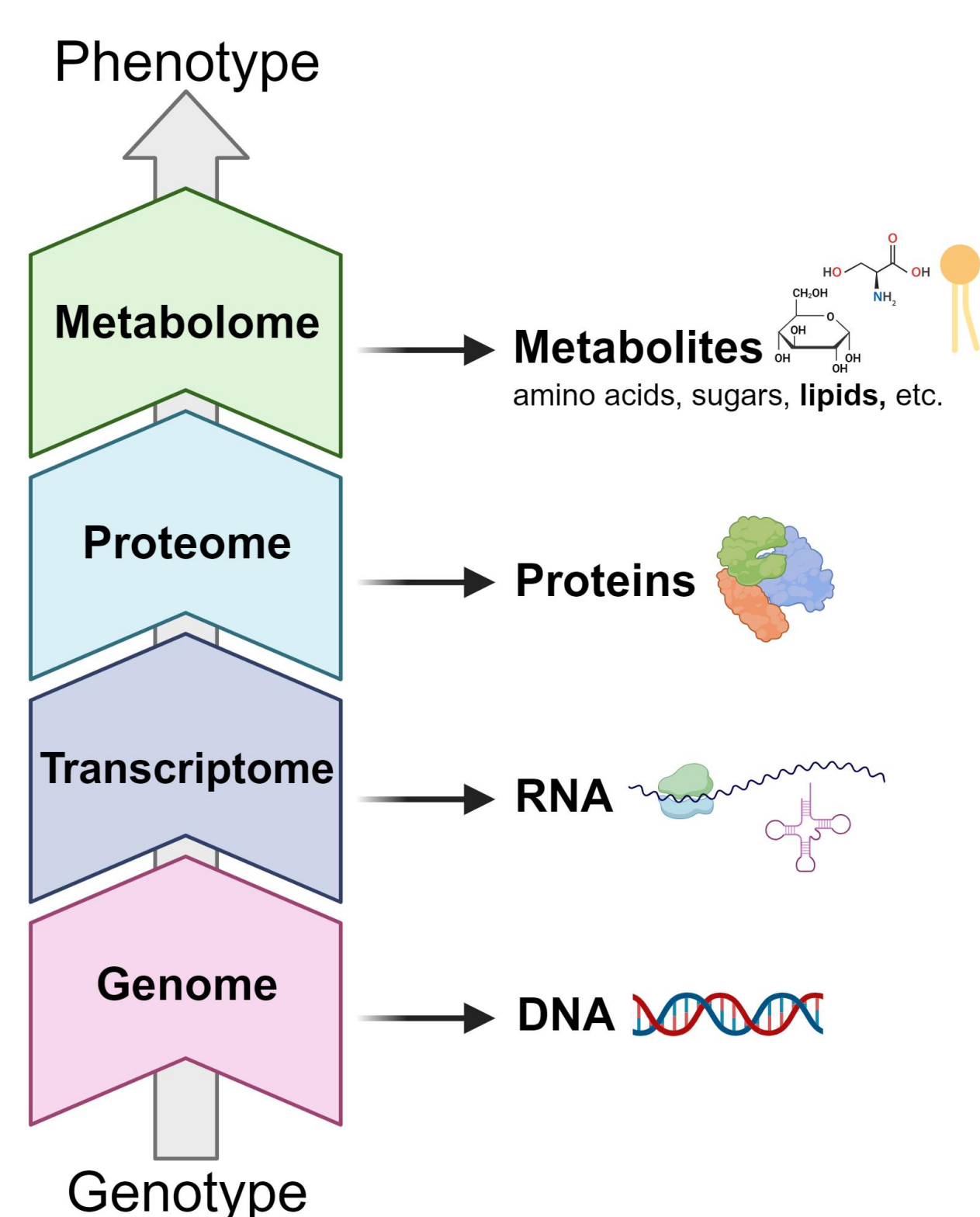


Background and Rationale

- Ovarian cancer (OC) remains highly lethal
- Symptoms overlap with benign gynecologic and GI conditions¹
- Avg. diagnostic delay is ~9 months in the U.S.²
- >70% diagnosed with late-stage OC, 5-year survival 10-30%³
- Lack of effective diagnostic tools for early-stage OC
- If OC is diagnosed at early stages, survival jumps to >90%⁴

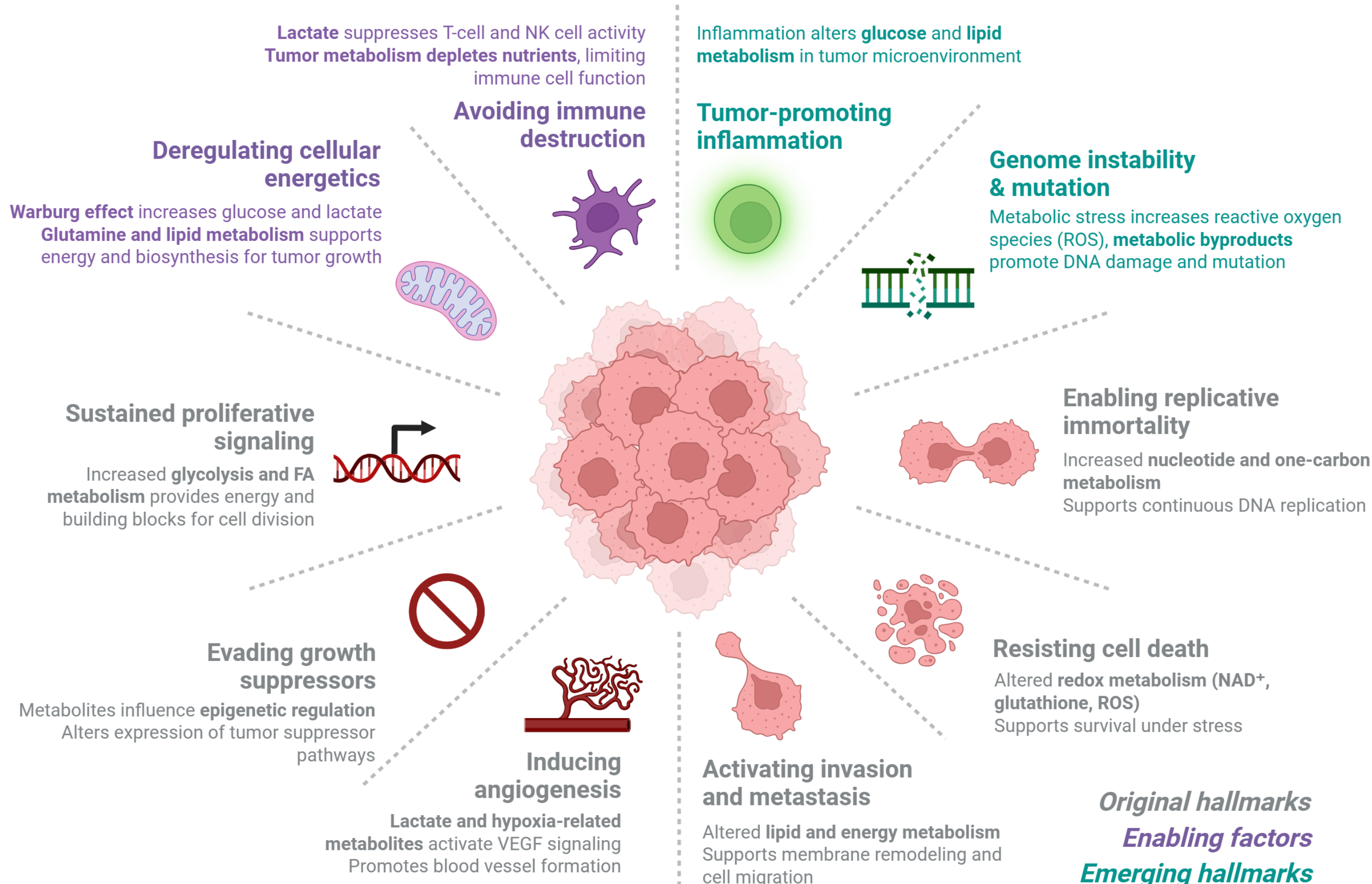
Altered metabolism is a cancer hallmark



- While genomics and transcriptomics reflect biological potential and proteomics reflects functional machinery, metabolomics captures the phenotype — the integrated output of tumor and host metabolism.
- MS-based metabolomics enables detection of subtle metabolic shifts, offering a powerful tool for early cancer detection.
- Metabolomic biomarkers have the potential to improve diagnostic accuracy and complement existing methods by detecting tumor-driven metabolic rewiring.

Metabolomics + Hallmarks of Cancer⁵

adapted from "Hallmarks of Cancer: The Next Generation" Hanahan & Weinberg 2011

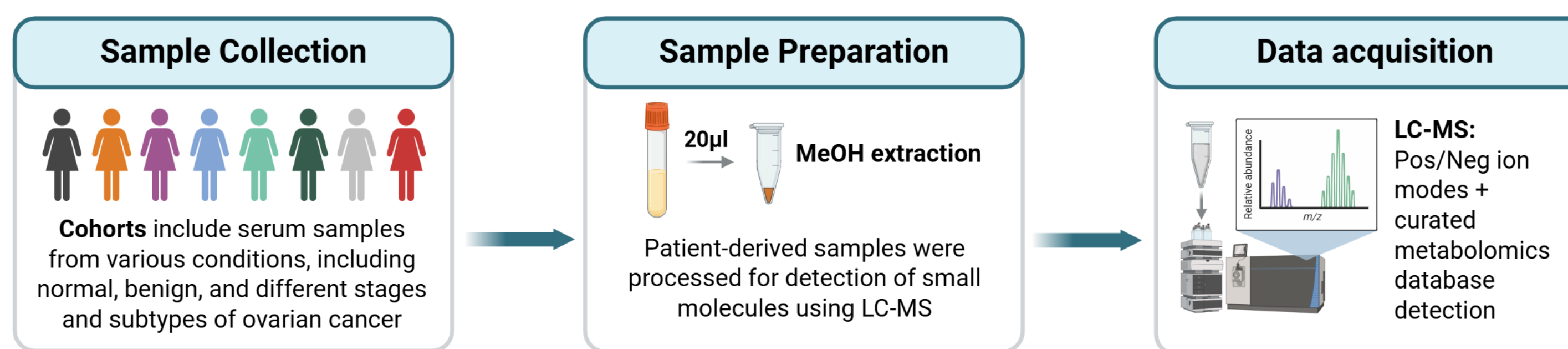


Study Design + Workflow

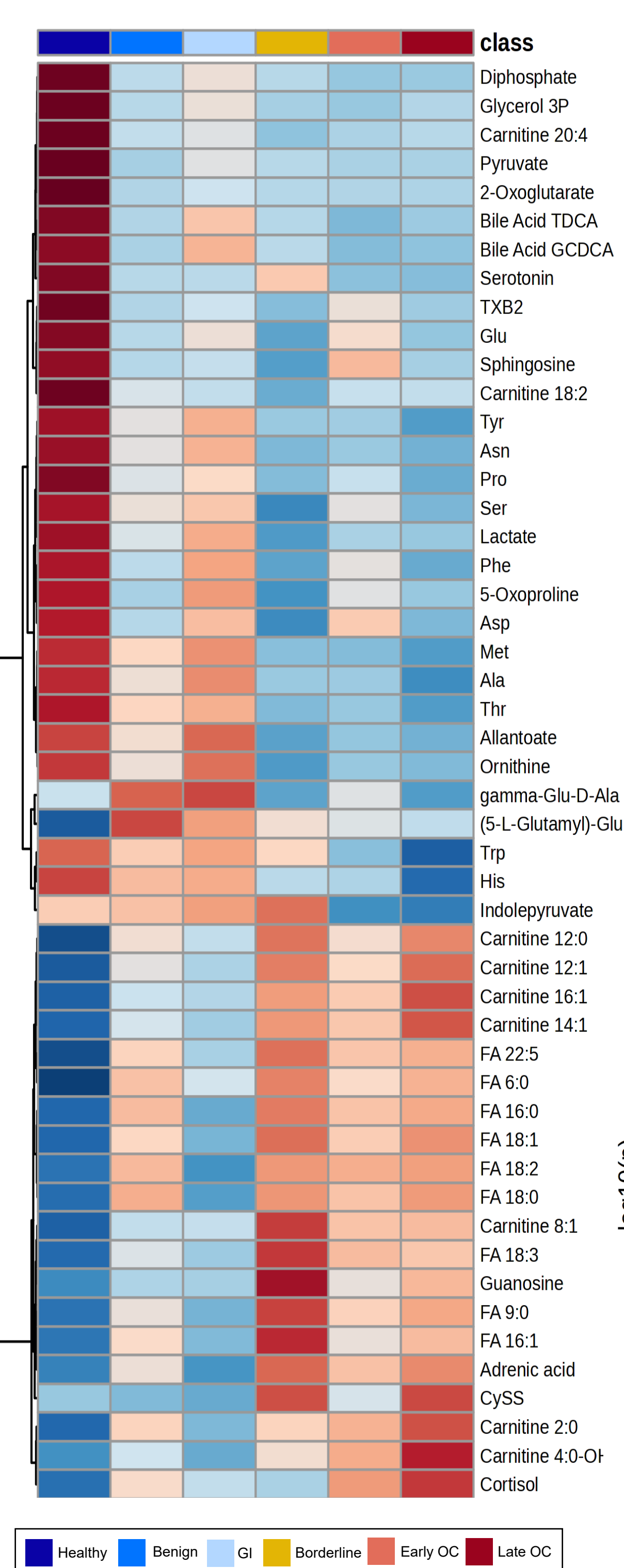
We conducted metabolomics analysis of two independent, clinically annotated cohorts. Cohort 1 was obtained from the University of Colorado Gynecologic Tissue and Fluid Bank + commercial vendors. Cohort 2 was collected from a prospectively enrolled symptomatic population (Manchester University NHS Foundation Trust + commercial vendors). Samples were blinded and cohorts processed independently.

Diagnosis	Group	Cohort 1	Cohort 2	Combined
Cancer	All OC	219	116	335
	Early-Stage OC	80	50	130
	Late-Stage OC	139	66	205
Non-Cancer	Normal/Healthy	82	149*	231
	Benign Gynecologic Conditions	168	116	284
	GI Disorders	50	0	50
	Borderline Tumors	25	19	44
Grand Total		544	400	944

*normal/healthy samples from cohort 2 comprise normal healthy donors and individuals seeking care for symptoms with no confirmed diagnosis



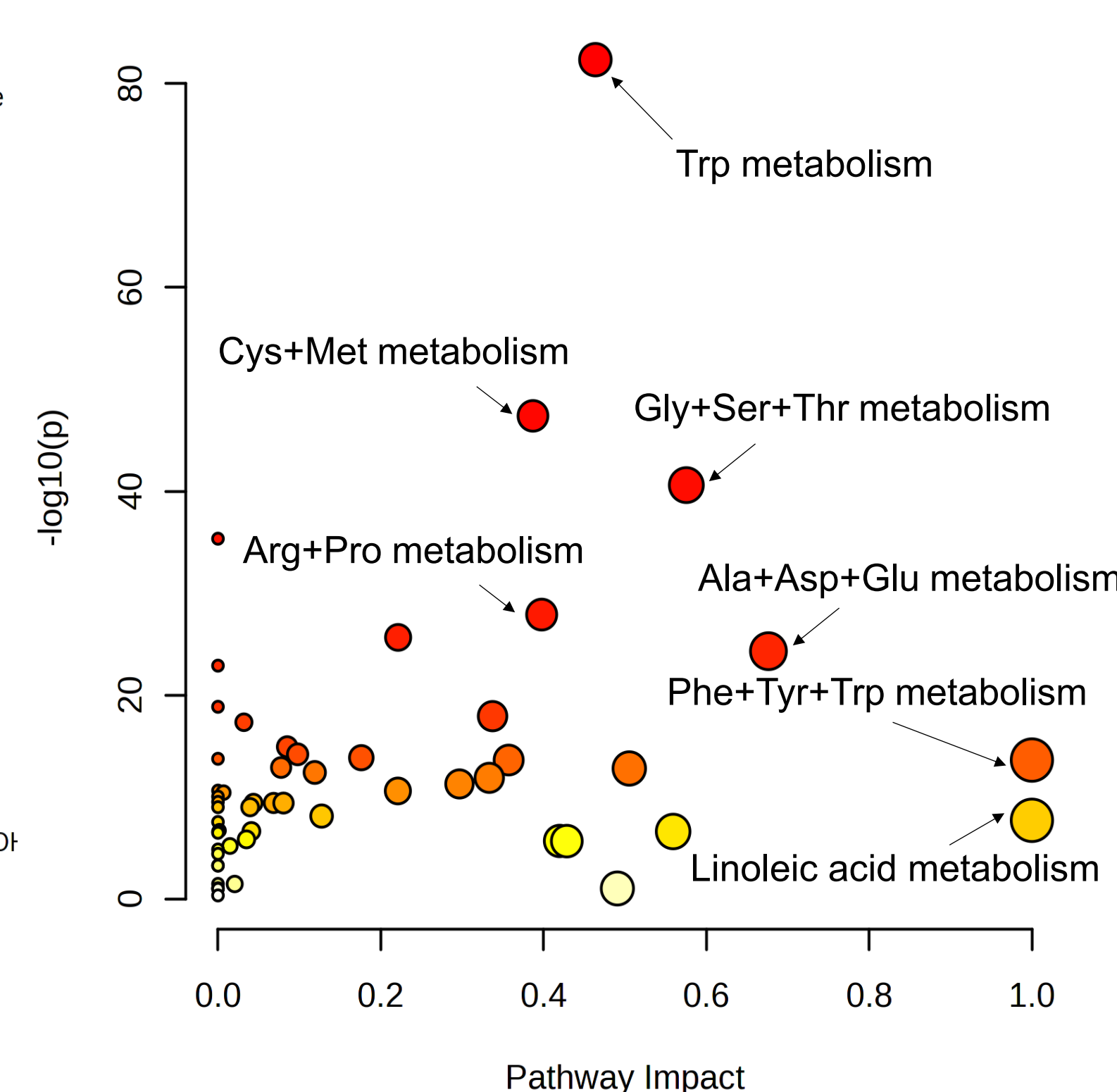
Metabolic Alterations in OC



We observed consistent alterations in several metabolic pathways. Amino acids were decreased while fatty acids and acyl-carnitines were increased in OC groups relative to non-OC groups.

Pathway analysis also revealed that amino acid and fatty acid metabolism were altered in OC relative to non-OC.

These pathways have previously been individually implicated in ovarian cancer biology and metabolic reprogramming.



Cross-cohort Reproducibility



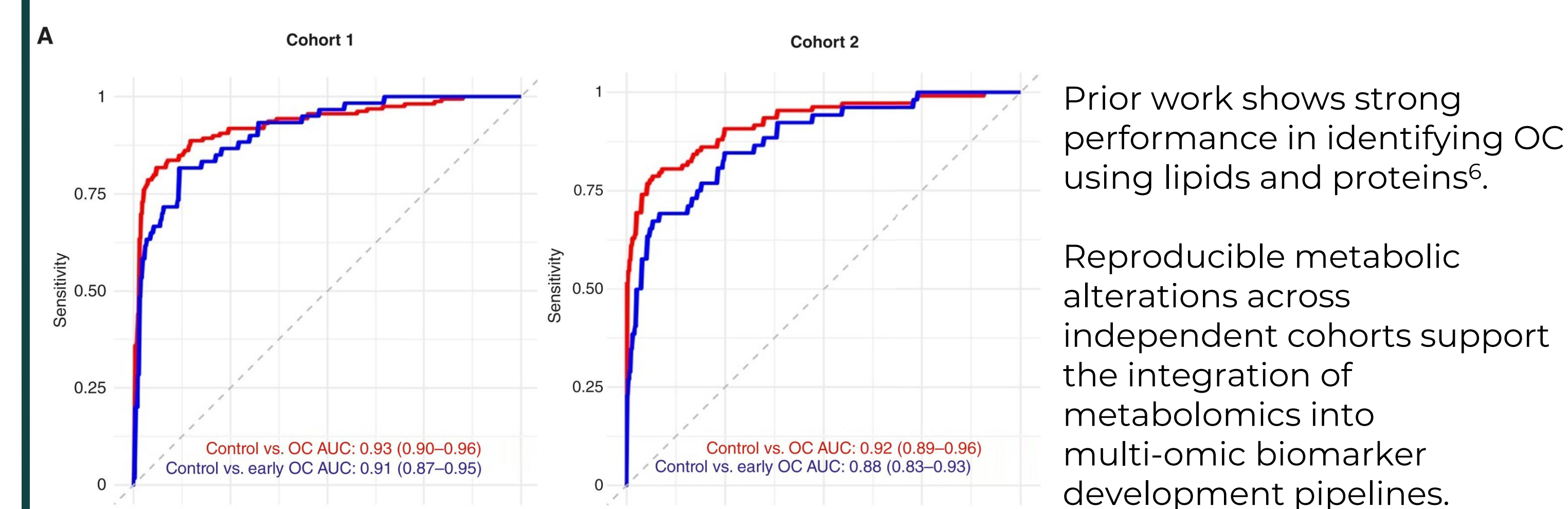
68% of significantly altered features maintained directionality across cohorts
 90% of consistent features mapped to key pathways identified in pathway analysis

Despite independent sample acquisition and analytical batches, metabolic alterations showed reproducible directional trends across cohorts.

Implications

- OC metabolic alterations are reproducible in symptomatic populations
- Pathway-level changes align with prior OC biology
- This supports biological signal rather than cohort-specific artifacts

The Power of Multi-omics



Prior work shows strong performance in identifying OC using lipids and proteins⁶.

Reproducible metabolic alterations across independent cohorts support the integration of metabolomics into multi-omic biomarker development pipelines.

Conclusions

- This study represents the largest metabolomics analyses of symptomatic OC cohorts to date.
- Serum metabolomics analysis identifies consistent pathway-level alterations in OC.
- Key metabolic pathways (amino acids, acyl-carnitines, fatty acids) show reproducible dysregulation across independent cohorts.
- Directional concordance across cohorts supports biological consistency rather than cohort-specific signal.
- Findings support continued investigation of metabolomics as part of multi-omic biomarker strategies.

(1) Huespenbecker SP, et al. Factors impacting time to OC diagnosis based on classic symptom presentation in the U.S. Cancer. 2021 Nov 15;127(22):4151-60. (2) Menon U, et al. Diagnostic routes and time intervals for OC in 9 international jurisdictions, findings from the International Cancer Benchmarking Partnership (ICBP). Br J Cancer. 2022 Sep 1;127(5):844-54. (3) Goff BA, et al. How are symptoms of ovarian cancer managed? Cancer. 2011;117(19):4474-23. (4) Howlander N, et al. SEER Cancer Statistics Review, NCI (5) Hanahan, Douglas et al. Hallmarks of Cancer: The Next Generation. Cell. Volume 144, Issue 5, 646 - 674. (6) Giles BM & Culp-Hill R, et al. Utilizing Lipidomics with Protein Biomarkers and Machine Learning for Early Detection of Ovarian Cancer in the Symptomatic Population. Cancer Research Communications (2023) 5 (9): 1516-1529