INTRODUCTION
Recent comprehensive genomic analyses of advanced stage, high grade serous ovarian carcinomas carried out by TCGA (1) have revealed their heterogeneous nature and provide an explanation for the failure of molecularly targeted drugs evaluated without consideration of the tumor molecular characteristics of the patients treated. Copy number (CN) alterations of mutants in RB, RAS-MAPK, and PI3K-AKT pathway genes occur in ~35%, 20%, and 35% of the tumors, respectively. Activation of these pathways may confer sensitivity to drugs that target them (e.g., PI3K or AKT inhibitors, MEK inhibitors, CDK inhibitors) as well as resistance to drugs (e.g., HER2 or EGFR inhibitors) that have not demonstrated significant activity in ovarian cancer clinical trials. A comprehensive evaluation of the genetic aberrations in treatable pathways may therefore inform the selection of appropriate drugs and combinations for each patient in the context of clinical trials.

MATERIALS AND METHODS
Patients and tumor specimens. The patients are an unselected population who sought molecular profiling assistance from The Clery Foundation between November, 2011 and March, 2013. Formalin-fixed, paraffin-embedded (FFPE) tissue specimens, pathology reports, and patient treatment histories were obtained under written informed consent.

DNA extraction and library construction. DNA was extracted from 40 μg of tissue using the Promega Maxwell 16 FFPE Plus LE DNA Purification kit and molecular barcoded-indexed ligation-based sequencing libraries were prepared (2) and constructed (2) from at least 50-200 ng sheared DNA (~100-400 bp). Solution-based hybrid capture (2) with a custom CancerSure panel hybridization kit was used to enrich all ~3300 exons of 182 cancer-related genes and 37 introns from 14 recurrently rearranged in cancer representing approximately 1.1 Mb of the human genome.

Illumina Sequencing and data analysis. The sequenced libraries were sequenced on an Illumina HiSeq 2000 platform using 400x paired-end reads. Median exon coverage was 1278X. Sequence data were mapped to the reference human genome, hg19, using the Burrows-Wheeler Aligner (3) and were processed using the publicly available GANNRCC tool (4). Germline base substitutions and indels were detected using custom tools optimized for mutation calling in heterogeneous tumor samples based on statistical modeling of Illumina sequencing data (5). Somatic base substitutions and indels were detected using custom tools optimized for mutation calling in heterogeneous tumor samples based on statistical modeling of Illumina sequencing data (5).

Figure 1. A. 182 cancer-related genes and rearrangements interrogated in this study

Figure 2. Sequencing of 182 genes: average of 2.8 'actionable' alterations in each serous ovarian tumor (median 3; range 0-7).

Figure 3. A. Genetic alterations in 29 high grade serous tumors

Figure 4. Genomic coverage of sequencing needs for specimens with KRAS, PIK3CA/SOX2, and ERBB2 amplifications or PTCH1 and FLT3 rearrangements.

Figure 5. A. Clustering of tumor specimens based on pathway alteration status. Tumors with at least one alteration in one of the following pathways were included: RB, RAS-MAPK, PI3K-AKT, RPTOR, mTORC1, mTORC2, MET, EGFR, IGF1R, FGF, PI3K, PTEN, AKT, ERBB2, FGFR, FGFR4, DDR, DDR2, DDR1, DDR3, homologous recombination, DNA repair, cell cycle progression, and oncogenesis.

Tumors from 75% of recurrent high grade serous ovarian cancer patients (18/24) contain ‘actionable’ or clinically relevant genetic alterations (Figure 5).

RAS-MAPK pathway alteration was observed in 33% of the tumors (Figure 5A), often in conjunction with RB-cyclin pathway or MYC family member alterations.

SUMMARY and CONCLUSIONS
1. Sequencing of exons from 182 genes associated with cancer etiology and progression identified a median of 3 alterations (range: 0-7) in high grade serous histology ovarian cancers (n=29).
2. 75% of the 24 recurrent serous ovarian tumors contained genetic alteration(s) in at least one pathway that is targeted by approved or clinically developed drugs.
3. The RAS-MAPK pathway was altered in 33% of the recurrent serous ovarian tumors--primarily due to focal NRAS and/or KRAS mutations or KRAS amplification. In such tumors, RB-cyclin pathway or MYC family alterations frequently occurred, suggesting combination therapy strategies with drugs targeting these pathways. Notably, the PI3K-AKT pathway was less frequently altered (i.e., 8%).
4. Rearrangement of the PTCH1 and FLT3 genes were observed in two specimens suggesting the potential for Hedgehog pathway and FLT3 inhibitor therapy in these patients.
5. Increased NF1 alterations and decreased frequency of PIK3CA, CCNE1, and MYC copy number changes were observed relative to the published TCGA analysis of primary tumors. As these studies used different sequencing and analytical methods, additional primary tumor specimens will be analyzed.
6. Additional studies with a larger sample size are required to confirm these observations suggesting that patient selection for clinical trials should be informed by comprehensive characterization of recurrent tumor specimens.

REFERENCES