Integrated Clinical and Genomic Models to Predict Optimal Cytoreduction in High Grade Serous Ovarian Cancer

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Research Article

Keywords: High grade serous carcinoma, Surgical cytoreduction, Prediction modeling

Posted Date: January 5th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1167716/v1>

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Abstract

Background: Advanced high grade serous (HGSC) ovarian cancer is treated with either primary surgery followed by chemotherapy or neoadjuvant chemotherapy followed by interval surgery. The decision to proceed with surgery either primarily or after chemotherapy is based on a surgeon's clinical assessment and prediction of an optimal outcome. Optimal surgery is correlated with improved overall survival. This clinical assessment results in an optimal surgery approximately 70% of the time. We hypothesize that this prediction can be improved by using biological tumor data to predict optimal cytoreduction.

Methods: With access to a large biobank of ovarian cancer tumors, we obtained genomic data on 83 patients encompassing gene expression, exon expression, long non-coding RNA, micro RNA, single nucleotide variants, copy number variation, DNA methylation, and fusion transcripts. We then used machine learning to incorporate this data with pre-operative clinical information to create predictive models which successfully predicted whether or not a patient's cytoreductive surgery would have an optimal outcome. These models were then validated within The Cancer Genome Atlas (TCGA) HGSC database.

Results: Of the 124 models created and validated, 21 performed at least equal if not better than our historical clinical rate of optimal debulking in advanced-stage HGSC as a control, 78%.

Conclusions: This is the first time tumor genomic data has been used to predict surgical outcome in ovarian cancer. Prospective validation of these models could result in improving our ability to objectively predict which patients will undergo optimal cytoreduction and, therefore, improve our ovarian cancer outcomes.

Background

Ovarian cancer continues to be one of the leading causes of cancer death for women in the United States. This is mainly because most cases are diagnosed at an advanced stage, either FIGO Stages III or IV.(1) Treatment in those stages of the disease consists of a combination of surgery and chemotherapy, either with cytoreductive surgery primarily or after 3 to 6 rounds of platinum-based chemotherapy.(2) The Gynecologic Oncology Group (GOG) has used the definition of optimal surgical resection as 'no residual lesions greater than 1 cm in maximal diameter' in ovarian cancer clinical trials dating back to 1996 and as recently as 2019.(3–8) If there is no visible residual disease then surgical resection is termed complete.(8) Overall survival is strongly associated with the amount of residual disease after surgery, with the best survival occurring after complete resection.(9–13)

Upfront surgical resection is preferred if an optimal surgical result can be achieved. However, if an optimal or complete resection of disease is not feasible, neoadjuvant chemotherapy has been used to improve the chances of optimal resection without compromising overall survival.(14–16) Surgeons have traditionally used clinical data such as physical examination, imaging studies, and functional status to determine the ability to successfully resect disease. In high-volume centers, this leads to an optimal surgical result in 70% of cases.(17) Numerous attempts have been made to try and improve the surgeon's ability to predict an optimal surgical outcome. Clinical data, such as CA-125, and imaging studies combined into predictive models have not yielded a formula which improves on an experienced surgeon's decision-making.(18–20) A laparoscopic scoring system has yielded an optimal result 88% of the time, but still requires a surgical procedure and an increased use of resources.(21) Some believe that tumor biology plays a major role in the likelihood of optimal surgical resection and that

molecular characterization can improve rates of optimal resection.(22) Previously published prediction models have not incorporated tumor biological data to account for the impact of tumor biology on surgical outcome.

We hypothesize that integrating genomic data from surgical specimens with clinical data will improve the prediction of optimal cytoreductive surgery in ovarian cancer over clinical decision-making. We leveraged the University of Iowa (UI) Biobank that harbors hundreds of high-grade serous cancers (HGSC) of tubo-ovarian or peritoneal origin. HGSC was chosen as it is the most common histological sub-type of ovarian cancer. We extracted DNA and RNA from these tumors and determined their genomic features: DNA methylation, gene expression, exon expression, micro RNA (miRNA), long non-coding RNA (lncRNA), single nucleotide variation (SNV), copy number variation (CNV) and fusion transcripts. Our objective was to create accurate prediction models that would discriminate which patients with HGSC will undergo optimal cytoreduction surgery using both clinical and genomic data. Then, we validated these models using The Cancer Genome Atlas (TCGA), an independent comprehensive genomic-clinical database.

Methods

We performed a retrospective, single institution cohort study in which we included all Stage III and IV patients with high grade serous ovarian cancer (HGSC) from 1990 to 2014 with available pre-operative and intra-operative clinical data. DNA and RNA were then extracted from tumor specimens and processed as detailed below to obtain the necessary genomic data. Clinical and genomic data were then combined to create predictive models using machine learning in order to identify criteria which accurately predicted an optimal surgical result.

Tissue samples and clinical outcome data were obtained from the Department of Obstetrics and Gynecology Gynecologic Oncology Biobank (IRB, ID#200209010), which is part of the Women's Health Tissue Repository (WHTR, IRB, ID#201804817). All of the tissues archived in the Gynecologic Oncology Biobank (herein termed Biobank) were originally obtained from adult patients under informed consent in accordance with University of Iowa IRB guidelines. Tumor samples were collected, reviewed by a board-certified pathologist, flash-frozen, and then the diagnosis was confirmed in paraffin at the time of initial surgery. All experimental protocols were approved by the University of Iowa Biomedical IRB-01.

The University of Iowa is a high-volume clinical center with gynecologic oncologists trained in extensive cytoreductive surgery, including upper abdominal debulking. All procedures were performed by board certified or board eligible gynecologic oncologists with the assistance of appropriate consulting services if necessary.

Clinical Data:

Clinical data was extracted from the electronic medical record. **Table 1** summarizes the baseline clinical and pathologic characteristics. Only data that was available prior to surgical intervention was used in the development of predictive models. This included age, BMI, Charlson Comorbidity index, CA-125, stage, use of neoadjuvant chemotherapy, and location of disease on imaging. Other clinical data was collected for the purposes of comparing outcomes and demonstrating the extent of surgical resection.

Clinical data from the University of Iowa Biobank was also used to determine the rate of optimal cytoreduction through time at our institution to act as our threshold for a successful predictive model.

Genomic Analysis

470 patients with ovarian cancer were identified in the database. Only patients with a primary diagnosis of advanced stage III and IV, high grade serous ovarian cancer were included. The flow diagram in Figure 1 summarizes patients included in this study.

RNA was then isolated from these tumor specimens. RNA extraction, processing and sequencing have been described previously.(23, 24) In brief, total cellular RNA was extracted from primary tumor tissue using the mirVana (Thermo Fisher, Waltham, USA) RNA purification kit. The RNA yield and quality were assessed with Trinean Dropsense 16 spectrophotometer and Agilent Model 2100 bioanalyzer. RNA quality was determined to be adequate if the sample had an RNA integrity number (RIN) of 7.0 or greater. Samples that were of adequate quality were then sequenced. 500ng of RNA was quantified by Qubit measurement (Thermo Fisher). RNA was then converted to cDNA and ligated to sequencing adaptors with Illumina TriSeq stranded total RNA library preparation (Illumina, San Diego, CA, USA). cDNA samples were then sequenced with the Illumina HiSeq 4000 genome sequencing platform using 150 bp paired-end SBS chemistry. All sequencing was performed at the Genome Facility at the University of Iowa Institute of Human Genetics (IIHG).

STAR was used to align the RNA-seq reads to the human reference genome (version hg38).(25) We then created BAM files after alignment. featureCount was used to measure gene expression.(26) The DESeq2 package was used to import, normalize, and prepare the gene counts for analysis.(27) Gene expression and miRNA expression were independently used for the association analysis. ENSEMBL was used to annotate single exons within the gene expression alignment analysis. Exon expression was then evaluated using the DEXSeq package.(28) BAM files for each sample were used for SNV discovery and base-calling against the human genome reference utilizing SAMtools and BCFtools for sorting and indexing. Results were filtered for duplicates, known non-synonymous single-nucleotide variants, and synonymous variants and then annotated with ANNOVAR. Gene CNV were estimated using SAMtools and superFreq.(29) BAM files were then used to identify lncRNA, as described previously.(30, 31) Lastly, fusion transcripts were determined using the STAR-Fusion suite from fastq files.(32) **Supplementary Figure S1** depicts each program used for RNA processing and the identification of various genomic components.

Genomic DNAs (gDNAs) were purified from frozen tumor tissues using the DNeasy Blood and Tissue Kit according to manufacturer's (QIAGEN) recommendations. Yield and purity were assessed on a NanoDrop Model 2000 spectrophotometer and by horizontal agarose gel electrophoresis. DNA methylation was determined by using bisulfite-converted gDNA processed on MethylationEPIC arrays. Details of this process have been previously published by our group.(33) The Illumina Infinium MethylationEPIC BeadChip Kit (Illumina) quantifies more than 850,000 methylation sites. Bisulfite-converted samples were denatured and neutralized, then isothermally amplified overnight. The product was fragmented enzymatically. Fragmented DNA was precipitated, resuspended and placed onto the Illumina methylationEPIC BeadChip and hybridized. The BeadChip was washed, extended and stained. The arrays were scanned with the Illumina iScan and methylation intensity was measured. Analysis was performed using the Minfi R statistical package.(34)

Statistical Analysis:

Only baseline clinical characteristics, before surgery, were included in the statistical analysis. Most genomic data were used as continuous variables, except SNV and fusion-genes, which were used as dichotomous variables. To select those variables most informative for prediction of cytoreduction, we used univariate analysis with ANOVA ($p < 0.05$), as implemented by the caret *R* package and detailed previously.(23) Significant predictive variables were

then used in a multivariate lasso regression prediction model. Thus, poorly annotated variables were removed from model construction.

Creation of prediction models of cytoreduction

Significant variables from the univariate analysis were then incorporated into multivariate lasso regression prediction models of optimal surgery. Initial models included only significant variables from one category of clinical or genomic data (i.e. lncRNA expression, miRNA expression, CNV, etc.). Variables were then progressively combined to create more complex prediction models. Multivariate prediction models were fit with *lasso* (least absolute shrinkage and selection operator) as implemented in the *glmnet* R package(35), and detailed previously. (23) Performances of prediction models were measured with area under the receiver operating characteristics curve (AUC) and 95% CI, and estimated with 1,000 replicates of ten-fold cross-validation to avoid over-fitting. Bias-corrected and accelerated bootstrap CI's were computed for each model. AUC of 0.5 indicates no predictive ability of a model and 1.0 represents perfect predictive performance.

Validation of predictive models

HGSC data from TCGA was used to validate the created predictive models.(36) We included only patients within the TCGA database that had at least 6 months of clinical follow-up, adequate data on disease status, and treatment received, including surgical outcome.(37) As with UI patients, only baseline clinical characteristics were included in the validation. Some clinical data was not available in TCGA patients, as this database was not designed for this particular study.

Also, DNA methylation analysis in TCGA was performed with an earlier chip with less features, the Illumina Infinium HumanMethylation27K BeadChip arrays.(37) Some of the significant features from prediction models containing DNA methylation were not able to be validated. BAM files from these HGSC TCGA patients underwent the same analysis pipeline as describe previously in *Genomic Analysis*.

Prediction models of cytoreduction constructed with UI data were validated in the TCGA HGSC dataset. When there was missing data in TCGA (either clinical or genomic), we constructed alternative models from our dataset with those variables available in TCGA. The R package, *pROC* was used to determine thresholds for our model when applied to the TCGA dataset.(38) Models yielding a sensitivity >90% were ranked from highest to lowest sensitivity, negative predictive value and AUC. A sensitivity threshold of over 90% will identify most of the patients that will achieve cytoreduction, with improvement over clinical algorithms. The best performing parameters were used to fit a final score of that model to the entire TCGA cohort.(23) Performances measured by AUC between 0.8-0.9 were considered 'very good'; performances between 0.9-1 were considered 'excellent.'

Results

Of 470 patients within our database, 405 patients were determined to have advanced stage HGSC. Of these, 273 underwent optimal cytoreduction and 132 received suboptimal cytoreduction. We successfully sequenced RNA from 83 available specimens from these patients, 52 underwent optimal cytoreductions and 31 suboptimal (Figure 1).

Pre-operative, intra-operative and post-operative clinical data were compared between the optimal and suboptimal groups. Significant differences between clinical characteristics for both groups are detailed in **Table 1**. We

computed differences between clinical features for the whole UI cohort of advanced stage HGSC patients (left side of the table) and those with genomic, RNA-seq and DNA methylation analyses (right side of the table). There was no difference in the proportion of patients with optimal surgery between the whole cohort and the subgroup that underwent genomic analysis (chi-square p-value = 0.402).

As a reference, we determined the rates of optimal cytoreduction at the UI since the establishment of the Biobank. Starting in 1985, the rate of optimal cytoreduction steadily increased. The 2011-2013 time period demonstrated a rate of optimal cytoreduction of 78%, with a 95% CI of 70%, 87% (Figure 2). Any prediction model that we should build will have to be above this 95% CI threshold to be considered an improvement. Moreover, based on 95% CIs, there have not been significant changes in optimal surgical resection rates since 1996.

Model construction:

The univariate analysis with ANOVA selected those variables which were more predictive of optimal surgery.

Supplementary Table S2 represents the variable reduction achieved with this initial univariate step. Only clinical data and fusion transcripts did not require this initial step. Significant genomic variables after the initial univariate step are depicted by heatmaps in **Supplementary Figure S3**. Heatmaps are separated by surgical outcome.

We created 124 prediction models of cytoreduction using the significant variables from the univariate analysis. The first set of models used only one type of genomic or clinical information. **Supplementary Table S4** shows resulting variables after the multivariate lasso regression analysis in models of prediction including only one type of data. Then, we combined different types of data to create models with 2 and then 3 categories of data (Figure 3). Models in the figure are ordered by types of data included (y axis) and by performance of the model, measured in AUC and its 95% CI (x axis). Models with more types of data (4 or more) only increased model complexity without any performance improvement.

Model validation:

These 124 models were then validated using data from the TCGA database. Not all variables for all types of data were available in TCGA. Therefore, we constructed new models from UI data with all variables that were available at TCGA and then we validated all models. AUC 95% CI of UI initial prediction models overlapped with validated TCGA models 57% of the time (Figure 4). Validated models with similar performances measured by AUC 95% CI, allowed us to further select those models that will be more robust across different datasets.

Overall, in 21 different prediction models of cytoreduction the 95% CI of AUC included or was over 87%, which is the upper limit of the 95% CI of our historical rate of cytoreduction. These models are represented in Figure 5, with AUC values and 95% CI in the x axis, and types of data in the y axis. Fusion transcripts expression (FT) was the most common category within these models, utilized in 13 of the 21 (62%) models. MicroRNA was the second-most common, present in 12 of 21 (57%) models, followed by lncRNA, 8 out of 21 (38%). The best models included MIR expression, lncRNA expression and/or clinical data.

Discussion

Optimal cytoreduction is consistently one of the strongest predictors of overall survival in the platinum-based chemotherapy era. There are several clinical factors known to affect the extent of surgical debulking, including surgical training and patient's performance status.(13, 14) In the present study we also add biological or genomic factors that predict cytoreduction accurately. Until now, precise prediction of an optimal surgical outcome with preoperative data was not possible, leading to suboptimal cytoreduction in 20-30% of surgeries. Additionally, while neoadjuvant chemotherapy has been shown to be non-inferior to primary debulking, there is evidence to indicate that: a) patients who undergo primary debulking with no residual disease may have improved survival and; b) those with residual disease ≤ 1 cm may have equivalent survival, compared to patients who receive neoadjuvant chemotherapy and have no residual disease at the completion of their surgery.(39) Given this information, it is imperative to identify those patients who can be successfully cytoreduced and triage them to primary cytoreductive surgery, if they are fit for the planned procedure. Our study is the first, to our knowledge, to incorporate large scale genomic data into models to predict optimal cytoreduction.

Previous prediction models have had minimal to modest success on improving preoperative ability to predict optimal cytoreduction. These models have used such pre-operative criteria as physical exam, CA-125 and HE-4 levels, CT scan evaluation with artificial intelligence, and ECOG performance status.(18–20) These methods generally fail to improve successful optimal cytoreduction beyond 70%. While the laparoscopic “scope and score” improves optimal cytoreduction rates to 88%, it still requires that the surgeon be prepared to perform an extensive cytoreduction, thus requiring the allocation of a significant amount of operating room time and resources. Additionally, it commits the patient to a surgical procedure, which while low risk, is not devoid of it. Ideally, this decision can be made without the cost and resource allocation of a surgical procedure. Moreover, while a recent National Cancer Database (NCDB) study from 2004 to 2015, determined that 73% of patients with advanced stage epithelial ovarian cancer in cancers centers around the US underwent primary cytoreduction surgery(39), only 23% (111 out of 488) of patients undergoing the laparoscopic “scope and score” would be offered a surgical cytoreductive procedure.(21) It seems that the improved rate of surgical outcomes with this laparoscopic algorithm may be at expense of a large proportion of patients not being offered primary cytoreduction.

We created 21 predictive models using a combination of genomic and clinical data whose 95% CIs met the threshold established to improve only clinical criteria for optimal surgical outcomes. This means that, following future prospective validation, these models have the potential to predict optimal cytoreduction with higher accuracy than the clinical decision-making of an experienced gynecologic oncology team. These models were also validated within the TCGA database, meaning that the model is capable of performing well in independent datasets.

One criticism of primary cytoreduction is the increase in morbidity and mortality inherent in an aggressive surgical procedure. Narasimhulu et al., has developed a prediction tool which significantly reduces perioperative morbidity and mortality by using simple selection criteria to determine which patients would best tolerate upfront debulking.(40) By reducing this morbidity, we improve the rate of optimal outcome in primary surgery. The clinical criteria used for this tool include age, performance status, albumin, surgical complexity, and stage. We incorporated these significant predictors of morbidity into the clinical portion of our prediction model with the exception of albumin, which was not reliably available in our cohort.

Each model incorporated different combinations of clinical and genomic data. Three of these models included only one category of data. Fusion transcripts and miRNA expression were the most common categories

represented, present in 62% (13/21) and 57% (12/21) of the models, respectively. The two best-performing models with the smallest confidence intervals incorporated miRNA and lncRNA +/- clinical data. Models using solely miRNA and lncRNA also produced high quality models. miRNA and lncRNA are known to be significant regulators in ovarian cancer, correlated with overall survival, and are relatively new categories of investigation for novel therapeutics.(31, 41, 42) We are only beginning to understand the extent of their importance. These results confirm our hypothesis that tumor biology can be used to inform our surgical decision-making.

The strengths of our study include a comprehensive analysis of genetic and epigenetic data on a single institution cohort of HGSC patients, treated with similar philosophy and with clinical and outcomes data available. The involvement of a vast array of genomic data provides a complete representation of the genome and its regulators to the extent of our current knowledge. Also, the resulting models were validated in an independent, comprehensive clinical-genomic database, TCGA, that informed which models were more robust and consistent, in order to further validate prospectively.

There are several limitations to our study. This is a retrospective study and thus is subject to some biases, particularly selection bias. Patients were chosen for surgery based on traditional clinical decision-making and this subset of patients were the ones included in our models. This could affect our models since these were the patients in whom surgery was already expected to be successful, creating a cohort of patients with a higher probability of an optimal outcome. In order to correct for this bias, prospective validation of our models must be performed using all patients assessed for cytoreduction. Another limitation is that our study was conducted with patients from a single institution. Ovarian cancer populations can have significant genomic variation and therefore our models may only be valid within the University of Iowa population.(43) Therefore, other institutions may require further refinement for these models to fit their patient populations. We chose the definition of optimal cytoreduction (≤ 1 cm) in agreement with GOG usage in multiple trials (3–8). There is a linear association regarding size of maximum residual disease and overall survival(11), but to create a classifier, or predictor, a dichotomous outcome for the model is preferred (44). Although all surgeons aspire to achieve complete cytoreduction (or R0), a classifier that predicts optimal cytoreduction to ≤ 1 cm should be used as an inclusion/exclusion criteria for trials, as has been done in the past.

Lastly, there is also the concern that resulting models would not translate into a simple test that could be performed easily, promptly and inexpensively in clinical settings before surgery. The best resulting models contained 2-3 types of genomic or clinical information. All these genomic parameters could be assessed by PCR-based analytical tests. PCR-based tests are cheap, fast and can be performed from small biological samples. Our study was performed in samples from an historical biobank, with flash frozen tissue that may have degraded over time. We expect to have better yields from fresh samples extracted exclusively to be applied to the model. In other gynecological tumor models, there was a high degree of concordance of molecular parameters and feasibility between pre-operative specimens and surgical specimens (45). We expect to see the same concordance with pre- and post-surgical HGSC specimens.

Conclusions

In summary, the results of our study are encouraging, despite some limitations due to the study design. Further work is needed to prospectively validate these models and to ascertain whether selection biases affected prediction model performances. Prospective validation in consecutive HGSC patients, using fresh tumor samples,

will further validate and select the best performing model, while optimizing it for rapid and inexpensive processing. Finally, this diagnostic tool could be assessed in a diagnostic trial, where specimens are collected before surgery to inform clinicians about potential cytoreduction options based on its performance. We foresee that this diagnostic tool will help surgeons to improve surgical outcomes, by increasing optimal cytoreduction rates, decreasing surgical morbidity, and with inclusion of the majority of advanced-stage HGSC patients.

Abbreviations

- HGSC – High grade serous carcinoma
- TCGA – The Cancer Genome Atlas
- GOG – Gynecologic Oncology Group
- UI – University of Iowa
- miRNA – Micro RNA
- lncRNA – long non-coding RNA
- SNV – single nucleotide variation
- CNV – copy number variation
- WHTR – Women's Health Tissue Repository
- RIN – RNA integrity number
- IIHG – University of Iowa Institute of Human Genetics
- gDNA – genomic DNA
- FT – fusion transcripts
- NCDB – National Cancer Database

Declarations

Ethics Approval and Consent to Participate: Tissue samples and clinical outcome data were obtained from the Department of Obstetrics and Gynecology Gynecologic Oncology Biobank (IRB, ID#200209010), which is part of the Women's Health Tissue Repository (WHTR, IRB, ID#201804817). All of the tissues archived in the Gynecologic Oncology Biobank (herein termed Biobank) were originally obtained from adult patients under informed consent in accordance with the University of Iowa Institutional Review Board guidelines. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for Publication: Not applicable

Availability of Data and Materials: Data for the prediction model has been submitted to the GEO at NCBI website: <https://www.ncbi.nlm.nih.gov/geo/>. Datasets with methylation data can be browsed by their accession number: GSE133556. Datasets with RNA-seq can be browsed by their accession number: GSE156699. The validation part of this study was performed in silico, with de-identified publicly available data. All data from TCGA is available at their website: <https://portal.gdc.cancer.gov/>. Software utilized by this study is also publicly available at Bioconductor website: <http://bioconductor.org/>.

Competing interests: Authors of this manuscript received funding from the National Institutes of Health NIH 5R01CA99908-18 (K. Leslie PI), Department of Defense OC190352 (K. Leslie PI), and the University of New Mexico P30CA118100 (PI C. Willman/A. Tomkinson).

Funding: This work was supported in part by the NIH 5R01CA99908-18 (K. Leslie PI), Department of Defense OC190352 (K. Leslie PI), and the basic research fund from the Department of Obstetrics & Gynecology at the University of Iowa. Also, this was supported in part by the American Association of Obstetricians and Gynecologists Foundation (AAOGF) Bridge Funding Award.

Author contributions: All authors contributed to the writing of this manuscript. In addition, Dr. Jesus Gonzalez-Bosquet is the principal investigator, providing conceptualization, supervision, methodology, and analysis. Funding acquisition was provided by Dr. Kimberly Leslie and Dr. Gonzalez-Bosquet. Dr. Eric Devor contributed methodology as well as supervision. Brian Smith is the principal statistician and contributed to methodology and conceptualization. Dr. Michael Goodheart contributed to supervision.

Acknowledgements: Not applicable

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Tables

Table 1: Patient characteristics and association with PCS outcome.

		All UI HGSC population			Samples with RNA-seq and DNA methylation		
		Optimal (N=273)	Suboptimal (N=132)	p-value	Optimal (N=52)	Suboptimal (N=31)	p-value
Preoperative characteristics	Age (mean)	61	61	0.742	62	58	0.204
	BMI (mean)	28.3	28.1	0.772	25.5	27.8	0.149
	Charlson Morbidity Index**						0.604
	Low (1-3)	44	19	Ref	8	6	Ref
	Medium (4-6)	182	87	0.738	31	16	0.548
	High (>6)	37	19	0.660	5	3	0.806
	Preop CA-125 (mean)	1,613	2,067	0.224	2,695	3,030	0.808
	Disease in Upper abdomen (Other than Omentum) by Imaging	203	101	0.638	34	20	0.936
	Large bowel	14	8	0.698	1	1	0.711
	Spleen	5	0	0.982	0	0	N/A
	Mesenteric LN	15	7	0.936	2	1	0.884
	Porta/Hepatis	21	17	0.097	2	1	0.884
	Ascites (upper abdomen)	115	56	0.954	20	8	0.241
	Other	90	52	0.204	12	11	0.225
	Disease in the Chest by Imaging	25	29	<0.001*	1	4	0.077
	Tumor	7	19	0.026*	1	2	0.313
	Pleural effusion	25	27	0.002*	1	2	0.313
	Neoadjuvant chemotherapy	22	9	0.700	10	4	0.450
Operative characteristics	FIGO Stage			0.001*			0.225
	III	224	89		40	20	
	IV	49	43		12	11	
	Hysterectomy	210 (77%)	79 (60%)	<0.001*	38 (73%)	18 (58%)	0.161
	Surgery to remove cervix	188 (69%)	63 (48%)	<0.001*	32 (62%)	13 (42%)	0.085
	Adnexectomy	263	125 (95%)	0.443	51	29 (94%)	0.313

		(96%)		(98%)			
	Omentectomy	260 (95%)	118 (89%)	0.031*	49 (94%)	29 (94%)	0.894
	Surgery large bowel	96 (35%)	35 (27%)	0.082	16 (31%)	9 (29%)	0.868
	Surgery small bowel	5 (2%)	3 (2%)	0.765	0 (0%)	1 (3%)	0.991
	Splenectomy	4 (1%)	0 (0%)	0.984	1 (2%)	0 (0%)	0.992
	Diaphragmatic stripping	8 (3%)	0 (0%)	0.977	5 (10%)	0 (0%)	0.992
	Para-aortic lymphadenectomy	56 (10%)	13 (21%)	0.009*	3 (6%)	3 (10%)	0.510
	Residual disease			0.981			0.988
	Microscopic	71	0		11	0	
	Macroscopic	202	132		41	31	
	Surgical complexity index [#]						
	Low	126	91	Ref	30	20	Ref
	Intermediate	140	41	<0.001*	20	11	0.685
	High	7	0	0.978	2	0	0.992
Outcomes	30-day mortality	4 (1.5%)	3 (2.3%)	0.558	0 (0%)	0 (0%)	N/A
	90-day mortality	8 (2.9%)	9 (6.8%)	0.071	0 (0%)	1 (1%)	0.991

*Statistically significant.

**Charlson Comorbidity Index is a measure of the prognostic burden of all associated morbidities to predict mortality and is the most validated measure of the prognostic impact of multiple chronic illnesses (www.charlsoncomorbidity.com).

[#]Surgical complexity score: score to predict surgical morbidity and 90-day mortality after primary debulking surgery for HGSC.

Figures

Figure 1

Flow of included patients in analysis.

Of the initial 470 HGSC patients, 405 were confirmed to be high-grade, advanced stage, of serous histology: **(a)** 273 underwent optimal PCS, and 132 underwent suboptimal PCS. 187 had frozen tumors in the biobank, and RNA-seq and DNA methylation was successfully processed in 83: **(b)** 52 underwent optimal PCS, and 31 underwent suboptimal PCS.

A Optimal cytoreduction RNA-seq patients: 62% (95% CI: 52-72%)

B

	Primary Debulking		Proportion of	95% CI
	Suboptimal	Optimal	optimal	
1987-1995	23	11	0.32	0.16, 0.49
1996-2000	26	60	0.70	0.60, 0.80
2001-2005	18	35	0.66	0.53, 0.79
2006-2010	37	77	0.68	0.59, 0.76
2011-2013	22	80	0.78	0.70, 0.87

Figure 2

Patients with optimal surgical outcomes at the University of Iowa from 1987-2013: **A.** There has been a steady increase in optimal surgical outcome during the study period, probably associated with improvements in operative and perioperative care. This improvement was statistically significant (p -value < 0.001). **B.** Proportions and 95% confidence intervals (CI) of optimal cytoreductive surgery by calendar year intervals. In the last available interval, 78% patient underwent optimal debulking (95% CI: 70%, 77%)

Figure 3

Performance of prediction models of treatment response.

A. The solid vertical bar represents the number of types of data: **1** (yellow): only one variable was included in the model; **2** (orange): combination of 2 types of variables. **B.** Panel with combination of 3 types of variables: solid vertical line with **3** tag (maroon).

Different performances on both panels are displayed in ascending order. The x axis is AUC as a percentage (0-100%). The red error mark displays the 95% confidence interval (CI). Overall, 120 models with different combinations of data were tested.

FT: Fusion transcripts; Met: DNA methylation; SNV: single nucleotide variation; CNV: gene copy number; DEXSeq: exon expression; IncRNA: long non-coding RNA; MIR: micro RNA, mRNA: gene expression. Graphics were generated with R package *ggplot*.

Figure 4

TCGA validation of prediction models of response.

A. The solid vertical bar represents the number of types of data (as per models constructed with UI data): **1** (yellow): only one variable was included in the model; **2** (orange): combination of 2 types of variables. **B.** Panel with combination of 3 types of variables: solid vertical brown line labeled “3”.

Different performances on both panels are displayed in ascending order. The x axis is AUC as a percentage (0-100%). The red error mark displays the 95% confidence interval (CI). Over 57% of TCGA models had an AUC 95% CI that overlapped with AUC 95% CI of UI models. Overall, 93 models were validated in TCGA

FT: Fusion transcripts; Met: DNA methylation; SNV: single nucleotide variation; CNV: gene copy number; DEXSeq: exon expression; IncRNA: long non-coding RNA; MIR: micro RNA, mRNA: gene expression. Graphics were generated with R package *ggplot*.⁶⁹

Figure 5

Performance of prediction models with selected variables from best initial models.

A. The solid vertical bar represents the number of types of data: **1** (yellow): only one variable was included in the model; **2** (orange): combination of 2 types of variables; **3** types of variables: solid vertical line with **3** label (maroon).

Different performances are displayed in ascending order. The x axis is AUC as a percentage (0-100%). The red error mark displays the 95% confidence interval (CI).

We included all predictions models with a 95% CI of AUC that included or was superior to 87%. Overall, 21 were selected with the potential to be superior to the clinical assessment.

FT: Fusion transcripts; Met: DNA methylation; SNV: single nucleotide variation; CNV: gene copy number; DEXSeq: exon expression; IncRNA: long non-coding RNA; MIR: micro RNA, mRNA: gene expression. Graphics were generated with R package *ggplot*.⁶⁹

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigureS1Pipelineofgenomicanalytics.jpeg](#)
- [SupplementaryTableS2Selectionofvariables.docx](#)
- [SupplementaryFigureS3Heatmapofselectedvariables.jpeg](#)

- [SupplementaryTableS4Specificvariables.xlsx](#)