Moving Beyond BRCA—Incorporating Molecular Assays into Ovarian Cancer Trials

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SUMMARY

PrOTYPE is a locked down assay validated to Institute of Medicine standards, using NanoString technology to classify high-grade serous ovarian cancer into four defined subgroups. Future directions will include prospective–retrospective analysis and prospective clinical validation to define the predictive role of this assay and its role in influencing treatment decisions.

See related article by Talhouk et al., p. 5411

In this issue of Clinical Cancer Research, Talhouk and colleagues report the development and validation of the predictor of high-grade serous ovarian carcinoma molecular subtype (ProTYPE) assay (1). This international collaborative effort has brought gene expression–based molecular subtyping to the point of clinical validation in high-grade serous ovarian cancer (HGSOC), developing an assay to Institute of Medicine standards, ready for incorporation into clinical and translational research trials.

To date, the potential for molecular subtyping to guide individual patient management in HGSOC has remained unrealized. For three decades, platinum-free interval has been the most widely used clinical predictor of response to treatment. This has been simple to understand, implement, and assess despite lacking biologic precision and being susceptible to observer specific interpretation bias. In recent years, *BRCA* mutation and homologous recombination deficiency (HRD) have been widely incorporated into treatment algorithms as a clinically important molecular stratification, predicting response to PARP inhibitors and sensitivity to platinum chemotherapy, beginning the complex work to unpack the biologic basis of platinum-free interval. Tothill and colleagues first described four phenotypically distinct transcriptional subtypes of high-grade ovarian cancer (C1/Mesenchymal, C2/Immunoreactive, C4/Differentiated, C5/Proliferative), by performing microarray gene expression profiling on a cohort of mixed high- and low-grade serous and endometrioid tumors of the ovary, peritoneum, and fallopian tube ($n = 289$; ref. 2). These expression subtypes were confirmed in a HGSOC cohort ($n = 489$) by cluster analysis integrating mRNA, miRNA expression, and DNA methylation data in The Cancer Genome Atlas project study ($n = 489$; ref. 3). A number of subsequent studies have repeatedly reproduced these subtypes and sought to further define and explore their predictive and prognostic relevance, broadly noting that the C1 subtype is characterized by stromal desmoplasia and worse outcomes, while the C2 subtype has more favorable outcomes, characterized by immune $CD3^+/CD8^+$ T-cell infiltrate and increased inflammatory cytokine expression. However, the clinical utility and integration of this molecular stratification as a predictive tool for treatment or prospective validation in trials has remained elusive, perhaps due to fragmentation of analytic methods, data used for subtype assignment across studies, and absence of a laboratory workflow that is compatible with formalin-fixed, paraffin-embedded (FFPE) and archival tissues.

Talhouk and colleagues directly address these prior technical limitations with this study, proposing a d*e facto* standard and validated assay which classifies HGSOC into these four subtypes (C1, C2, C4, C5), using methodology that can be applied in a single patient setting using pathology-standard fixed tissues. Utilizing two independent and parallel approaches, the study team derived and internally validated algorithms for molecular subtype prediction using published gene expression data from a pure cohort of 1,650 tumors with confirmed HGSOC histology. These models were then applied to NanoString data obtained from a large ($n = 3,829$) cohort of HGSOC from the Ovarian Tumor Tissue Analysis Consortium. These data were refined to establish the PrOTYPE assay; a 55-gene classifier, able to predict gene expression subtype with >95% accuracy.

Molecular prediction assays incorporating NanoString technology have been successfully integrated into treatment algorithms in other tumor types, and there is opportunity to learn from this prior experience when considering the next steps in clinical validation of the ProTYPE assay. In breast cancer, the ProSIGNA assay uses NanoString technology to estimate the risk of distant recurrence of hormone receptor–positive breast cancer, to inform personalized decision making regarding adjuvant treatment (4). This assay was validated using highly specific inclusion criteria and a large cohort, to predict 10-year distant recurrence-free survival. In non–small cell lung cancer, transcript-based assays using NanoString technology have been developed, but not fully validated, to detect clinically actionable *ALK, RET*, and *ROS1* fusion genes using FFPE tissue (5). Compared with other profiling methods, NanoString technology may be more practical and cost-effective, as the methodology is rapid, sensitive, and allows direct quantification of mRNA without reverse transcription or amplification steps, making the test less sensitive to preanalytic variables such as fixation effects and applicable for use in degraded clinical samples (**Fig. 1**).

The authors propose that the clinical-grade PrOTYPE assay is fully "locked down" and ready for integration into clinical trials as well as research applications. As an initial step, there would be value in the initial retrospective validation of the ProTYPE assay using prospectively collected tissue and clinical data accessed through collaborative clinical trial networks potentially through Gynecologic Cancer Inter Group. Retrospective data can generate observations regarding the biologic rationale for embedding biomarker stratification into

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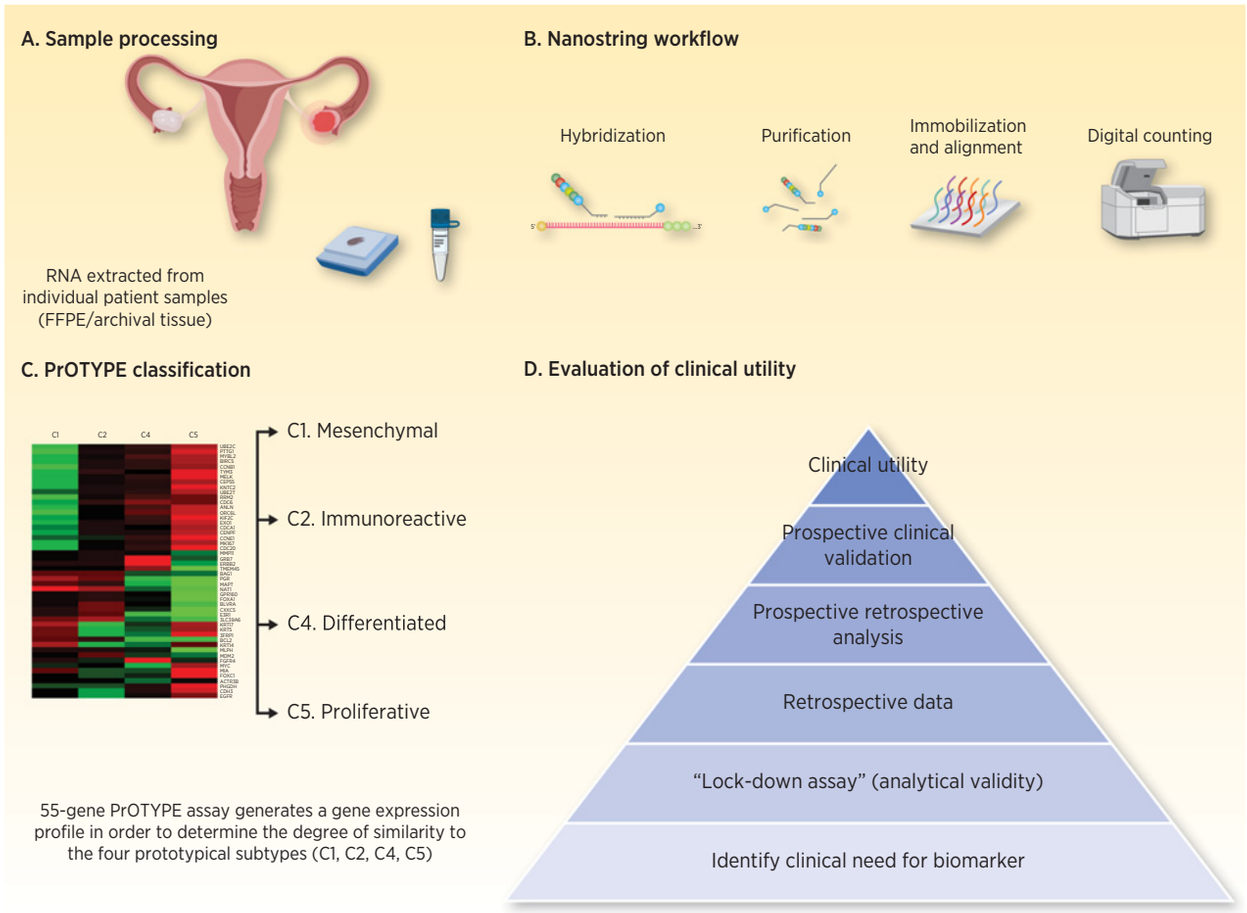

Figure 1.

PrOTYPE assay for subtype classification of high-grade serous ovarian cancer. **A,** RNA is extracted from individual patient samples using pathology-standard fixed tissues. **B,** NanoString workflow involves (i) Hybridization—target mRNA is hybridized with fluorophore-labeled reporter and biotinylated capture probe pairs; (ii) Purification—excess probes are washed away with two-step magnetic bead purification; (iii) Immobilization and Alignment—tripartite structure is bound to surface of sample cartridge and reporters aligned by electric current; (iv) Digital Counting—sample is scanned by digital analyzer. The level of expression is measured by counting the number of codes for each mRNA. **C,** 55-gene PrOTYPE assay is used to generate a gene expression profile which is compared with the four prototypical high-grade serous ovarian cancer subtypes (C1,C2,C4,C5) to determine the degree of similarity. **D,** To establish the clinical utility of a biomarker, steps include identifying the clinical need for the biomarker, locking down the assay, generating hypotheses regarding the biological role of the biomarker through retrospective data, followed by prospective–retrospective analysis. Prospective clinical validation is essential to defining the clinical utility of a biomarker.

prospective trial design. In this study, the authors reported associations between subtype and clinical–pathologic parameters, observing that the C1 subtype may be more commonly detected at metastatic extra-adnexal sites, and potentially able to predict risk of macroscopic residual disease after cytoreductive surgery. Embedding this classification system as an integrated or integral biomarker is the next essential step to confirm a potential predictive role, and importantly, make treatment decisions based on the assay. For example, it would allow us to refine and optimize patient selection for secondary cytoreductive surgery in the context of the recently reported positive AGO DESKTOP III/ENGOT-ov20 and SOC1/SGOG-OV2 studies. To answer these types of questions, the biologic behavior of the biomarker-specified cohort must also be reestablished, as it may differ from expectations based on retrospective data. This underscores the importance of integrating a control group into prospective validation studies. Prior experience in ovarian cancer has demonstrated the utility of embedding biologically plausible integral biomarker stratification into clinical trial design (**Table 1**). Understanding the difference between an integral assay (where the biomarker is used to stratify, choose treatment, or determine eligibility) and an Integrated assay (where the biomarker is used for hypothesis generation or testing) has critical implications for study design and interpretation of results.

Table 1. Examples of integral biomarkers incorporated into ovarian cancer clinical trial design.

Biomarker	Treatment	Example of clinical trial
BRCA mutation and HRD	PARP inhibitors	ENGOT-OV16/NOVA (NCT01847274)
Folate receptor (FRα)	FRα targeting through antibody–drug conjugate (mirvetuximab)	MIRASOL (NCT04209855)
TP53 mutation	Wee-1 inhibitor (adavosertib)	NCT02101775

Subtype stratified care has tremendous potential to enable a more nuanced approach to treatment and transform outcomes in HGSOC. As novel therapeutic options expand, there is an urgent need to identify and validate the frustratingly elusive biomarkers which may be predictive of response to treatments such as angiogenesis inhibitors, and immune modulators. Prospective studies are ongoing to assess the validity of subtype stratified care using the ProTYPE assay, including a trial of pembrolizumab in recurrent C2 ovarian cancer (NCT03732950); a trial targeting reactive stromal features of C1 ovarian cancer with bevacizumab, atezolizumab, and cobimetinib (NCT03363867); and targeting stem-like features of C5 ovarian cancer with vinorelbine (NCT03188159). The ProTYPE assay will also be assessed as part of the multicenter umbrella study INOVATe (Individualised Ovarian Cancer Treatment through Integration of Genomic Pathology into Multidisciplinary Care).

It must be recognized that classifying HGSOC has been difficult in the context of extreme genomic and spatial heterogeneity. The clinical validation of the ProTYPE assay therefore has to be in a well-defined population, taking steps to mitigate the impact this heterogeneity could have on the study results. This is particularly important as the authors report that subtype classification using ProTYPE may vary depending on whether an adnexal or other site is sampled. In addition to this spatial heterogeneity, acquired resistance is common in HGSOC, and real-time detection of resistance mechanisms remains an ongoing clinical challenge. Further prospective validation studies will be needed to delineate how the ProTYPE assay may assist in the classification of HGSOC at different time points across the trajectory of the disease at diagnosis, recurrence, and progression. Another key question remains how the ProTYPE assay relates to other HRD assays as there was insufficient information available from this dataset to capture the relevance of *BRCA1* disruptions and HRD on subtype classification and prognosis.

Integrating subtype stratification into clinical decision making represents a continuation of the paradigm shift in precision therapy for ovarian cancer, which started really only in the past decade, initially with distinct pathways for histologic subtypes, incorporation of BRCA mutation, and most recently HRD status. To be an effective biomarker for decision making, its clinical utility for treatment selection has to now be defined in clinical trials in women with HGSOC to establish the role of this assay in guiding individual patient treatment decisions.

Disclosure of Potential Conflicts of Interest

A.M. Oza is on the steering committee of GlaxoSmithKline, AstraZeneca, Clovis, Tesaro, and Merck (uncompensated), and is P.I. on clinical trials for AstraZeneca, GlaxoSmithKline, and Clovis. No potential conflicts of interest were disclosed by the other authors.

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