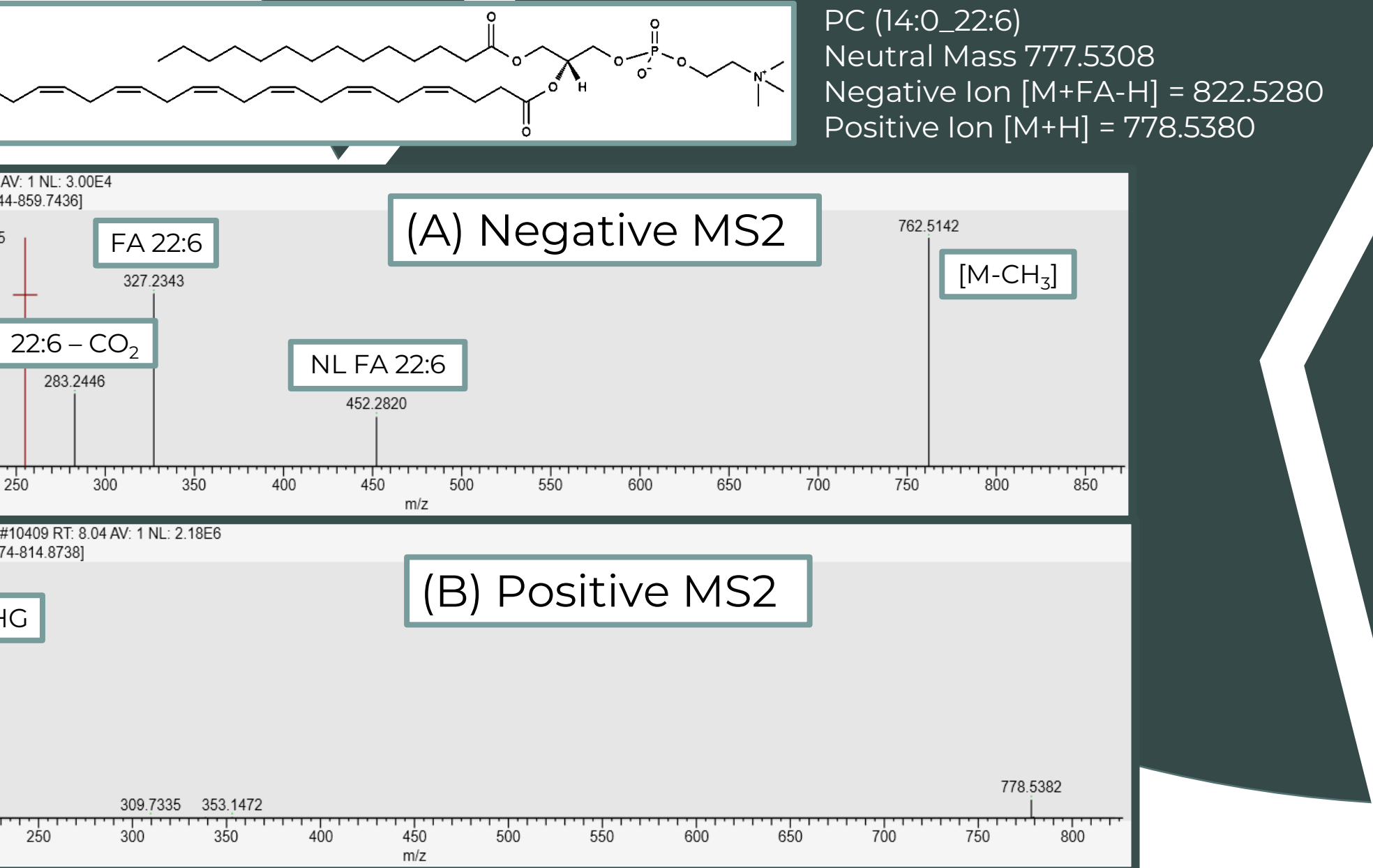
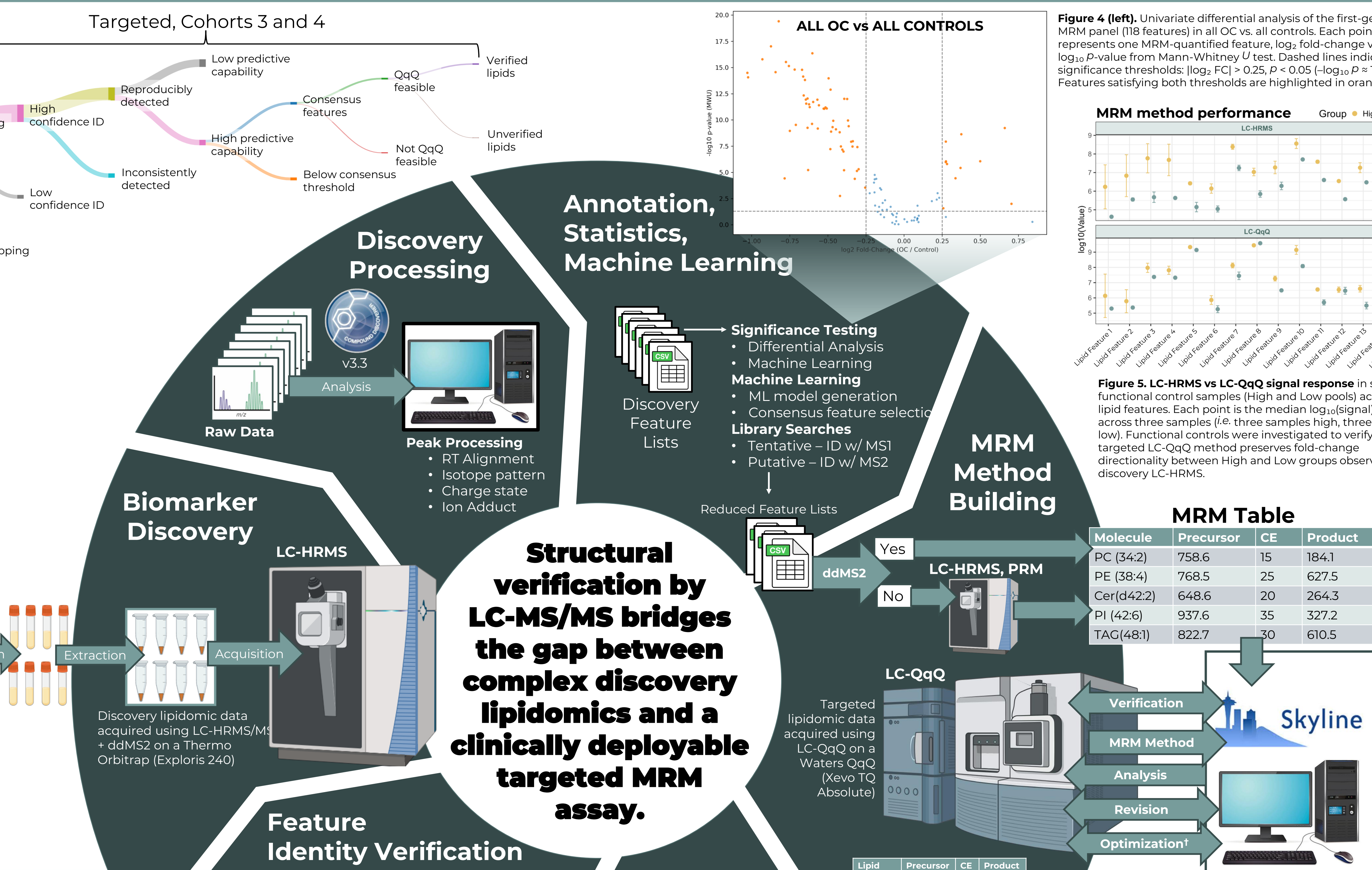


Figure 2. Discovery Lipidomics Annotation Workflow and Identification Confidence. Confidence levels for identifying lipid features in a biomarker discovery experiment using high resolution MS analysis with MS2 fragmentation. Library searches within compoundDiscoverer yield Level 3 and in some cases Level 2 identifications^{10,12,13}. The six structural annotation tiers derive from Level 2 (putative ID) matches. Additional analytical approaches (advanced fragmentation, ion mobility, chiral chromatography) progressively resolve isomeric structure. Ideally, DDA methods yield several Level 2 IDs that can be promoted to Level 1 via reference standards.

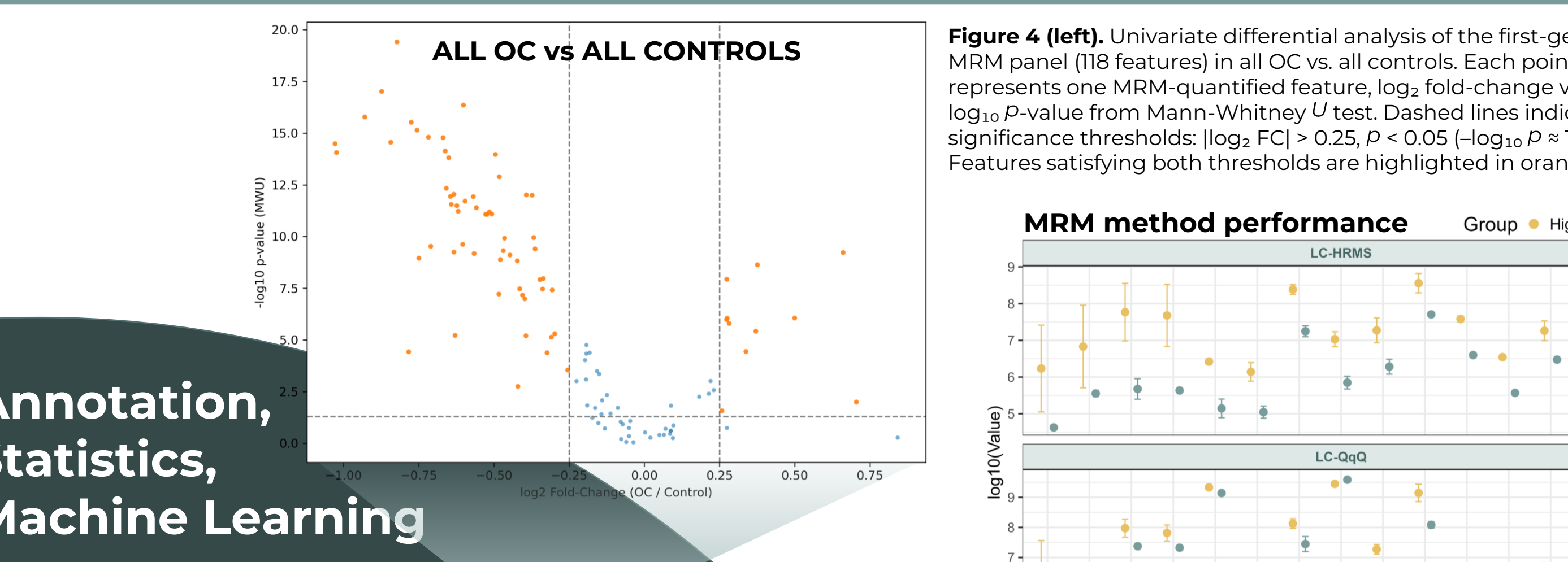
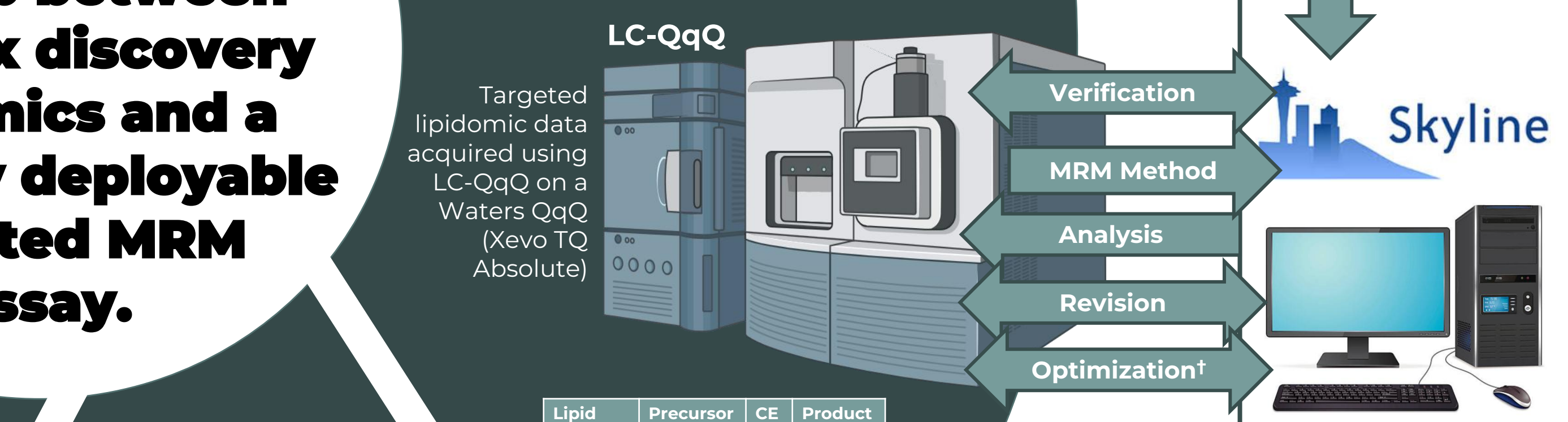


Figure 4 (left). Univariate differential analysis of the first-gen MRM panel (118 features) in all OC vs. all controls. Each point represents one MRM-quantified feature, \log_2 fold-change v. $-\log_{10} P$ -value from Mann-Whitney U test. Dashed lines indicate significance thresholds: $|\log_2 FC| > 0.25$, $P < 0.05$ ($-\log_{10} P \approx 1.30$). Features satisfying both thresholds are highlighted in orange.

Molecule	Precursor	CE	Product
PC (34:2)	758.6	15	184.1
PE (38:4)	768.5	25	627.5
Cer(d4:22)	648.6	20	264.3
PI (42:6)	937.6	35	327.2
TAG(48:1)	822.7	30	610.5



Lipid	Precursor	CE	Product
PE (36:2)	744.551	15	603.55
PE (36:2)	744.552	25	603.55
PE (36:2)	744.553	40	603.55
PE (36:2)	744.554	55	603.55

Figure 6 (left). Retention-time (RT) alignment between LC-HRMS and LC-QqQ. Deuterated standards spanning the chromatographic gradient (see Table 2) were analyzed on both instruments under matched LC conditions. Linear regression of LC-QqQ RT against LC-HRMS RT yielded $R^2 = 0.9994$, indicating near-unity slope. The fitted relationship is applied to translate LC-HRMS RTs of biomarkers identified in discovery into scheduled MRM windows on the LC-QqQ, ensuring accurate peak detection.

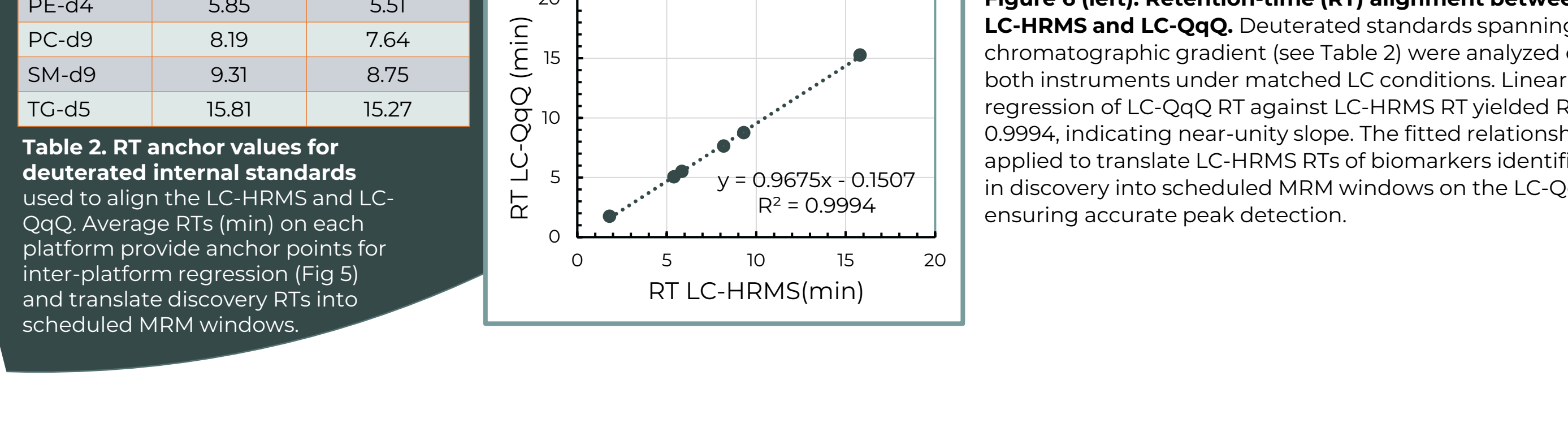


Figure 2 (right). MS² confirmation of PC(14:0_22:6) by dual-polarity HCD (NCE 30) on LC-HRMS. (A) Negative-mode MS² of [M+HCOO]⁻ (m/z 822.5280) yields demethylated precursor [M-CH₃]⁻ at m/z 762.51, sn-1 fatty-acyl carboxylate [FA 14:0-H]⁻ at m/z 227.20, sn-2 fatty-acyl carboxylate [FA 22:6-H]⁻ at m/z 327.23, a lyso-fragment at m/z 452.28 corresponding to neutral loss of sn-2 (22:6) acyl chain, and PC head-group anion at m/z 168.04. (B) Positive-mode MS² of [M+H]⁺ (m/z 778.5380) is dominated by the diagnostic phosphocholine head-group cation [C₁₄H₂₅N₂O₂P]⁺ at m/z 184.07. The head-group ions in both polarities, sn-specific acyl carboxylate anions, and sub-5 ppm mass accuracy on diagnostic fragments support confident identification of PC(14:0_22:6).