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A 3'UTR KRAS-variant as a biomarker of poor outcome and platinum chemotherapy resistance in ovarian cancer

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A 3'UTR *KRAS*-variant as a Biomarker of Poor Outcome and Platinum Chemotherapy Resistance in Ovarian Cancer

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Abstract: A 3'UTR *KRAS*-variant as a Biomarker of Poor Outcome and Platinum Chemotherapy Resistance in Ovarian Cancer

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Purpose: Ovarian cancer has a poor prognosis, yet pathologic and clinical data do not accurately predict which patients will ultimately succumb to their disease. We previously reported an association between *rs61764370*, a germline functional variant in the 3'UTR of the *KRAS* oncogene, and epithelial ovarian cancer (EOC) risk. Here we evaluate this variant as a biomarker of clinical outcome and chemotherapy resistance in EOC.

Patients and Methods: Four groups of EOC patients with complete clinical data were genotyped for the *KRAS*-variant and analyzed: Sporadic EOC patients (n=451); *BRCA* mutant EOC patients (n=79); EOC patients treated with neoadjuvant chemotherapy (n=122), and; EOC patients treated adjuvantly with platinum-based chemotherapy after cytoreductive surgery (n=292).

Results: The *KRAS*-variant predicts for significantly worse survival for EOC patients over 55 years-old by multivariate Cox regression analysis (HR=1.71, 95% CI=1.09 – 2.69, p = 0.02). However, for the subgroup of EOC patients with known *BRCA* mutations, the *KRAS*-variant did not predict altered outcome (HR=0.994, CI=0.28-3.56, p=0.99). *KRAS*-variant positive EOC patients respond poorly to neoadjuvant carboplatin and paclitaxel chemotherapy, having significantly more residual disease remaining after surgery (OR=26.27, CI=1.56- 441.83, p=0.0232). In addition, EOC patients that harbor the *KRAS*-variant are more likely to be resistant to adjuvant platinum chemotherapy (OR=2.86, CI=1.13-7.23, p=0.026).

Conclusions: These findings expand the potential importance of the *KRAS*-variant in EOC, from acting as a marker of risk to being a biomarker that predicts worse outcome, perhaps due to its association with platinum resistance. These data may ultimately help lead to treatment optimization and improved outcome for *KRAS*-variant positive EOC patients.

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Introduction

Ovarian cancer is the 5th leading cause of cancer deaths in women. In 2010 there were 21,880 new cases and 13,850 deaths (1). Early symptoms are vague, and approximately 75% of patients have stage III or stage IV disease at the time of diagnosis. For those patients with ovarian adenocarcinoma, the 5 year survival rate is 37% for those with stage III disease and 25% for those with stage IV disease (2). Randomized trials conducted by the Gynecologic Oncology Group have demonstrated that prognosis worsens in epithelial ovarian cancer with older age, higher grade, higher stage, and malignant cytology (3). Serial monitoring of CA-125 levels is currently used in patient follow-up, but is not without controversy given its low sensitivity in early disease (4). As such, further identification and study of molecular markers is key to providing a more thorough assessment of cancer risk, patient prognosis and response to therapy.

Demographics

Although the incidence of ovarian cancer is lower than the incidence of uterine cancer (12.9 cases per 100,000 women per year vs. 23.5 cases per 100,000 women per year, respectively), ovarian cancer is the deadliest gynecologic malignancy (5). Indeed, the 5-year survival rate for patients diagnosed between 1999 and 2006, regardless of stage, was 45.6% in ovarian cancer compared to 82.7% in uterine cancer. This difference is at least in part attributable to the stage at diagnosis, as 62% of ovarian cancer patients have metastatic disease at the time of diagnosis, while only 8% of uterine cancer patients present with metastatic disease (5). Among patients with ovarian cancer, older age,

advanced stage at diagnosis, ascites volume, and the amount of residual disease were all significant predictors of reduced overall survival (6).

As recorded by the SEER database the median age of diagnosis of ovarian cancer is 63 years-old, and 68.6% presented over the age of 55. Although ovarian cancer affects all ethnicities, it is most common among Caucasian patients and least common in Asian women (13.5 cases vs. 9.8 cases per 100,000 women per year, respectively) (5).

BRCA Mutations

Approximately 10 – 15% of ovarian cancers occur in patients with *BRCA1/2* mutations (7) (8). There is a higher risk of ovarian cancer in *BRCA1* patients. By age 70, the average risk of diagnosis ranges from 40 – 60 % in *BRCA1* patients (9) (10). By contrast, in *BRCA2* patients, the average risk of diagnosis ranges from 11 - 27% by age 70 (11) (12). *BRCA1* patients also tend to be younger at the time of diagnosis (7).

Up to 40% of Ashkenazi Jewish patients with epithelial ovarian cancer are estimated to carry a *BRCA1* or *BRCA2* mutation (13). Ashkenazi Jewish women with a *BRCA1* or *BRCA2* mutation have a 16% risk of ovarian cancer diagnosis by age 70 (14).

Diagnosis

As previously noted, over 75% of ovarian cancer patients present with stage III or stage IV disease. Survival declines significantly with advanced stage. Five-year survival rates for patients who are treated with cytoreductive surgery and chemotherapy are as follows: 93% in stage I disease; 70% in stage II disease; 37% in stage III disease; and 25% in stage IV disease (2). In the majority of cases there is a delay of at least four

months from symptom onset to presentation to a physician (15) (16) (17). Indeed, a survey of 1725 ovarian cancer patients by Goff et al reported that 95% of patients experienced symptoms prior to diagnosis. The most common symptoms were: abdominal, such as increased abdominal size or bloating; gastrointestinal, such as nausea, indigestion, or constipation; and pain. Symptoms were not limited to patients with advanced disease at the time of diagnosis, as 89% of patients with stage I or II disease reported symptoms, compared to 97% of patients with stage III or IV disease (18).

A higher number of symptoms was significantly associated with a delay in diagnosis (mean of 2.0 months from presentation to diagnosis in patients with 2 symptoms vs. 10.7 months in patients with 6 or more symptoms, $p = 0.001$), as well as younger age (mean age of 53 years old in patients with 2 symptoms vs. 46 years old in patients with 6 or more symptoms, $p = 0.001$) and a treatment for an incorrect diagnosis (21% of patients with 2 symptoms vs. 50% of patients with 6 or more symptoms, $p = 0.001$). Of note, patients who were diagnosed earlier were significantly more likely to have received diagnostic tests such as a pelvic exam, abdominal pelvic CT scan, and CA-125 levels.

Diagnosis is also complicated by the vague nature of the symptoms. A prospective case-control study consisting of 1709 control patients and 128 patients with a pelvic mass demonstrated that many patients who do not have cancer may report similar symptoms. However, patients with cancer were more likely to have a higher median number of symptoms (8 symptoms vs. 4 symptoms in control patients). There was also a significant difference in the median number of recurring symptoms (4 symptoms in cancer patients vs. 2 symptoms in control patients, $p = 0.01$) (19).

Patients in the survey by Goff et al were diagnosed by family practitioners, internists, or obstetrician-gynecologists. While there was no significant difference in mean time to diagnosis between these specialties, obstetrician-gynecologists were significantly more likely to diagnose patients with stage I or stage II disease compared to family practitioners or internists. Indeed, 29% of patients diagnosed by obstetrician-gynecologists had stage I or stage II disease compared to 18% of patients seen by family practitioners or internists ($p = 0.009$). A retrospective review of 533 ovarian cancer patients also found that overall survival increased when patients saw a gynecologist first ($p < 0.05$) (20). This difference may be due to initial interventions, as obstetrician-gynecologists were also more likely to perform a pelvic exam and order other diagnostic tests, such as CA-125 levels. For example, a pelvic exam was performed on 94% of patients seen by an obstetrician-gynecologist compared to 50% seen by a family practitioner and 43% seen by an internist, $p = 0.001$ (18).

Treatment

The standard treatment for epithelial ovarian cancer consists of cytoreductive surgery, which includes surgical staging, and a platinum-based chemotherapy regimen.

Surgery

Surgical staging and pathology results are key to an accurate diagnosis and will also influence treatment decisions and prognosis (21) (22) (23). A prospective randomized trial demonstrated that patients who had cytoreductive surgery after receiving three cycles of platinum based chemotherapy had improved overall and progression-free

survival compared to patients who only received chemotherapy (24). Furthermore, patients who have optimal cytoreduction at the time of surgery, which is typically defined as residual tumor mass less than or equal to 1 cm in diameter, have improved overall survival (25) (26) (27) (28). A meta-analysis consisting of 81 cohorts containing a total of 6,885 patients with stage III or stage IV disease showed a statistically significant correlation between the degree of cytoreduction and the log median survival time ($p<0.001$). This correlation remained statistically significant even after controlling for factors such as patient age, disease stage at diagnosis, and platinum dose-intensity (27).

Chemotherapy

The standard chemotherapy regimen for epithelial ovarian cancer is a platinum-containing agent in combination with another drug, such as a taxane. The most common regimen currently used to treat EOC is carboplatin and paclitaxel (29) (30).

Cisplatin was approved by the FDA for use in ovarian cancer in 1978 (31). An early trial demonstrated improved response rates and progression-free survival with cisplatin combination therapies; however there were no significant differences in overall survival (32). In a larger trial by O'Mura et al containing 440 patients treated with doxorubicin and cyclophosphamide with or without cisplatin, those patients with palpable disease at the start of the trial who received cisplatin had improved progression-free and overall survival. However, when survival results for patients with non-palpable disease were combined with patients with palpable disease, the difference in survival was not significant (33). These studies were complicated by their recruitment, in that cisplatin based therapies were used primarily as salvage therapy, instead of first-line treatment.

While the use of cisplatin increased in ovarian cancer, it was also associated with significant toxicity, including nephrotoxicity, neurotoxicity and gastrointestinal distress. As such, the development of carboplatin in the 1980s represented an important new treatment option. Unlike cisplatin, carboplatin is associated with myelosuppression but carries a much lower risk of nephrotoxicity or neurotoxicity (31).

The efficacy of platinum-based chemotherapy has since been demonstrated in several trials. An early meta-analysis by Aabo et al studied 37 trials containing a total of 5667 patients. There was no significant difference in cisplatin compared with carboplatin (HR 1.02, $p = 0.74$). Adding platinum to a chemotherapy regimen improved overall survival by 5% at both 2 (45-50%) and 5 (25-30%) years (HR = 0.88, $p = 0.02$) (34). Numerous randomized trials have also demonstrated that carboplatin and paclitaxel provide a survival benefit equivalent to cisplatin and paclitaxel, but with fewer side effects (35) (36) (37).

Platinum Resistance

While some patients are platinum resistant, developing recurrence within 6 months of treatment, the majority of patients initially respond to platinum-based therapy but ultimately develop resistance (38). Among platinum-sensitive patients, who by definition recur more than 6 months after the end of treatment, the time to recurrence also has a significant impact on response to additional platinum based chemotherapy (39). A study by Gore et al found that while 53% (10/19) patients who recurred more than 18 months after the end of treatment responded to additional platinum-based treatment, only 17% (6/35) of patients who recurred within 18 months after the end of therapy had a

significant response (p = 0.006) (40). There was also a significant difference in median overall survival between the two groups (486 days for patients who recurred after 18 months vs. 221 days for patients who recurred within 18 months, p = 0.026). There is no standard second line therapy, and treatment approaches need to be individualized based on clinical factors. An improved understanding of the biologic differences in tumor behavior would allow subsequent treatments to have a more rational approach (41) (42) (43).

Interestingly, *BRCA1* or *BRCA2* mutant ovarian cancers are more sensitive to platinum-based chemotherapy. A matched case-control study found that ovarian cancer patients with *BRCA* mutations were more likely to respond to platinum based chemotherapy - - 81.8% of *BRCA*-positive patients had a complete response, compared to 43.2% of nonhereditary EOC patients (Fisher's exact test P = 0.004). *BRCA* patients also had a higher rate of response to second- and third-line therapies. This difference in platinum sensitivity in turn impacts overall survival. A multivariate Cox regression model which controlled for factors such as stage and age at diagnosis demonstrated a significantly higher risk of death in patients with nonhereditary EOC compared to *BRCA* mutant EOC patients (HR 4.539, 95% CI 1.83 – 11.24, p = 0.001) (44).

Neoadjuvant chemotherapy

Neoadjuvant chemotherapy has typically been applied to patients whose medical comorbidities prevent surgery or to patients whose disease burden is too substantial for optimal cytoreduction. These patients have similar overall and progression-free survival compared to patients who undergo the standard regimen of cytoreductive surgery

followed by chemotherapy (45). A retrospective analysis by Schwartz et al also found similar progression-free and overall survival in patients with intra-abdominal disease who received neoadjuvant chemotherapy compared to the standard regimen. This is significant because patients who received neoadjuvant chemotherapy tend to have a poorer performance status and a higher disease burden at the time of diagnosis (46).

Other studies have demonstrated improved overall survival in patients receiving neoadjuvant chemotherapy (47) (48). Kuhn et al demonstrated that patients with an ascites volume of over 500ml who received neoadjuvant chemotherapy had improved cytoreduction results ($p = 0.04$) and overall survival (median overall survival 42 months vs. 23 months, $p = 0.007$) (48). A retrospective review by Hou et al noted a statistically significant difference in progression-free and overall survival in patients with extra-abdominal disease who received neoadjuvant chemotherapy compared to patients who were treated with cytoreductive surgery followed by chemotherapy (49).

MicroRNAs and Cancer

MicroRNAs (miRNAs) are a class of non-protein coding single-strand RNAs ~22 nucleotides in length which negatively regulate multiple gene targets. Since the first miRNA was discovered in the roundworm *Caenorhabditis elegans* over fifteen years ago (50), more than 700 miRNAs have been identified in the human genome. MiRNAs inhibit gene expression either through the RNAi pathway which leads to mRNA degradation, or by binding to the 3' untranslated region ("UTR") of mRNA and blocking protein production during translation (51) (52). As miRNAs regulate hundreds of mRNAs, mutations in the miRNA itself or in its binding site could be associated with

malignant transformation or disease progression. Oncomirs, which are miRNAs associated with cancer, may function as tumor-suppressor genes or oncogenes. Tumor-suppressor genes include *let-7* in lung cancer, *mir-125b* in breast cancer, and *miR-15a* in B cell chronic lymphocytic leukemia (53). Oncogenes include *miR-155* in breast cancer (54), *miR-21* in glioblastoma (55), and *miR-155* in Burkitt and Hodgkin lymphoma (56). In addition, miRNAs have been found to predict prognosis, as well as response to therapy (57) (53).

The miRNA *let-7* family, which functions as tumor suppressors, negatively regulates the *RAS* pathway and HMGA2 (58). Deregulation of the *let-7* family occurs in several cancers, including lung, colon, breast, ovarian, pancreatic, and prostate (59).

KRAS-variant

The *let-7* family of miRNAs acts as tumor suppressors. It has been demonstrated in lung cancer that *let-7* is reduced in cancer tissue, and *RAS* is elevated (60). The Weidhaas lab previously identified a germline single nucleotide polymorphism, *rs61764370*, in the *let-7* complementary site 6 in the *KRAS* 3' UTR region (61). To assess the impact of the *KRAS*-variant on *KRAS* expression, A549 cells, a lung cancer cell line, were transfected with a luciferase reporter containing the *KRAS*-variant in the *KRAS* 3' UTR and with a luciferase reporter containing the wild-type *KRAS* 3'UTR. There was increased *KRAS* expression in the cells transfected with the *KRAS* variant than in cells transfected with the wild-type *KRAS* 3' UTR. Therefore, the *KRAS*-variant disrupts the binding of *let-7* to *KRAS*, leading to increased *KRAS* expression.

The frequency of the variant allele (referred to as the *KRAS*-variant) was 18.1 – 20.3% in NSCLC patients compared to 5.3% in healthy controls from 46 world populations. Moreover, in patients with a moderate smoking history, defined as less than 41 pack years, the *KRAS*-variant was associated with a 1.4 - 2.3 fold increased risk of NSCLC (OR 1.4, CI 1.1 – 1.7, p = 0.01; OR 2.3, CI 1.1 – 4.6, p = 0.02) (61).

The *KRAS*-variant has also been shown to predict outcome and response to therapy in other cancers. In patients with oral squamous cell cancers, the *KRAS*-variant was a statistically significant predictor for reduced overall survival (HR 2.7, 95% CI 1.4 – 5.3) (62). Among patients with irinotecan-refractory metastatic colorectal cancer treated with anti-epidermal growth factor inhibitor therapy, overall survival and progression-free survival were both significantly decreased in patients with the *KRAS*-variant (63).

The KRAS-variant and Ovarian cancer

In ovarian cancer, miRNA expression patterns can distinguish between not only cancer tissue and ovarian tissue, but also different ovarian cancer histotypes (64). MiRNAs were also shown to alter response to treatment - - specifically, upregulation of *mir-214*, which targets *PTEN*, was associated with cisplatin resistance. Decreasing levels of *mir-214* rendered the cancer cells susceptible to cisplatin in vitro (65).

Given the importance of *KRAS* in solid tumors, several cancer populations were tested for the *KRAS*-variant. The *KRAS*-variant was present in 25% of patients with epithelial ovarian cancer, compared to less than 18% in control populations or other cancerous populations with solid tumors. Case control analyses also demonstrated an

increased ovarian cancer risk in *KRAS*-variant patients (OR 2.46, CI 1.14 – 5.29, p = 0.020) (66).

To further assess the impact of the *KRAS*-variant on ovarian cancer risk, ovarian cancer patients with a family history consistent with hereditary breast and ovarian cancer syndrome ("HBOC") were tested. In addition to their own histories of ovarian cancer, HBOC patients have at least one other case of ovarian or breast cancer in a first- or second- degree relative. Patients who tested negative for the *BRCA1* or *BRCA2* mutation were classified as "uninformative" HBOC patients. There was a lower frequency of the *KRAS*-variant in *BRCA1* and *BRCA2* HBOC patients compared to uninformative patients. Indeed, the KRAS-variant was present in 61% of the uninformative patients with ovarian cancer, a frequency which was significantly higher than in ovarian cancer patients without a family history ($p < 0.001$) (66).

The family profiles of *KRAS*-variant patients were also different than *BRCA1*- or *BRCA2*- patients. Specifically, the patients in *KRAS*-variant HBOC families were more likely to be older at the time of diagnosis, non-Jewish, and to have a family history of lung cancer. As such, the *KRAS*-variant represents a new mechanism for identifying patients at elevated risk of epithelial ovarian cancer.

Summary

In summary, epithelial ovarian cancer continues to have a poor overall prognosis despite aggressive surgery and chemotherapy regimens. The *KRAS*-variant is an important new predictor of ovarian cancer risk. Given its prevalence in EOC, we

hypothesize that the *KRAS*-variant also impacts overall survival and response to platinum-based chemotherapy.

Statement of Purpose

This project will assess the impact of the 3'UTR *KRAS*-variant on overall survival and response to platinum-based chemotherapy in ovarian cancer.

First, we will statistically compare overall survival in *KRAS*-variant and wild-type non-*BRCA* mutant ovarian cancer patients in both univariate and multivariate analyses. We hypothesize that patients with the *KRAS*-variant will have reduced overall survival compared to wild-type patients.

Second, we will statistically compare the overall survival of *BRCA* mutant patients. An association between the *KRAS*-variant and *BRCA1* EOC patients has been previously noted (66). To control for this difference between *BRCA1* and *BRCA2* EOC patients, we will assess survival of each group separately as well. Of note, *BRCA* mutants typically have improved response to platinum based chemotherapy. Therefore, although we hypothesize that patients with the *KRAS*-variant will have reduced overall survival; this result may not be as significant as the difference in survival in non-*BRCA* mutant patients.

Third, we will assess response to platinum-based chemotherapy in *KRAS*-variant patients and wild-type patients by comparing the rate of optimal cytoreduction in patients who received neoadjuvant chemotherapy. We hypothesize that patients with the *KRAS*-variant will have a higher rate of suboptimal cytoreduction when controlling for age, stage, histology, and grade.

Fourth, we will compare the rate of platinum resistance in *KRAS*-variant and wild-type EOC patients who received the standard therapy of cytoreductive surgery followed by adjuvant platinum-based chemotherapy. We hypothesize that *KRAS*-variant patients

will have a higher rate of platinum resistance, defined as recurrence within 6 months of completion of chemotherapy. We also propose that *KRAS*-status will be a significant predictor of platinum resistance in a multivariate regression controlling for residual disease, age, stage, grade and tumor histology.

Methods

Survival analysis cohorts

Clinical data and DNA from women diagnosed with invasive epithelial ovarian cancer without known *BRCA* mutations were included from the following three institutions under individual IRB approvals: 1) Yale New Haven Hospital (*n*=194); 2) Turin, Italy #1 (*n*=198) (67); 3) Brescia, Italy #2 (*n*=59) (66). Patients diagnosed between 1998 and 2009 were included in the analysis. Information was collected on patient demographics, including age, race, and family history, as well as pathologic data such as stage, grade, and histology. Patients with unknown tumor histology were excluded from the analysis, as it was not possible to rule out the presence of a borderline tumor in those patients.

Documented *BRCA* mutant epithelial ovarian cancer cases were collected from the following two cohorts: 1) Yale New Haven Hospital (*n*=17); 2) City of Hope Comprehensive Cancer Center (*n*=62). As *BRCA1* and *BRCA2* mutations have been shown to independently influence survival in ovarian cancer, we evaluated the impact of the *KRAS*-variant on these groups separately.

As not all Stage I ovarian cancer patients receive chemotherapy, and substage information was not available for patients with Stage I tumors, these patients were excluded from the overall survival analyses for both non-*BRCA* and *BRCA* mutant patients. Only patients treated with chemotherapy were included in this analysis. We included women treated with neoadjuvant chemotherapy, and counted date of pathological diagnosis as the start date.

A total of 451 patients with wild type *BRCA* or not tested for *BRCA* mutations and 79 patients with documented *BRCA* mutations were included in the survival analyses. Overall survival time was measured as time from primary cytoreductive surgery or first administration of chemotherapy, whichever was earlier, to date of death or last visit.

Neoadjuvantly treated ovarian cancer patients

An IRB-approved review of the pathologic and treatment records from Yale New Haven Hospital between 1996 and 2010 was done to identify women with epithelial ovarian cancer treated with neoadjuvant chemotherapy followed by cytoreductive surgery (n=122). This cohort of patients received chemotherapy as a primary treatment due to tumor burden that was too extensive for optimal surgical debulking at presentation. Only patients diagnosed between 1998 and 2009 and treated with six cycles of carboplatin and paclitaxel were included in this analysis. Following chemotherapy, patients underwent cytoreductive surgery and additional adjuvant treatment. The following information was collected for these patients: age, race, ethnicity, *BRCA* status, family history, chemotherapy given prior to surgery, surgery performed, residual disease after surgery, stage, tumor histology, histologic grade and subsequent adjuvant chemotherapy. Optimal cytoreduction was defined as residual disease measuring less than 1cm remaining after surgery, while suboptimal cytoreduction was defined as residual disease measuring greater than or equal to 1cm at the completion of surgery. Only women operated on at Yale New Haven Hospital by the same group of surgeons were included, to avoid bias in surgical skill as a factor impacting residual disease.

Patients for analysis of platinum resistance

Platinum resistance was defined as progression-free survival (PFS) of less than 6 months from the completion of platinum containing adjuvant chemotherapy to the date of recurrence. The progression-free survival interval was available for women from three groups of patients: Italy #1, Italy #2, and Yale-New Haven Hospital patients (*n*=292), which included some of the patients analyzed for survival with additional patients who were not included in that analysis. This cohort underwent surgical staging and cytoreductive surgery prior to treatment with platinum-based chemotherapy. The following information was collected for these patients: age at diagnosis, *KRAS*-variant status, stage at diagnosis, histology, grade, progression-free survival, and date of death or last visit. Importantly, information on residual disease following cytoreductive surgery was also analyzed.

DNA Extraction

As previously shown by Chin et al, the *KRAS*-variant does not appear to be somatically acquired nor does it require a loss of heterozygosity. As such, DNA samples could be collected from tumor, blood or sputum samples. DNA extraction was performed at each institution using the techniques described below.

At Yale, DNA extraction was performed by FK Keane with assistance from S Nallur. E Ratner and T Paranjape assisted with the collection and processing of fresh frozen tissue samples.

Fixed Formalin Paraffin Embedded ("FFPE") tissue samples

FFPE samples were provided by the Yale New Haven Hospital Department of Pathology. DNA was isolated from FFPE tissue samples using the Ambion RecoverAll™ kit. First, 1 ml 100% Xylene was added to the sample and incubated in a 50°C water bath for 3 minutes. The sample was centrifuged at maximum speed for 2 minutes, the xylene was discarded, and the pellet was washed twice with 1 ml of 100% ethanol. After the pellet was air dried for 1 hour, 200 ul of Digestion Buffer and 4 ul of Protease were added. The entire sample was incubated overnight in a 50°C water bath. Following incubation, an Isolation Additive/ ethanol mixture consisting of 240ul of the Isolation Additive and 550 ul 100% ethanol was added to each sample. A filter cartridge was placed in a collection tube, and then 700ul of the sample was pipetted onto the filter cartridge. The tube was centrifuged at 10,000xg for 30 seconds, and the flow through was discarded. The remaining volume of the sample solution was added to the filter cartridge, centrifuged at 10,000xg for 30 seconds and the flow-through was discarded. The filter cartridge was washed with 700ul of Wash 1, centrifuged at 10,000xg for 30 seconds, and the flow-through was discarded. Next, 500ul of Wash 2/3 was added to the filter cartridge, centrifuged, and the flow through was discarded. The filter cartridge and collection tube were centrifuged for an additional 30 seconds and the remaining flow-through was discarded. Next, the filter cartridge was transferred to a new collection tube, and 60ul of Elution Solution preheated to 95°C was added to the cartridge, incubated at room temperature for 1 minute and then centrifuged at maximum speed. The sample was stored at -20°C.

Fresh Frozen Tissue samples

Fresh frozen tissue samples were collected and ground with a pestle in a 1.5ml tube while on dry ice. Next, 180ul of Buffer ATL was added followed by 20 ul Proteinase K. The sample was vortexed and incubated in a 56°C water bath for approximately 2 hours until all tissue was lysed. The sample was vortexed for 15 seconds, a 400ul 50:50 mixture of Buffer AL and 100% ethanol was added, and the sample was vortexed again. The mixture was pipetted onto a DNeasy Midi spin column in a collection tube, and then centrifuged for 1 minute at 8000rpm. The flow through and collection tube were both discarded after this step, as well as the Buffer AW1 and Buffer AW2 steps. After 500ul Buffer AW1 was added, the sample was centrifuged at 8000rpm for 1 minute. Next, 500ul Buffer AW2 was added to the column and the sample was centrifuged at 14000rpm for 3 minutes. The column was placed in a 1.5ml tube and 200ul Buffer AE was added to the column. After incubating at room temperate for 1 minute, the tube was centrifuged at 8000rpm for 1 minute. The column was discarded and the eluate was stored at -20°C.

Sputum samples

Sputum samples were collected using the Oragene-DNA kit and processed according to DNA Genoteck instructions. Sputum samples were stored with the Oragene-DNA solution after collection and were incubated overnight in a 50°C water bath prior to processing. Following incubation the entire sample was transferred to a 15 ml centrifuge tube. A volume of Oragene-Purifier (equivalent to 1/25th of the original sample) was added and mixed by vortexing for 10 seconds. The sample was incubated on ice for 10 minutes and then centrifuged for 20 minutes at a speed of 4,000xg. The

supernatant was transferred to a fresh tube and the pellet was discarded. An equal volume of 100% ethanol was added to the supernatant and mixed by inversion. Following incubation at room temperature for 10 minutes the sample was centrifuged for 15 minutes at a speed of 4,000xg. The supernatant was removed and discarded and 250ul of 70% ethanol was added to the pellet. After standing at room temperature for 1 minute, the ethanol was removed. The pellet stood at room temperature for approximately 1 hour. The DNA was rehydrated by adding 300 – 500ul of TE solution and then transferred to a 1.5ml tube. The sample remained at room temperature overnight and then was stored at -20°C.

Blood samples

Finally, DNA was extracted from blood samples using the QIAamp Blood Midi Kit. Each blood sample was divided and processed in two batches. First, 200ul Qiagen Protease was added to a 15ml centrifuge tube. Between 1 and 2 ml of blood were added to each tube and mixed by inversion. Following the addition of 2.4ml of Buffer AL, each tube was inverted 15 times and then shaken for 1 minute. After the sample was incubated in a 70°C water bath for 10 minutes, 2 ml 100% ethanol was added and the tube was inverted 10 times. Half of the sample pipetted onto a QIAamp Midi Column in a 15 ml centrifuge tube. The tube was centrifuged at 1850xg for 3 minutes, the flow-through was discarded, and the remaining half was added to the column. The sample was centrifuged again at 1850xg for 3 minutes and the flow-through was discarded. After 2ml of Buffer AW1 was added to the column, the sample was centrifuged at 4500xg for 1 minute. 2ml Buffer AW2 was added to the column and then the sample was centrifuged at 4500xg for

15 minutes. The column was placed in a new 15ml tube, and 300ul Buffer AE was added. After incubating at room temperature for 5 minutes, the sample was centrifuged at 4500xg for 2 minutes. The column was discarded and the eluate was stored at -20°C.

Detection of the KRAS-variant

The variant allele was detected using a primer specific to the *KRAS*-variant and a TaqMan PCR assay. First, 50-60ng of DNA in 9 ul dH2O was added to the wells of a 96-well plate. DNA from at least two known heterozygous variant, homozygous variant and homozygous wild-type samples were included on each plate. A master mix was prepared with 10ul of Taqman® Genotyping Master Mix for every 1ul of Taqman probe. Next, 11ul of this master mix was added to each well of the 96 well plate. The plate was centrifuged at 3000rpm for 1 minute and then run on the Applied Biosystems 7900HT Real-Time PCR System. Less than 3% of populations carry two copies of the variant (61). As such, patients who carried at least one copy of the variant allele were classified as *KRAS*-variant carriers. Assays were performed by FK Keane with guidance from T Paranjape.

Statistics

To assess the significance of demographic variables, a χ^2 test or a two-sided Fishers's exact test was used for categorical variables. A *t* test was used for continuous variables, such as age and follow-up time.

The overall survival time of *KRAS*-variant and wild-type patients was compared using the Kaplan-Meier method (68), and the statistical significance of the survival

curves was determined by the log-rank test (69). A Cox proportional hazards regression model (70) was used to assess the impact of the *KRAS*-variant and demographic and prognostic variables, including age, stage, grade, and histology, on overall survival.

Multivariate logistic regression analyses (71) were used to determine the impact of the *KRAS*-variant and other demographic and prognostic factors on the probability of suboptimal cytoreduction. Multivariate logistic regression analyses (71) were used to assess the association of the *KRAS*-variant and other prognostic factors on the probability of platinum resistance.

The statistical analyses described above were performed by F.K. Keane using SAS 9.1.3 (SAS Institute Inc., Cary, NC) with guidance from Lingeng Lu and Yanhong Deng.

Results

Survival in KRAS-variant positive EOC patients

We evaluated the association of the *KRAS*-variant with overall survival in EOC patients who had received surgery and platinum-based chemotherapy (*n*=451). The clinicopathologic parameters for this cohort are presented in **Table 1**.

Table 1. Clinicopathologic parameters for non-*BRCA* mutant EOC patients

Variable name	Wild type (n=348)	KRAS variant (n=103)	P value
Age (standard deviation)	60.44 (11.97)	58.77 (11.59)	0.2144
Stage			0.7747
1	52 (14.94)	15 (14.56)	
2	21 (6.03)	6 (5.83)	
3	194 (55.75)	52 (50.49)	
4	78 (22.41)	29 (28.16)	
Unknown	3 (0.86)	1 (0.97)	
Grade			0.0420
Well differentiated	29 (8.33)	14 (13.59)	
Moderately differentiated	60 (17.24)	8 (7.77)	
Poorly differentiated	228 (65.52)	74 (71.84)	
Unknown	31 (8.91)	7 (6.80)	
Histology			0.2230
Serous	202 (58.05)	52 (50.49)	
Endometrioid	37 (10.63)	16 (15.53)	
Undifferentiated	7 (2.01)	0 (0.00)	
Clear Cell	21 (6.03)	10 (9.71)	
Mucinous	17 (4.89)	2 (1.94)	
Carcinosarcoma	13 (3.74)	7 (6.80)	
Mixed	20 (5.75)	6 (5.83)	
Unknown	31 (8.91)	10 (9.71)	
Center			0.3417
Yale New Haven Hospital	156 (44.83)	38 (36.89)	
Italy #1	147 (42.24)	51 (49.51)	
Italy #2	45 (12.93)	14 (13.59)	
Follow up Time in months	38.84 (30.17)	35.96 (29.45)	0.3924

Because we have previously found that the *KRAS*-variant is not associated with early onset EOC, we evaluated the impact of the *KRAS*-variant on survival in women who developed EOC over the age of 55 (*n*=248). For this subset of EOC patients overall survival was significantly reduced in *KRAS*-variant positive patients compared to variant negative patients as shown by Kaplan-Meier curves (**Figure 1**, median survival of 30.00 months in KRAS-variant patients, vs. median survival of 58.50 months in wild-type patients, log rank p = 0.0103).

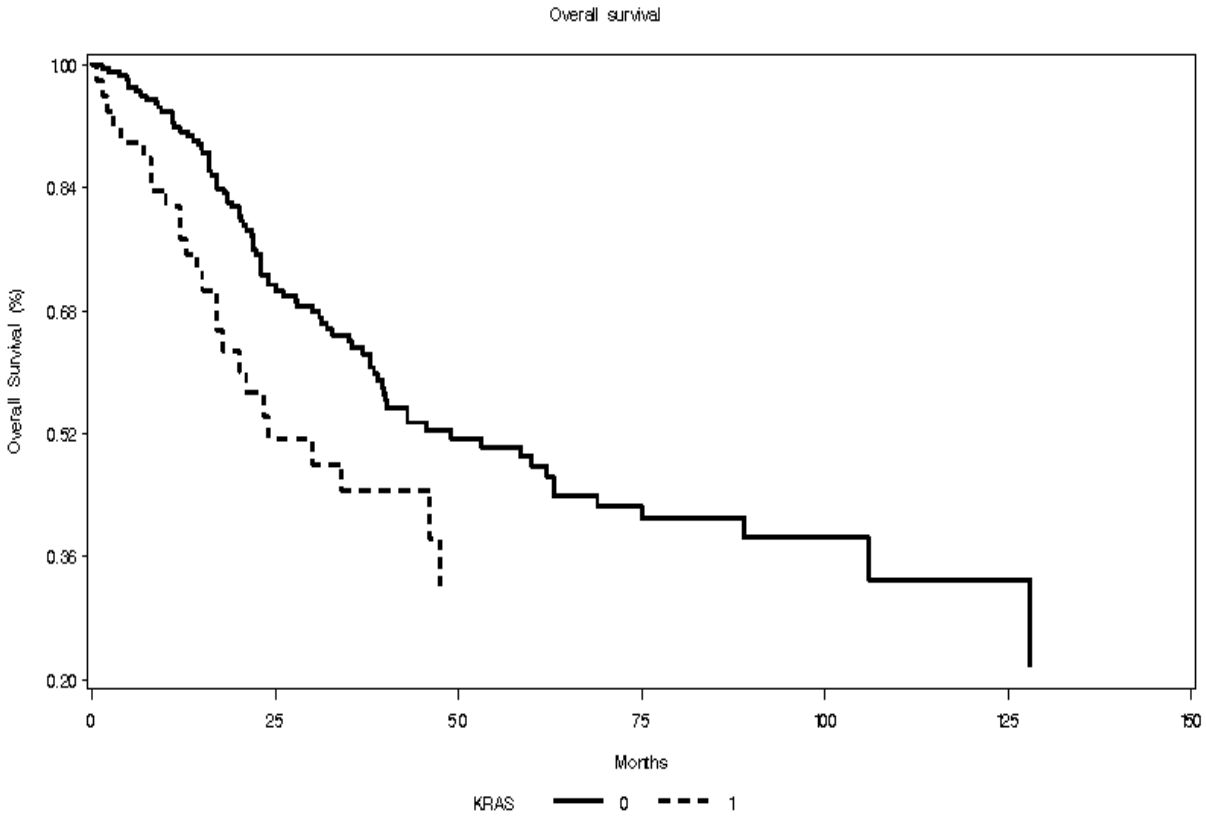

Figure 1: The *KRAS*-variant predicts significantly worse overall survival for epithelial ovarian cancer patients over 55 years-old. Overall survival for ovarian cancer patients with and without the *KRAS*-variant is compared using Kaplan Meier curves. There is significantly worse outcome for *KRAS*-variant positive ovarian cancer patients. Log rank p = 0.0103

The *KRAS*-variant was also a predictor of poor outcome for women over 55 years-old in a Cox proportional hazards multivariate model (**Table 2,** *KRAS*-variant HR = 1.71, 95% CI=1.09 – 2.69, p = 0.0204).

Table 2: The *KRAS*-variant Predicts Worse Survival for Ovarian Cancer Patients over 55 years-old

Variable	HR	95% CI	p value
KRAS status	1.71	1.09 – 2.69	0.0204
Stage	1.57	1.13 – 2.18	0.0078

HR: hazards ratio obtained from Cox proportional Hazards multivariate analysis
CI: confidence interval
Studies Included: Yale New Haven Hospital, Italy #1, Italy #2
Note: Age, Grade, and Histology were not statistically significant and were therefore excluded from the final analysis.

Survival in KRAS-variant BRCA positive EOC patients

We evaluated the impact of the *KRAS*-variant on survival in EOC patients carrying deleterious *BRCA1* or *BRCA2* mutations. Clinicopathologic parameters for these patients are presented in **Table 3**.

Table 3. Clinicopathologic parameters for *BRCA* mutant EOC patients

Variable name	Wild type (n=69)	KRAS variant (n=10)	P value
Age	52.77 (10.20)	52.60 (12.47)	0.9623
Stage			0.1771
1	5 (7.25)	2 (20.00)	
2	8 (11.59)	2 (20.00)	
3	51 (73.91)	5 (50.00)	
4	5 (7.25)	1 (10.00)	
Grade			0.5275
Well differentiated	2 (2.90)	1 (10.00)	
Moderately differentiated	13 (18.84)	1 (10.00)	
Poorly differentiated	49 (71.01)	8 (80.00)	
Unknown	5 (7.25)	0 (0.00)	
BRCA status			0.7206
BRCA 1	51 (73.91)	7 (70.00)	
BRCA 2	18 (26.09)	3 (30.00)	
Center			0.6808
Yale New Haven Hospital	16 (23.19)	1 (10.00)	
City of Hope	53 (76.81)	9 (90.00)	

Note: Histology information was not available for City of Hope patients

There was no significant difference in survival between those with and without the *KRAS*-variant in a multivariate analysis using a Cox proportional hazards model (**Table 4**, *KRAS*-variant HR = 0.99, 95% CI: 0.28-3.56, p = 0.99). However, the number of patients available for the analysis was low (*n*=79). Because we had previously seen an association of the *KRAS*-variant with *BRCA1* but not *BRCA2* mutations, we evaluated the impact of the *KRAS*-variant on survival separately for EOC patients with *BRCA1* mutations. Again there was no significant difference in survival in *KRAS*-variant positive versus negative patients in a multivariate analysis using a Cox proportional hazards model (*KRAS*-variant HR = 0.61, 95%CI: 0.14-2.76, p = 0.52). There were too few patients with *BRCA2* mutations for analysis.

Table 4: The *KRAS*-variant and Overall Survival in *BRCA* mutant patients

Variable	HR	95% CI	P value
All *BRCA* mutant patients (n = 72)			
KRAS status	0.994	0.28 – 3.56	0.9921
Age	1.028	0.996 – 1.06	0.0832
Stage	3.369	1.475 – 7.691	0.0039
***BRCA1* mutant patients (n = 54)**			
KRAS status	0.610	0.14 – 2.76	0.5202
Stage	3.748	1.43 – 9.80	0.0071

HR: hazards ratio obtained from Cox proportional Hazards multivariate analysis
CI: confidence interval
Studies Included: Yale New Haven Hospital, City of Hope

The KRAS-variant and response to neoadjuvant chemotherapy

To gain insight into the cause of the worse survival in *KRAS*-variant positive ovarian cancer patients, we evaluated the impact of *KRAS*-variant positivity on response to platinum chemotherapy. We included women with EOC who were treated at Yale-New Haven Hospital with neoadjuvant chemotherapy followed by surgical cytoreduction (n = 122), and used residual disease after surgery (cytoreduction) as a marker of patient response to neoadjuvant carboplatin and paclitaxel chemotherapy. The *KRAS*-variant allele was present in 22.13% of patients, a frequency which is similar to the prevalence of the *KRAS*-variant in EOC noted by Ratner et al (66). The mean ages of the *KRAS*-variant patients (n=27) and the wild-type (non-*KRAS*-variant) patients (n=95) were similar (62.67 ± 13.57 years old vs. 63.84 ± 12 years old in wild-type patients), and the majority of the patients were Caucasian (96.3% of *KRAS*-variant patients vs. 92.6% of wild-type patients). More *KRAS*-variant patients were classified as Stage IV compared to wild-type patients (85.2% vs. 53.7%, p=0.01). Complete clinicopathologic parameters of patients who received neoadjuvant chemotherapy are presented in **Table 5**.

Table 5. Clinicopathologic parameters of patients receiving neoadjuvant chemotherapy

Variable name	Wild type (n=95)	KRAS variant (n=27)	P VALUE
Age (standard deviation)	63.84 (12.00)	62.67 (13.57)	0.6636
Race			0.6832
Caucasian	88 (92.63)	26 (96.30)	
Other	7 (7.37)	1 (3.70)	
Stage			0.0106
2	1 (1.05)	0 (0.00)	
3	42 (44.21)	4 (14.81)	
4	52 (53.68)	23 (85.19)	
Unknown	1 (1.05)	0 (0.00)	
Grade			0.1923
Well differentiated	2 (2.11)	0 (0.00)	
Moderately differentiated	12 (12.63)	0 (0.00)	
Poorly differentiated	69 (72.63)	24 (88.89)	
Unknown	12 (12.63)	3 (11.11)	
Histology			0.6138
Serous	72 (75.79)	18 (66.67)	
Endometrioid	2 (2.11)	0 (0.00)	
Undifferentiated	3 (3.16)	0 (0.00)	
Clear Cell	4 (4.21)	2 (7.41)	
Mucinous	1 (1.05)	0 (0.00)	
Carcinosarcoma	1 (1.05)	1 (3.70)	
Mixed	5 (5.26)	3 (11.11)	
Unknown	7 (7.37)	3 (11.11)	
Neoadjuvant Chemotherapy			0.2014
Carboplatin/ Paclitaxel	83 (88.87)	20 (74.07)	
Carboplatin/ Taxotere	1 (1.05)	1 (3.70)	
Carboplatin/ Cyclophosphamide	8 (8.42)	5 (18.52)	
Other	3 (3.16)	1 (3.70)	
Neoadjuvant cycles completed:			0.2328
2	2 (2.11)	0 (0.00)	
3	4 (4.21)	2 (7.41)	
4	17 (17.89)	2 (7.41)	
5	3 (3.16)	4 (14.81)	
6	66 (69.47)	19 (70.37)	
7	2 (2.11)	0 (0.00)	
9	1 (1.05)	0 (0.00)	
Follow up time	31.54 (27.52)	37.22 (36.56)	0.3834

Optimal cytoreduction was defined as less than 1cm of residual disease remaining at the completion of surgery, and suboptimal cytoreduction was defined as greater than 1cm of residual disease left at the completion of surgery. We found that 20.0% of *KRAS*-variant patients were suboptimally cytoreduced, compared with only 2.0% of wild-type patients (**Figure 2)**.

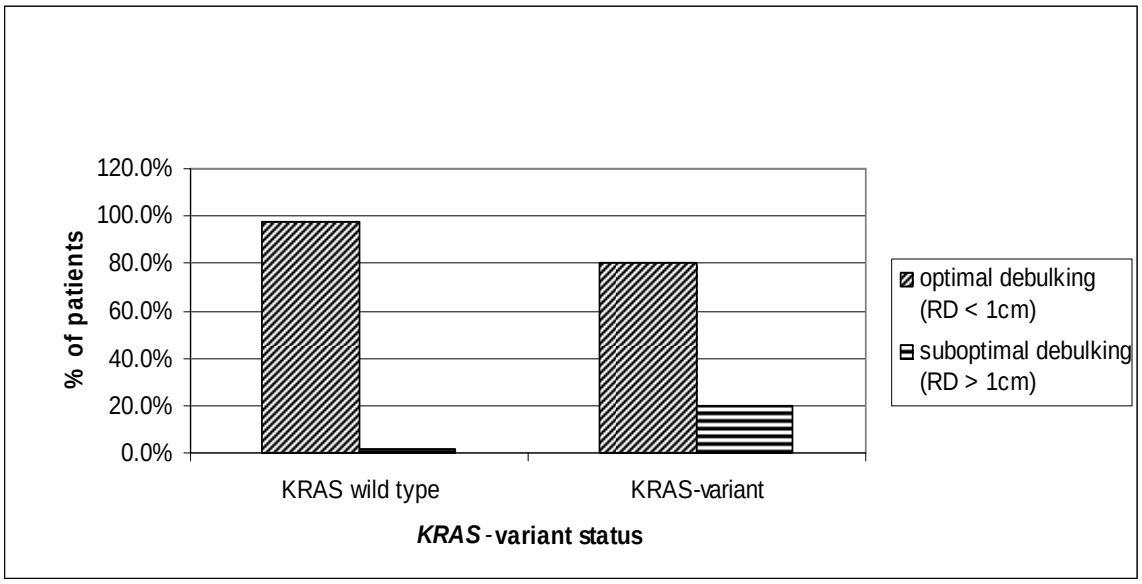

Figure 2: Surgical debulking in Stage III and IV patients treated with six cycles of neoadjuvant carboplatin and paclitaxel

The *KRAS*-variant was significantly associated with residual disease greater than 1 cm (suboptimal cytoreduction) after neoadjuvant chemotherapy and surgery in a multivariate logistic regression controlling for age and histology (**Table 6,** OR = 26.27, 95% CI: 1.56 – 441.83, p = 0.0232).

Table 6. The *KRAS*-variant Predicts Suboptimal Debulking after Neoadjuvant Chemotherapy

***KRAS*-variant Genotype**	**Univariate**			**Multivariate[3]**		
	OR[1]	95% CI[2]	p	OR	95% CI	p
All						
Wild-type (*n*=58)	1.00			1.00		
Variant (*n*=15)	14.25	1.36 – 149.01	0.027	26.27	1.56 – 441.83	0.0232

1. OR: odds ratio obtained from logistic regression
2. CI: confidence interval
3. Multivariate: adjusted for age and histology.

The KRAS-variant is associated with platinum resistance

To better evaluate if the increase in residual disease after neoadjuvant carboplatin and paclitaxel chemotherapy seen in *KRAS*-variant positive EOC patients was due to chemotherapy resistance and not simply desmoplasia of the tumors, we directly assessed platinum resistance in adjuvantly treated EOC patients with known *KRAS*-variant status (*n*=292). The median ages of the *KRAS*-variant patients (*n*=68) and the wild-type patients (*n*=224) were similar (56.97 ± 10.21 years old vs. 58.83 ± 11.87 years old in wild-type patients). The majority of both *KRAS*-variant and wild-type patients had Stage III or Stage IV disease (69.1% vs. 69.6% in wild-type patients). Importantly, we found that in this cohort, optimal cytoreduction before chemotherapy was no different between *KRAS*-variant positive and wild-type patients (60.29% vs. 59.24%). Complete clinicopathologic parameters for patients included in the platinum resistance analysis are presented in **Table 7**.

Table 7. Clinicopathologic parameters for platinum resistance analysis

Variable name	Wild type (n=224)	KRAS variant (n=68)	P VALUE
Age	58.83 (11.87)	56.97 (10.21)	0.1179
Stage			0.9830
1	49 (21.88)	16 (23.53)	
2	19 (8.48)	5 (7.35)	
3	134 (59.82)	40 (58.82)	
4	22 (9.82)	7 (10.29)	
Grade			0.1593
Well differentiated	27 (12.05)	14 (20.59)	
Moderately differentiated	42 (18.75)	8 (11.76)	
Poorly differentiated	140 (62.50)	44 (64.71)	
Unknown	15 (6.70)	2 (2.71)	
Histology			0.2746
Serous	107 (47.77)	29 (42.65)	
Endometrioid	36 (16.07)	15 (22.06)	
Undifferentiated	27 (12.05)	7 (10.29)	
Clear Cell	14 (6.25)	8 (11.76)	
Mucinous	19 (8.48)	2 (2.94)	
Carcinosarcoma	11 (4.91)	5 (7.35)	
Mixed	9 (4.02)	1 (1.47)	
Unknown	1 (0.45)	1 (1.47)	
Platinum response			0.0262
Sensitive	210 (93.75)	58 (85.29)	
Resistant	14 (6.25)	10 (14.71)	
Cytoreductive surgery			0.1115
Optimal cytoreduction (<1cm residual disease)	125 (55.80)	41 (60.29)	
Suboptimal cytoreduction (>1cm residual disease)	86 (38.39)	27 (39.71)	
Unknown	13 (5.80)	0 (0.00)	
Center			0.1934
Yale New Haven Hospital	44 (19.64)	7 (10.29)	
Italy #1	147 (65.63)	51 (75.00)	
Italy #2	33 (14.73)	10 (14.71)	
Follow up Time	44.33 (35.24)	34.35 (22.33)	0.0283

Platinum resistance, defined as disease recurrence within 6 months of receiving platinum based chemotherapy, was significantly more prevalent in *KRAS*-variant carriers

than in wild-type patients (14.71% vs. 6.25%, $p < 0.0307$). The *KRAS*-variant was a statistically significant predictor for platinum resistance in a multivariate logistic regression analysis controlling for residual disease remaining after cytoreductive surgery, stage, and grade (**Table 8**, OR = 2.86, 95% CI: 1.13 – 7.23, p = 0.0264).

Table 8. The *KRAS*-variant Predicts Platinum resistance

***KRAS*-variant Genotype**	**Univariate**			**Multivariate[3]**		
	OR[1]	95% CI[2]	p	OR	95% CI	p
All						
Wild-type (*n*=224)	1.00			1.00		
Variant (*n*=68)	2.59	1.09 – 6.12	0.0307	2.86	1.13 – 7.23	0.0264

1. OR: odds ratio obtained from logistic regression
2. CI: confidence interval
3. Multivariate: adjusted for stage, grade, and residual disease
Studies: Yale, Italy #1, Italy #2

Discussion

Our data supports the hypothesis that the *KRAS*-variant is associated with reduced overall survival in EOC patients who do not carry a *BRCA* mutation, as well as a higher risk of suboptimal cytoreduction and platinum resistance. There was no significant difference in survival among *BRCA* patients, although this will need to be confirmed with a larger patient cohort.

Although the association of the *KRAS*-variant with risk of EOC is strongest in patients from high-risk breast and ovarian cancer families(66), our data demonstrates that the *KRAS*-variant is an important marker of poor survival in nonhereditary EOC patients, as well.

Overall Survival in non-BRCA mutant patients

The identification of reduced overall survival in nonhereditary EOC patients represents an important step in the understanding of the role of the *KRAS*-variant. Indeed, the KRAS-variant was a significant predictor of reduced overall survival in patients over 55 years old in both a Kaplan-Meier analysis and in a Cox proportional hazards multivariate analysis controlling for stage. Age, grade and histology were not significant predictors of reduced overall survival by the Cox proportional hazards model and were therefore excluded from the final analysis.

The significant association of the *KRAS*-variant with poor survival for women over age 55 may be due to the unintentional inclusion of *BRCA* mutant patients or patients with non-epithelial tumors in the larger cohort of ovarian cancer patients studied,

both of whom would most likely be found in the group of women diagnosed under the age of 55.

Alternatively, these findings may instead reflect some true underlying biology of the *KRAS*-variant, which appears not to be associated with early onset tumors, and may instead be affected or accelerated by the aging process. The association of the *KRAS*-variant with diagnosis at older age was previously demonstrated in non-*BRCA* mutant HBOC families (66). This hypothesis requires additional validation. Of note, given the reduced survival of patients with the *KRAS*-variant, it is important for case-control studies to avoid delays in patient enrollment. Significant delays would likely lead to inadvertent underrepresentation of patients with the *KRAS*-variant.

Overall Survival in BRCA mutant patients

The *KRAS*-variant did not predict for reduced survival in *BRCA* mutant patients. In both a univariate analysis and a Cox proportional hazards multivariate regression controlling for age and stage there was no significant difference in the risk of death. This may be secondary to the low sample size. However, this observation may also reflect the fact that *BRCA* mutations are associated with platinum-sensitivity (44) and this effect may be downstream of any resistance caused by the *KRAS*-variant to platinum agents. Studies are underway to determine if the *KRAS*-variant predicts altered resistance to other chemotherapeutic agents in the background of *BRCA* mutations.

Of note, there were no *BRCA2* patients with the *KRAS*-variant who died during the analysis period. This precluded a comparison of overall survival in *BRCA2* patients through a Kaplan-Meier analysis.

Response to neoadjuvant chemotherapy

Our data demonstrated that *KRAS*-variant patients were more likely to be suboptimally cytoreduced than wild-type patients. Importantly, all patients included in this analysis had received six cycles of platinum-based combination chemotherapy prior to neoadjuvant surgery. Furthermore, the surgeries were all performed by gynecologic oncologists at Yale New Haven Hospital. This is important for two reasons. First, patients who are operated on by a gynecologic oncologist instead of a gynecologist or a general surgeon have improved overall survival (72) (73). This difference is likely due to more accurate surgical staging by gynecologic oncologists (21) as well as an increased likelihood of optimal surgical debulking (73) (74). General surgeons who have not received training in cytoreductive surgery tend to achieve optimal cytoreduction in 25% or less of cases (75) (27). By contrast, gynecologic oncologists at tertiary care centers have been shown to achieve optimal cytoreduction in up to 76% of cases (76) (77). Of note, these studies defined optimal cytoreduction as less than 2cm of residual disease remaining at the end of cytoreductive surgery. The acceptable level of residual disease for optimal cytoreduction has since been revised to less than or equal to 1cm. The current standard of 1cm was used to evaluate cytoreduction in our analysis.

Second, even in a tertiary care setting, optimal debulking varies with surgeon philosophy - - defined as how frequently a surgeon employs radical procedures such as diaphragm stripping and bowel resection. In other words, increasing aggressiveness was associated with a higher rate of optimal debulking (78). By limiting the analysis to patients treated by one department, Gynecologic Oncology at YNHH, we reduced

discrepancies in surgeon philosophy which may have been present if numerous departments or hospitals were included.

In addition to the above restrictions, we also controlled for age, stage, histology and grade in a multivariate logistic regression. As such, we were able to accurately assess the impact of *KRAS*-status on the rate of optimal cytoreduction. The elevated rate of suboptimal debulking in *KRAS*-variant patients compared to wild-type patients raises questions as to the ideal chemotherapy regimen for *KRAS*-variant patients. Further study will be required to determine if patients would benefit from altering the current standard regimen.

Platinum resistance

The data demonstrated a significant association of the *KRAS*-variant with platinum resistance in non-*BRCA* mutant EOC patients. This association held even when controlling for stage, grade, and residual disease remaining after cytoreductive surgery. The risk of platinum resistance, as well as the association of suboptimal cytoreduction with the *KRAS*-variant, suggests that the reduced overall survival in *KRAS*-variant patients may be secondary to resistance to current chemotherapy regimens.

The current approach to tailor cancer treatment has been to use assays that measure tumor-acquired mutations to try and predict tumor response to different therapeutic regimens. This approach is inherently limited by the inability to confirm which mutations, of which there are many, truly drive tumor biology for an individual's tumor.

By contrast, the germline *KRAS*-variant appears to lead to biologically and behaviorally similar tumors, allowing sub-classification of these tumors, and affords the potential in the near future to use this marker to tailor and optimize patient treatment. Indeed, several studies have documented the association of polymorphisms with response to chemotherapy, progression-free survival and overall survival. In colorectal cancer, polymorphisms in drug metabolism genes have been associated with reduced overall survival (79) as well as impaired treatment response (80) and toxicity (81). A retrospective review of NSCLC patients receiving platinum-based chemotherapy demonstrated that polymorphisms in DNA repair genes were associated with reduced overall survival (82). Further study will be necessary to determine the ideal chemotherapy regimen for *KRAS*-variant EOC patients.

Conclusions

These data demonstrate the potential application of the *KRAS*-variant as a biomarker for poor outcome in epithelial ovarian cancer. The poor survival of patients with the *KRAS*-variant may be associated with resistance to platinum-based chemotherapy, as *KRAS*-variant patients had a significantly higher risk of suboptimal cytoreduction and platinum resistance. Further understanding of the mechanism of the *KRAS*-variant in tumor biology and response to chemotherapy will require additional work in cancer initiation models, which is ongoing. Regardless, identification of inherited variants that are biomarkers of outcome is an exciting advance in clinical oncology and will hopefully lead to more effective treatment options and improved patient survival.

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