Objectives
Relapse is frequent for patients with high grade serous ovarian cancer (HGSOC) after chemotherapy. Methods to interrogate the clonal composition of minimal residual disease are needed to better understand the mechanisms of HGSOC relapse. To address this unmet need, we developed and validated a novel cfDNA-based approach for tracking on-treatment clonal evolution using tumor-informed structural variants (SVs).

Methods
Genomic DNA (gDNA) was isolated from cryopreserve multisite pre-treatment HGSOC biopsies and matched germline samples and whole genome sequencing (WGS) was performed. cfDNA was isolated from contemporary pre-treatment plasma. High-confidence SVs were identified from multisite WGS using a consensus method of five published SV callers. Tumor-informed bespoke quantitative PCR primer/probe sets were designed to interrogate SVs breakpoints in tumor gDNA and matched cfDNA.

Results
Optimization was first performed using synthetic cfDNA generated from ovarian cancer cell lines. SV breakpoint-spanning PCR assay exhibited 90% sensitivity and 89% specificity in detection of WGS-informed SVs. 11 validated SVs breakpoint-spanning PCR assays had specific amplifications in synthetic cfDNA. 8 SVs breakpoint-spanning custom TaqMan probe assays for qPCR (real-time PCR) and ddPCR (digital droplet) had significantly increased sensitivity and superior specificity. When cutoff value of negative was set to “undetected” in qPCR and 0 copies/ul in ddPCR, the sensitivity and specificity of custom TaqMan probe assays can still be 100%. The performance of this method was next evaluated using pre-treatment samples from 2 HGSOC patients. After screening 18 SVs by tumor-informed SV breakpoint-spanning PCR assay, 8 SVs custom TaqMan probe qPCR assays and 6 SVs custom TaqMan probe ddPCR assays demonstrated 100 % sensitivity and specificity in multisite biopsy samples. 1 SV custom TaqMan probe ddPCR assay measured 0.947 copies/ul in cfDNA and 0 copies/ul in matched germline control. Altogether, our multi-layer screening approach yields the “perfect” SV breakpoint-spanning assays that are ready to apply for SVs detection in cfDNA.

Conclusions
Detection of SVs from pre-treatment cfDNA using tumor-informed breakpoint-spanning ddPCR is feasible and may enable a novel and sensitive method for monitoring on-treatment disease burden.